HANDBOOK OF FRUIT AND VEGETABLE FLAVORS

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Summary: "This book provides a comprehensive reference on the flavor science and technology of fruits and vegetables, spices, and oils. Beginning with an introduction on the specific flavors and their commercialization, the book then details the technical aspects including biology, biotechnology, chemistry, physiochemistry, processing, analysis, extraction, commodities, and requirements for application as food additives. Regulatory considerations are discussed in relation to sanitation and safety in a flavor manufacturing establishment" – Provided by publisher.
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PREFACE

For the last 50 years, our knowledge of the science and technology of food flavors has increased tremendously. To distribute the information, publishers have released many professional reference books on the subject. In general, most of the books have some discussion on the flavors of fruits and vegetables, though several of them specifically address these two important groups of food commodities. Information on the flavors of fruits and vegetables is essential to the work of government, academia, and industry. This book is an updated reference treatise on the flavors of fruits and vegetables. It includes 55 chapters, with 31 on the flavor of fruits and 24 on that of vegetables, covering the following topics:

- biology, chemistry, and biochemistry
- biotechnology and genetic engineering
- analytical methodology
- processing technology
- fresh and processed commodities
- products derived from processed fruits and vegetables
- regulatory consideration

There are several professional books on the subject matter and the preference for any particular one depends on the needs of the users. Although many topics are included in this volume, we do not claim that the coverage is comprehensive.

This work is the result of the combined efforts of more than 70 individuals from industry, government and academia worldwide. They represent the expertise of professionals from 18 countries including Belgium, Brazil, China, Canada, Croatia, Cuba, France, India, Indonesia, Israel, Italy, Japan, Kuwait, Malaysia, Mexico, Portugal, Spain, Turkey, and the United States. The editorial team consists of 12 established experts in the flavors or processing of fruits and vegetables. 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 in many respects. It is our sincere hope and belief that it will serve as an essential reference on the flavors of major plant foods.

We wish to thank all the contributors for sharing their expertise throughout our journey. We also thank the reviewers for giving their valuable comments leading to

improvements in the contents of each chapter. In addition, we thank members of the production team at John Wiley and Sons for their time, effort, advice, and expertise. All these professionals are the ones who made this book possible. You are the best judge of the quality of their work and we trust that you will benefit from the fruits of their labor.

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LIST OF ABBREVIATIONS

AECA	aroma extract concentration analysis
AEDA	aroma extraction dilution analysis
APCI-MS	atmospheric pressure chemical ionization–mass spectrometry
DAD	diode array detection
DSA	descriptive sensory analysis
GC	gas chromatography
GC-FTIR	gas chromatography–Fourier transform infrared spectroscopy
GC-MS	gas chromatography–mass spectrometry
GC-O	gas chromatography–olfactometry
HPLC	high-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography diode array detection
HPLC-DAD-MS/MS-ESI	high-performance liquid chromatography–diode array detection–mass spectrometry/mass spectrometry–electrospray ionization
HRGC	high-resolution gas chromatography
HRGC-MS	high-resolution gas chromatography–mass spectrometry
HS	headspace
HSE	headspace extraction
HSSE	headspace sorptive extraction
LC	liquid chromatography
LLE	liquid-liquid extraction
OPLC	optimum performance laminar chromatography
PTR-MS	proton transfer reaction mass spectrometry
SBSE	stir bar sorptive extraction
SDE	simultaneous distillation-extraction
SDEV	simultaneous distillation-extraction under vacuum
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SPE	solid phase extraction
SPME	solid phase microextraction
SPME-GC	solid phase microextraction–gas chromatography

SPME-GC-MS	solid phase microextraction–gas chromatography–mass spectrometry
SSF	solid-state fermentation
TD-GC–MS	thermal desorption–gas chromatography–mass spectrometry
TLC	thin-layer chromatography
UAE	ultrasound-assisted extraction
VHS	vacuum headspace

SECTION A: FRUIT FLAVORS

PART I

FRUIT FLAVORS: BIOLOGY, CHEMISTRY, AND PHYSIOCHEMISTRY

CHAPTER 1

Fruits and Fruit Flavor: Classification and Biological Characterization

YUEMING JIANG[1] and JUN SONG[2]

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Fruit has always been a part of the human diet and is an important nutritional source, with high water content (70–85%) and a relatively high amount of carbohydrates but low contents of fat (less than 0.5%) and protein (<3.5%). It usually contains many useful vitamins as well as minerals, dietary fiber, and antioxidants (Goff and Klee 2006; Knee 2002). From 2002 to 2007, there has been a steady increase in fruit production with 2.67% each year, partly in response to population growth and living standard improvement in most countries and effective encouragement by government health agencies of fruit consumption. In 2007, a total amount of 318.6 million tons of fruit was produced in the world, which is equivalent to 48.2 kg per capita of production and a fruit consumption of 12 kg per capita (Euromonitor 2008; http://faostat.fao.org).

In this chapter, the botanical information, characterization, importance, and production of fruits are briefly reviewed. The chapter provides general information about fruit and draws comparisons between fruit and fruit flavor. Flavor characterization is also discussed in detail.

CLASSIFICATION OF FRUITS

There are different ways to classify fruit (Table 1.1). Generally speaking, the outer, often edible layer in fleshy fruits is the pericarp, which develops from the ovary wall of the flower and surrounds the seeds. While the seeds are akin to the egg development in the ovary of a fowl, the pericarp may be assumed as the uterus. However, a small number of fruits do not fit into this description. For example, in most nuts, the edible part is the seed but not the pericarp. In addition, many edible vegetables such as cucumber and squash are common pericarp and are botanically considered as fruits. In this chapter, the use of the term "fruit" will not refer to these vegetable

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TABLE 1.1. Types of Fruit

True Berry	Pepo	Hesperidium	False Berry	Aggregate Fruit	Multiple Fruit	Other Accessory Fruit
Black currant	Pumpkin	Orange	Banana	Blackberry	Pineapple	Apple
Red currant	Cucumber	Lemon	Cranberry	Raspberry	Fig	Peach
Gooseberry	Melon	Lime	Blueberry	Boysenberry	Mulberry	Cherry
Pomegranate		Grapefruit		Hedge apple		Strawberry
Avocado						
Kiwifruit						
Grape						

fruits. In some fruits such as lychee and longan, the edible portion is actually an aril. From the botanical point, fruits can be classified into simple fruits, aggregate fruits, and multiple fruits on the basis of anatomical attributes.

Simple Fruits

Simple fruits are formed from a single ovary and may contain one to many seeds, which have developed as part of the fruit. Simple fruits can be divided into two groups: fleshy pericarp—berries, drupes, and pomes; and dry pericarp—nuts. Types of fleshy and simple fruits are berry (red currant, gooseberry, and avocado), stone fruit or drupe (plum, cherry, peach, apricot, olive), false berry—epigynous accessory fruits such as banana and cranberry, and pome—accessory fruits such as apple and pear. In contrast to fleshy and simple fruits, in nuts, it is the stony layer that surrounds the kernel of pecans and is removed when eating.

Aggregate Fruit

Aggregate fruits are formed from a single compound flower and contain many ovaries. Examples include strawberries, raspberries, and blackberries. An aggregate fruit or etaerio develops from a flower with numerous simple pistils. An example is the raspberry, whose simple fruits are termed as drupelets because each is like a small drupe attached to the receptacle. In some bramble fruits (such as blackberry), the receptacle is elongated and part of the ripe fruit, which makes the blackberry an aggregate-accessory fruit. The strawberry is also an aggregate fruit, in which the seeds are contained in achenes.

Multiple Fruit

Multiple fruits, such as pineapple, fig, and mulberry, are formed from the fused ovaries of many separate but closely clustered flowers. There are also many dry multiple fruits, for example, tulip tree (multiple of samaras), sweet gum (multiple of capsules), sycamore and teasel (multiple of achenes), and magnolia (multiple of follicles).

As described above, fruits can be summarized into eight types: (1) berry—simple fruit and seeds developed from a single ovary, (2) pepo—berries where the skin is

hardened, (3) hesperidium—berries with a rind, (4) false berries—epigynous fruit made from a part of the plant other than a single ovary, (5) compound fruit—from several ovaries in either a single flower or multiple flowers, (6) aggregate fruit—multiple fruits with seeds from different ovaries of a single flower, (7) multiple fruit—fruits of separate flowers packed closely together, and (8) other accessory fruit—where the edible part is not generated by the ovary. Another common way to classify fruits is based on growing regions such as temperate zone fruits, subtropical fruits, and tropical fruits (Kader 2002).

SPECIES, VARIETIES, AND BIOLOGICAL CHARACTERISTIC OF MAJOR FRUITS

The major fruits, such as apple, pear, grape, strawberry, citrus, banana, and mango, currently contribute the most of the total world production. About two-thirds of the major fruits produced worldwide are consumed as fresh fruit.

As discussed above, fruits are classified mainly on the basis of the ovary characteristic. In biology, fruit species can be classified by their botanical origin. In this following section, species, varieties, biological characteristic, and production of major fruits are briefly reviewed.

Apple

The genus *Malus* belongs to the Rosaceae family and forms with its closely related fruit (*Pyrus* and *Cydonia*) and ornamental (*Amelanchier*, *Aronia*, *Chaenomweles*, *Cotoneaster*, *Crateagus*, *Pyracantha*, *Sorbus*) genera, the subfamily Maloideae. Nowadays, *Malus* × *domestica* Borkh has been widely applied for apples.

World apple production reached 66 million tons in 2007 (Euromonitor 2008). Apple production is dominated by cultivars, such as "Delicious," "Gold Delicious," "McIntosh," "Jonathan," "Cox's Orange Pippin," "Granny Smith," and "Braeburn." In Asia, these varieties often replace the local varieties selected from the native species *Malus prunifolia* and its cultivated species *Malus asiatica*. China's enormous growth in apple production is entirely due to the introduction of the "Fuji" cultivar.

Banana

Banana belongs to the genus *Musa* in the family Musaceae, order Zingiberales. The family Musaceae comprises two genera viz., *Musa* and *Ensete*. The genus *Musa* comprises all the edible bananas and plantains with over 50 species. Bananas are perennial monocotyledonous herbs that grow well in humid tropical and subtropical regions. The origin of banana is traced back to Southeast Asia in the jungles of Malaysia, Indonesia, or the Philippines. Banana originated from two wild diploid species namely, *Musa acuminate* Coll and *Musa balbisiana* Coll. *M. acuminate* is native of the Malay Peninsula and adjacent regions, while *M. balbisiana* is found in India eastward to the tropical Pacific.

Bananas are the fourth world's most important food crop after rice, wheat, and maize, with production of 73 million tons in 2007 (Euromonitor 2008). The majority

of the banana crops are grown in the tropical and subtropical zones. From a consumer perspective, bananas are nutritious with a pleasant flavor and widely consumed throughout the world. India is the world's leading producer of banana and plantain, followed by Brazil and China.

Grape

The *Vitis vinifera* L. grape is one of the oldest cultivated plants and is thought to have originated in the region between the Mediterranean and the Caspian Sea. Cultivars of the vine slowly spread eastward across southern Asia and westward around the Mediterranean Sea. The Germplasm Resources Information Network (http://www.ars-grin.gov) of the United States Department of Agriculture describes the genera and 43 species, 5 natural hybrids, and 15 varieties of species in *Vitis*. *V. vinifera* is the most successfully used grape species with thousands of wine, table, and raisin grape cultivars grown throughout the world's temperate zones.

Grapes are now grown in more than 90 countries of the world and become the world's largest fruit crop with a total production of 69 million tons (Euromonitor 2008). The countries with the greatest acreage are Spain, France, Italy, Turkey, China, and the United States. The leading countries for the production of table grapes consumed as fresh fruit are China, Turkey, Italy, Chile, and the United States.

Citrus Fruit

Citrus, belonging to the family Rutaceae, is one of the world's most important fruit. Citrus can be eaten as a fresh fruit, processed into juice, or added to dishes and beverages. The major types of edible citrus include citron (*Citrus medica* L.); pomelo or shaddock (*Citrus grandis*); tangerine, mandarin, or satsuma (*Citrus reticulata* Blanco); limes (*Citrus aurantifolia* L.); sour orange (*Citrus aurantium* L.); sweet oranges (*Citrus sinensis* [L.] Osbeck); lemon (*Citrus limon* L.); and grapefruit (*Citrus paradisi* Macfad.). Brazil, the United States, and China are the three largest citrus producers in the world.

Strawberry

Strawberry belongs to the genus *Fragaria*. The genus is comprised of 32 species. Historically, several *Fragaria* species and novel hybrids have been brought into cultivation in different parts of the world, including *Fragaria chiloensis* in South America, and *Fragaria moschata* and *Fragaria vesca* in Europe. However, strawberry (*Fragaria* × *ananassa* Duch) is one of the most widely grown small fruits in the world. The large modern fruit of today was developed in the early 18th century by the cross between the wild strawberry *F. chiloensis* and *Fragaria virginiana*.

Globally, a large part of the cultivated area is located in Europe, followed by Asia and North and Central America. In 2004, a total production of strawberry reached to 2.4 million tons in the world. The United States is the world's leading strawberry producer with China, Spain, and Korea. Some countries like Turkey, Morocco, and Egypt have strongly increased their production.

Peach

Peach belongs to the Prunoideae subfamily of the family Rosaceae. In temperate regions, the family ranks third place in economic importance. The genus *Prunus* is characterized by species that produce drupes known as "stone fruit." The edible portion of the fruit is a juicy mesocarp. There are three major groups of cultivars: nectarines, freestone peaches, and clingstone peaches. All commercial varieties of peach are *Prunus persica* (L.) Batsch, including nectarines differing from peach in the absence of pubescence ("fuzzless") on the fruit surface. Peaches originated in China, with a cultivation history of over 4000 years. Peach is grown in all continents except Antarctica, and world peach production has increased steadily in recent year.

Pear

Pear species belong to the genus *Pyrus*, the subfamily Maloideae (Pomoideae) in the family Rosaceae. There are about 22 primary species in the *genus*, all of which originate in either Asia or Europe. The pear has been cultivated in China for at least 3000 years. There are two major species, European pear (*Pyrus communis* L.) and Asian pear (*Pyrus pyrifolia* L.), which are commercially cultivated. The first species to be domesticated was *P. pyrifolia* (Burm.) Nakai because the wild type is edible but without selection. Later, the hardy northern Chinese type *Pyrus ussuriensis* Maxim probably became cultivated after selection from the wild type. Natural hybridization between these two wild species likely occurred in China to produce the modern "Ussuri" cultivars in northern China. In other parts of the world, cultivated pears have been derived from *P. communis* L., while *P. communis* var. pyraster and/or *P. communis* var. caucasica were probably the ancestors of the common pear of Europe, but "French" cultivars may be complex hybrids of these two.

Pear is the third important temperate fruit after grape and apple. Asia produces the most, followed by Europe, North and Central America, and South America. Among countries, China produced the most, followed by the United States, Italy, and Spain. Pears can be consumed as fresh fruit, fruit juice, cube for fruit salad, canned product, and dry fruit. About 80% of the total pear production is destined for fresh consumption.

Mango

The genus *Mangifera*, belonging to the dicotyledonous family "Anacardiaceae," originates in the Indo-Burma region. Almost all the edible cultivars of mango are the single species *Mangifera indica* L., which originated in the Indian subcontinent. The few other species that contribute edible fruits are *Mangifera caesia*, *Mangifera foetida*, and *Mangifera odorata*, which are confined to the Malaysian region.

Mango is a very important tropical fruit and popularly known as the "apple of the tropics." Mango is commercially grown in over 103 countries of the world. The major growing countries in the world are India, China, Mexico, Pakistan, Indonesia, Thailand, the Philippines, Brazil, Australia, Nigeria, and Egypt (http://faostat.fao.org). There are more than 1000 varieties of mango under cultivation, but only a few of them are grown on a commercial scale.

Papaya

Papaya (*Carica papaya* L.) belongs to family Caricaceae, which consists of six genera including *Carica* a monotypic genus, *Jacaratia* (7 species), *Jarilla* (3 species), *Cylicomorpha* (2 species), *Horovitzia* (1 species), and *Vasconcellea* (21 species). *Carica* is the only genus of Caricaceae containing the domesticated species *papaya*, which is by far the most economically important and has a wide distribution throughout the tropics and subtropics of the world. Papaya probably originated in the lowland of Central America between southern Mexico and Nicaragua, and is now cultivated in many tropical and subtropical regions.

Papaya is a major tropical fruit grown commercially in India, Brazil, Mexico, Australia, Hawaii, Thailand, South Africa, the Philippines, Indonesia, and China. In recent years, intensive improvements and selections have given rise to a large number of papaya varieties, such as "Kapoho Solo," "Sun Rise," "Sun Set," "Waimanalo," "Kamiya" (United States), "Pusa Delicious," "Pusa Nanha," "Pusa Dwarft," "Surya" (India), "Cavite Special" (the Philippines), "Sainampueng," "Kak Dum" (Thailand), and improved "Peterson," "Guinea" and "Gold and Sunnybank" (Australia).

Pineapple

Pineapple is a perennial monocot belonging to the family of Bromeliaceae, subfamily Bromelioideae. The Bromelioideae comprises 56 genera with more than 2000 species, which are classified into three subfamilies: Pitcarnioideae, Tillandsioideae, and Bromelioideae. This last subfamily shows a tendency toward the fusion of floral parts, a trait most developed in *Ananas*. Many distinctions, particularly those related to fruit size and fertility, appear to be the direct result of human selection in the course of domestication.

Pineapple is the third most important tropical fruit after bananas and mangoes and has been cultivated in South America since the 15th century. Owing to its attractive sweet flavor, pineapple is widely consumed as fresh fruit, processed juice, and canned fruit, and is used as an ingredient in exotic foods. Five countries, Thailand, the Philippines, Brazil, China, and India, contribute to the major production in the world.

Plum

Plums belong to subfamily Prunoideae of the family of Rosaceae. *Prunus* species are divided into three major subgenera: *Prunophora* (plum and apricots), *Amygdalus* (peaches and almonds), and *Cerasus* (sweet and sour cherries). The subgenus *Prunophora* is divided into two main sections: *Euprunus* groups (plum species) and *Armeniaca*, which contains the apricot species. Plum has been domesticated independently in Europe, Asia, and America. In Europe, *Prunus domestica* L. is the most important source of fruit cultivars and has been grown for over 2000 years. In Asia, the Japanese plum *Prunus salicina* L. originates from China where it has been cultivated since ancient times. In north America, the third plum domestication source, a wide range of native species, such as *Prunus americana* Marsh., *Prunus hortulana* Bailey, *Prunus angustifolia* Marsh., and *Prunus maritima* Marsh., are present. The

major production of plum is located in Europe and Asia. In Europe, Germany is the leading producer.

FRUIT FLAVOR

The consumption of fresh fruit is dependent on the fruit quality (Baldwin et al. 2007; López et al. 2007). The quality of fresh fruit includes many aspects such as appearance, color, texture, flavor, and nutritional value (Kader 2002; Song 2007). Among them, flavor is one of the most important quality traits for fresh fruit (Dirinck et al. 1989; Dull and Hulme 1971; Maarse 1991; Reineccius 2006). Fruit flavor is made up of sugars, acids, salts, bitter compounds such as alkaloids or flavonoids, and aroma volatiles (Dirinck et al. 1989; Salunkhe and Do 1976; Song and Forney 2008). The flavor of fresh fruit is determined by taste and aroma (odor-active compounds). The contribution of odor-active compounds to the fruit flavors has gained increasing attention because these compounds are important for the characteristic flavors of fruits (Baldwin 1993, 2002b; Brückner 2008). The present chapter refers specifically the term "flavor" to the volatile compounds. Volatile compounds in fruits are diverse, consisting of hundreds of different chemical compounds comprising only 10^{-7}–10^{-4} of the fresh fruit weight (Berger 2007; Brückner 2008). Although these volatile compounds are produced in trace amounts, they can be detected by human olfaction. The diversity is partially responsible for the unique flavors found in different fruit species. The importance of volatile production in fruit related to its influencing factors has been intensively investigated and/or reviewed (Baldwin 2002; Dixon and Hewett 2000; Fellman et al. 2000; Forney et al. 2000; Song 2007; Song and Forney 2008).

Classification of Volatile Compounds in Fruit Flavor

Chemical Structure Various types of fresh fruits produce distinct volatile profiles. Volatile compounds, which are produced by fresh fruits, are mainly comprised of diverse classes of chemicals, including esters, alcohols, aldehydes, ketones, lactones, and terpenoids (Table 1.2). However, some sulfur compounds, such as *S*-methyl thiobutanoate, 3-(methylthio) propanal, ethyl 2-(methylthio) acetate, ethyl 3-(methylthio) propanoate, and 3-(methylthio) propyl acetate, also contribute to the flavor of fruit such as melons (Song and Forney 2008). Although an overwhelming number of chemical compounds have been identified as volatile compounds in fresh fruit, only a fraction of these compounds have been identified as impact compounds of fruit flavor based on their quantitative abundance and olfactory thresholds (Cunningham and Barry 1986; Schieberle et al. 1990; Wyllie et al. 1995).

Biogenesis Volatile compounds forming the fruit flavor are produced through many metabolic pathways during fruit ripening and postharvest storage, and depend on many factors related to the species, variety, climate, production, maturity, and pre- and postharvest handling. For most fruits, volatile production is closely related to fruit ripening. As direct products of a metabolic pathway or as a result of interactions between pathways or end products, volatile compounds can be classified by the biogenesis: fatty acids (FAs), amino acids, glucosinolates, terpenoid, phenol, and related compounds (Berger 2007). However, from the point of chemical

TABLE 1.2. Volatile Compounds Present in Fruit Flavor

Esters	Alcohols	Aldehydes	Ketones	Lactones	Terpenoids
Butyl acetate	Benzyl alcohol	Acetaldehyde	2,3-Butanedione	γ-Butyrolactone	β-Caryophyllene
Butyl butanoate	Butan-1-ol	Benzaldehyde	β-Damsenone eucalyptol	γ-Decalactone	1,8-Cineole
Butyl hexanoate	(*E*)-cinnamyl alcohol	(*E*)-cinnamaldehyde	Eugenol	δ-Decalactone	Citral
Butyl-2-methyl butanoate	1-Hexanol	(*E*,*E*)-2,4-decadienal	2-Heptanone	γ-Dodecalactone	β-Damascenone
Butyl propanoate	(*E*)-2-hexenol	Hexanal	4-(*p*-Hydroxyphenyl)-2-butanone	δ-Dodecalactone	Dihydroedulan
Ethyl acetate	(*Z*)-3-hexenol	(*E*)-2-hexenal	3-Hydroxy-2-butanone	γ-Jasmolactone	Farnesyl acetate
Ethyl butanoate	1-Octanol	(*Z*)-3-hexenal	β-Ionone	γ-Octalactone	Geraniol
Ethyl 9-decenoate	(*Z*)-6-nonenol	(*Z*)-3-hexenal	Linalool	δ-Octalactone	Hotrienol
Ethyl hexanoate	Hexan-1-ol	Nonanal	6-Methyl-5-heptene-2-one		α-Ionone
Ethyl 2-methylbutanoate	(*Z*,*Z*)-3,6-nonadienol	(*Z*)-6-nonenal	Nerolidol		β-Ionone
Ethyl 3-methylbutanoate	1-Phenylethanol	(*E*,*Z*)-2,6-nonadienal	1-Octen-3-one		Limonene
Ethyl 2-methylpropanoate	2-Phenylethanol	(*E*)-2-nonenal	2-Pentanone		Linalool
Ethyl 2-methylbutanoate		Phenylacetaldehyde	(*Z*)-1,5-octadien-3-one		Myrtenol
Ethyl propanoate			Terpenes		Nerol
Ethyl 2-methylpropanoate					α-Phellandrene
Ethyl nonanoate					α-Pinene
(*E*)-2-hexenyl acetate					β-Pinene

(*E*)-3-hexenyl acetate
Hexyl acetate
Hexyl butanoate
Hexyl propanoate
Hexyl-2-methyl butanoate
Methyl acetate
Methyl cinnamate
Methyl butanoate
Methyl hexanoate
Methyl nonanoate
Methyl octanoate
Methyl-2-methylbutanoate
Methyl-3-methylbutanoate
2-Methylbutyl acetate
3-Methylbutyl acetate
2-Methylpropyl acetate
(*Z*)-6-nonenyl acetate
(*Z*,*Z*)-3,6-nonadienyl acetate
Pentyl acetate
Benzyl acetate
Propyl acetate
Propyl-2-methyl butanoate

Terpinen-4-ol
α-Terpineol
Terpinolene
α-Farensene

characterization, volatiles can be classified as esters, alcohols, aldehydes, ketones, lactones, and terpenoids (Table 1.2).

Volatile Compounds Formed from FAs FAs are precursors for a large number of volatile compounds. Many of them are important character-impacted aroma compounds that are responsible for fresh fruit flavors. Those compounds are usually having straight-chain carbons ranged from C_1 to C_{20}. Degradation of FAs occurs mainly by the three different oxidative routes: (1) α- and β-oxidation, (2) oxidation by the lipoxygenase pathway, and (3) autoxidation. The formation of flavors via β-oxidation is exemplified by considering flavor formation in pears (Jennings 1967). The widest variety of flavor compounds formed from lipids arises via lipoxygenase activity. Many of the aliphatic esters, alcohols, acids, and carbonyls found in fruits are derived from the oxidative degradation of linoleic and linolenic acids (Reineccius 2006). In addition, some of the volatile compounds derived from enzyme-catalyzed oxidative breakdown of unsaturated FAs may also be produced by autoxidation (Chan 1987). Autoxidation of linoleic acid produces the 9- and 13-hydroperoxides, whereas linolenic acid also produces 12- and 16-hydroperoxides (Berger 2007). Hexanal and 2,4-decadienal are the primary oxidation products of linoleic acid, while autoxidation of linolenic acid produces 2,4-heptadienal as the major product. Further autoxidation of these aldehydes leads to the formation of other volatile products (Chan 1987). As an alternative to the membrane catabolism, a hypothesis of low rate of de novo FA biosynthesis (free FA hypothesis) was proposed as the limiting factor for the aroma biosynthesis in fruit harvested too early (Song and Bangerth 2003). This hypothesis is also supported by the evidence that a close relationship between low aroma volatile production, low free FA, and low ATP content in apple fruit (Song and Bangerth 2003; Tan and Bangerth 2001). Either oxidative degradation of FAs or newly biosynthesized free FAs are precursors responsible for the formation of straight-chain esters in many fruits, but their role in flavor formation needs to be clarified.

Volatile Compounds Formed from Amino Acid Metabolism Amino acid metabolism generates aromatic, aliphatic, and branched-chain alcohols, acids, carbonyls, and esters that are important to fruit flavor (Reineccius 2006). Some volatile compounds can be produced by the action of enzymatic systems on amino acids. The major types of volatile compounds formed from the interaction of amino acids and sugars include aldehydes, alkyl pyrazines, alkyl thiazolines and thiazoles, and other heterocycles from the Strecker degradation (Maarse 1991). Amino acids are precursors for some branched aliphatic compounds such as 2-methyl-1-butanol and 3-methyl-1-butanol that are formed during the amino acid catabolism. These compounds can be further synthesized to form esters, which are important volatile compounds in many fruits with distinct "fruity" odor. As they share the same precursor pyruvate, which is generated from glycolysis, the interaction between FAs and branched amino acids is another important factor in the volatile biosynthesis of fruits. As apple fruits ripen, there is a great production of volatile compounds from branched amino acid pathway (Song 1994).

Volatile Compounds Formed from Carbohydrate Metabolism A large variety of volatile flavors can be traced to carbohydrate metabolism (Berger 2007). As the

photosynthetic pathways involve turning CO_2 into sugars that are metabolized into other plant needs, for example, lipids and amino acids, nearly all plant flavors come indirectly from carbohydrate metabolism. However, there are few flavor constituents that come directly from carbohydrate metabolism (Reineccius 2006).

Volatile Compounds Derived from Terpenoid Terpenoids are widely distributed among fruits. There are two main types of terpenoids that may contribute significantly to the fruit flavor: (1) monoterpenes and sesquiterpenes and (2) irregular terpenes mainly produced by catabolistic pathways and/or autoxidation (Berger 2007). The monoterpenes and sesquiterpenes are mainly formed by anabolic processes and, therefore, are present in intact plant tissue. However, the formation of some irregular terpenes cannot be explained by anabolic pathways in some fruits. These terpenoids are primarily oxidation-degraded products of the carotenoids.

Phenols and Related Compounds A large number of volatile phenols and related compounds occur in fruits, some of which are potent aroma compounds (Berger 2007). The majority of volatile phenols and related compounds are formed mainly through the shikimic acid pathway and are present either as free aglycones or bound glycosides that can be liberated by enzymatic hydrolysis. Generally, the volatile phenols and related compounds are benzene-substituted derivatives with methoxy and phenolic groups, often with an allyl, a vinyl, or an aldehyde group. Common flavor compounds of this group are eugenol, vanillin, myristicin, apiole, elemicin, and benzaldehyde.

VOLATILE COMPOUNDS AND THEIR BIOLOGICAL CHARACTERISTIC OF MAJOR FRUITS

As described above, lipids, carbohydrates, proteins, and amino acids are enzymatically converted to volatile compounds. The characterization of fruit volatiles can be very complicated due to various influencing factors such as cultivars, fruit maturity, postharvest treatment, fruit sample (either intact fruit, slices, or homogenized samples), and analytic techniques (Berger 2007; Brückner 2008; Cunningham and Barry 1986). Volatiles can be classified as "primary" or "secondary" compounds, indicating whether they were present in intact fruit tissue or produced as a result of tissue disruption (Drawert et al. 1969). It should be pointed out that analysis of volatiles from either intact or disrupted fruit tissues will influence the aroma profiles and final aroma interpretation. This following section reviews overall flavor characterization of volatile compounds reported for some major fruits published in the past few years. The listed volatile compounds are those that are produced by fruit at a full ripe or close to consumption stage and summarized from different methodologies. In the following section, volatile compounds of major fruits are summarized in Table 1.3.

Apple

More than 300 volatile compounds have been identified in apple fruit (Dirinck et al. 1989). Only a few of these volatiles have been identified as important active

TABLE 1.3. Volatile Compounds of Major Fruits

Fruit Name	Volatile Compounds	Reference
Apple	Alcohols, aldehydes, 1-butyl acetate, butyl 2-methylbutanoate, β-damascenone, ethyl butanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 2-methylbutyl acetate, *n*-hexanal, 1-hexanol, hexen-1-ol, hexyl acetate, hexyl butanoate, hexyl 2-methylbutanoate, hexyl propanoate, ketone, 2-methylbutanoate, methyl 2-methylbutanoate, propyl 2-methylbutanoate, pentyl acetate, 1-propyl propionate, *trans*-2-hexenal, and *trans*-2-hexen-1-ol	Dixon and Hewett (2000), Flath and others (1969), Cunningham (1985)
Banana	Alcohols, amyl butanaote, butyl butanaote, esters, and isoamyl acetate	Jayanty and others (2002), Maciel and others (1986)
Citrus fruit	Acetaldehyde, acetoin, carvone, β-damascenone, (*E*,*E*)-2,4-decadienal, decanal, diacetyl, dodecanal, elinalool, ethanol, ethyl acetate, ethyl butanoate, ethyl propanoate, ethyl-2-methyl propanoate, ethyl-2-methyl butanoate, ethyl hexanoate, ethyl-3-hydroxy hexanoate, ethyl octanoate, ethyl decanoate, geranial citronellal, hexanal, (*E*)-2-hexenaln, (*E*)-2-hexen-1-ol, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, limonene, methyl butanoate, 3-methyl butanol, neral, nonanal, (*E*)-2-nonenal, (*Z*)-2-nonenal, 1-penten-3-one, 1-octanol, octanal, 1-octen-3-one, β-sinensal, α-terpincol, and terpinen-4-ol	Berger (2007), Berry and others (1983)
Strawberry	Butyrates, butyl acetate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, dimethyl-4-methoxy-3(2H)-furanone, γ-decalactone, γ-dodecalactone, ethyl butanoate, ethyl cinnamates, ethyl hexanoate, ethyl 3-methylbutanoate, ethyl propanoate, farnesyl acetate, furaneol, furaneol-β-glucoside, geraniol, 2-heptanone, hexanal, (*E*)-2-hexenal, hexyl acetate, linalool, methyl cinnamates, methyl and ethyl acetates, methyl anthranilate, methyl butanoate, methyl 2-methylbutanoate, methyl hexanoate, mesifurane, methional, propionates, and 1-octen-3-one	Forney and others (2000), Hakala and others (2002), Sanz and others (1994), Whitaker and Evans (1987)

TABLE 1.3. ***Continued***

Fruit Name	Volatile Compounds	Reference
Peach	Benzaldehyde, benzyl alcohol, γ-caprolactone, *cis*-3-hexenyl acetate, β-damascenone, γ-decalactone, (*E*,*E*)-2,4-decadienal, δ-decalactone, γ-decalactone, dimethyl disulfide, γ-dodecalactone, δ-dodecalactone, ethyl acetate, ethyl butanoate, ethyl octanoate, γ-decalactone, hexanal, (*Z*)-3-hexen-1-yl acetate, (*E*)-2-hexen-1-ol, (*Z*)-3-hexenal, γ-jasmolactone, linalool, methyl octanoate, γ-octalactone, δ-octalactone, 6-pentyl α-pyrone, and terpinolene	Aubert and Milhet (2007), Berger (2007), Horvat and others (1992), Narain and others (1990), Visai and Vanoli (1997)
Pear	Butyl acetate, butyl butanoate, hexyl acetate, ethyl hexanoate, ethyl octanoate, ethyl (*E*)-2-octenoate, ethyl (*E*,*Z*)-2,4-decadienoate, methyl (*E*,*Z*)-2,4-decadienoate, and pentyl acetate	Argenta and others (2003), Kahle and others (2005), Rapparini and Predieri (2003), Rizzolo and others (1991)
Grape	Methyl anthranilate	Rosilllo and others (1999), Whitaker and Evans (1987)
Mango	Camphene, butan-1-ol, car-3-ene, β-caryophyllene, *p*-cymene, *cis*-hex-3-en-1-ol, α-copanene, cyclohexane, dimethylcyclohexane, 1,1-diethoxyethane, ethanol, ethylcyclohexane, ethyl butenoate, ethyl dodecanoate, ehtyl decanoate, ethyl octanoate, α-fenchene, 2-furfural, hexanal, α-humulene, hydrocarbon, limonene, 1-methylpropan-1-ol, methylcyclohexane, 3-methylbutan-1-ol, myrcene, α-phellandrene, β-phellandrene, α-pinene, β-pinene, sabinene, sabinyl acetate, toluene, γ-terpinene, α-terpinolene, and xylene	MacLeod and Snyder (1985), Macleod and Troconis (1982), Malundo and others (2001), Pino and Mesa (2006)
Papaya	Linalool, ethyl acetate, phenylacetonitrile, benzyl isothiocynate, methyl butanoate, ethyl butanoate, 3-methylbutanol, benzyl alcohol, α-terpineol, and butanol	Almora and others (2004), Flath and others (1990), Heidlas and others (1984), Pino and others (2003)
Pineapple	Acetoxyacetone, *p*-allyl phenol, γ-butyrolactone, β-hydroxyhexanoic acids, 4-methoxy-2,5-dimethyl-2(H)-furan-3-one, methyl esters of β-hydroxybutyric, γ-octalactone, 2-propenyl hexanoate, sesquiterpene, 1-(*E*,*Z*)-3,5-undecatriene, and 1-(*E*,*Z*,*Z*)3,5,8-undecatetraene	Berger and others (1983, 1985), Takeoka and others (1991), Tokitomo and others (2005)
Plum	Benzaldehyde, (*E*,*E*)-2,4-decadienal, δ-decalactone, γ-decalactone, ethyl nonanoate, (*Z*)-3-hexenal, linalool, methyl cinnamate, and δ-octalactone	Maarse (1991), Horvat (1992)

odor compounds being responsible for the characteristic aroma in most apple cultivars, such as β-damascenone, butyl, isoamyl, and hexyl hexanoate, along with ethyl, propyl, and hexyl butanoates (Cunningham 1985). The most abundant volatile components are esters, alcohols, aldehydes, ketones, and ethers, while esters are the principal compounds responsible for fruity odor (Fellman et al. 2000; Plotto et al. 2000). For example, ethyl 2-methylbutanoate, 2-methylbutyl acetate, and hexyl acetate contribute mostly to the characteristic aroma of "Fuji" apples, while ethyl butanoate and ethyl 2-methylbutanoate are the active odor compounds in "Elstar" apples, and ethyl butanoate, acetaldehyde, 2-methyl-1-butanol, and ethyl methylpropanoate in "Cox Orange" (Acree et al. 1984; Berger 2007; Echeverria et al. 2004). Ethyl 2-methylbutanoate also has a direct impact on "Granny Smith" apple flavor (Lavilla et al. 1999).

Banana

The major volatile compounds in banana fruit are identified as alcohols and esters, including amyl acetate, isoamyl acetate, butyl butyrate, and amyl butyrate. Esters predominate in the volatile fraction of banana fruit. Based on the combined analytic chemistry with sensory analysis, penten-2-one, 3-methylbutyl, and 2-methylpropyl esters of acetate and butyrate have been identified as the most important banana fruit aroma (Berger et al. 1986). Isopentyl acetate and isobutyl acetate are also known as the most important impact compounds of banana aroma. The concentrations of acetates and butanoates increased during ripening of banana fruit (Jayanty et al. 2002). In addition, isoamyl alcohol, isoamyl acetate, butyl acetate, and elemicine were detected by olfactometric analyses as characteristics of banana odor (Boudhrioua et al. 2003).

Citrus

Citrus volatiles have been extensively examined over the past several decades. As the most foods of commercial interest, the volatile components of citrus juice have been known for some time. Table 1.2 lists the volatile compounds present in citrus juice, which were detected by gas chromatography (GC)–olfactometry. Esters are important as they are responsible for the flavor characteristic (Berger 2007), while the major esters are ethyl esters of C_3 to C_4 organic acids. Linalool is by far the most important alcohol. However, ketones, carvone, diacetyl, and acetoin are off-flavors. Thus, the key flavor compounds in fresh citrus fruit still need to be identified.

Strawberry

Over 360 different volatile compounds have been identified in strawberry fruit (Maarse 1991). Strawberry aroma is composed predominately of esters with alcohols, ketones, lactones, and aldehydes being present in smaller quantities (Forney et al. 2000). Strawberries contain primarily straight esters, which comprise primarily of methyl, and ethyl acetates, butanoates, and hexanoates. Esters provide an aroma characteristic to the fruit (Gomes da Silav and Chavees das Neves 1999). Terpenoids and sulfur compounds may also have a significant impact on the characteristic

aroma of strawberry fruit (Dirinck et al. 1981). The most important aroma compounds in strawberry fruit include ethyl cinnamates, methyl cinnamates, 2,5-dimethyl-4-hydroxy-3(2H)-furanone,furaneol,furaneol-beta-glucoside,dimethyl-4-methoxy-3(2H)-furanone (mesifurane), methyl and ethyl acetates, propionates, and butyrates, which are responsible for fruity flavor. A number of terpenes also contribute to the flavor of strawberry fruit.

Peach

Approximately 100 volatile compounds have been identified in peaches, including alcohols, aldehydes, alkanes, esters, ketones, lactones, and terpenes (Aubert et al. 2003; Visai and Vanoli 1997). The major volatile compounds are identified as ethyl acetate, *cis*-3-hexenyl acetate, methyl octanoate, ethyl octanoate, γ-decalactone, benzyl alcohol, γ-caprolactone, and δ-decalactone. Among them, lactones, particularly γ-decalactone and δ-decalactone, have been reported as character-impacted compounds in peaches and are associated with C6-aldehydes, aliphatic alcohols, and terpenes, which are responsible for fruity characteristics (Derail et al. 1999; Engel et al. 1988; Horvat et al. 1990; Narain et al. 1990). Nectarines produce less volatiles in total but more esters, linalol, and terpinolene and have more fruity and floral aroma notes than peaches (Visai and Vanoli 1997).

Pear

More than 300 volatile compounds have been identified in pear, including aldehydes, alcohols, esters, ketones, and sulfur compounds (Rapparini and Predieri 2003). The most important character-impacted compounds of pears are listed in Table 1.3. Methyl and hexyl esters of decadienoate are the character-impacted compounds of the European pear (Argenta et al. 2003; Kahle et al. 2005; Rapparini and Predieri 2003). Other volatile esters, for example, hexyl acetate, 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, and ethyl hexanoate possess strong pear-like aroma (Rapparini and Predieri 2003). Ethyl octanoate and ethyl (*E*)-2-octenoate contribute to sweet or fruity odors in pears, while a high concentration of 2,4-decadienoates in fruit flesh is accepted by consumers (Rizzolo et al. 1991). In addition, hexanal, 2-methylpropyl acetate, ethyl acetate, hexyl acetate, 3-methylbutyl 2-methylbutanoate, ethyl butanoate, and butanol are identified as impact volatiles in "Conference" pears (Rizzolo et al. 2005).

Grape

The flavor of grapes is made up of volatile alcohols, aldehydes, esters, acids, terpenols, and carbonyl compounds. Grape may be divided into aromatic and non-aromatic varieties. Free terpenols, for example, linalool and geraniol, have been identified as major aroma compounds in both red and white grapes (Rosilllo et al. 1999). Octanoic acid and alcohols, particularly 2-phenylethanol, are recognized after crushing (Rosilllo et al. 1999). In addition, esters and aldehydes were also reported in "Aleatico" grapes (Bellincontro et al. 2009). Fruit flavor is highly correlated with consumer likings in table grapes.

Mango

Mango possesses a very attractive flavor characteristic. About 270 volatile compounds from mango fruit were identified. However, application of distillation extraction in combination with active odor value (aroma threshold) technologies exhibits that monoterpenes such as α-pinene, myrecene, α-phelladrene, σ-3-carene, *p*-cymene, limone and terpinolene, esters including ethyl-2-methylpropanaote, ethyle-butanoate, as well as (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, methyl benzoate, (*E*)-β-ionone, decanal, and 2,5,-dimethyl-4-methoxy-3(2H)-furanone are the most important compounds contributing to mango flavor (Pino and Mesa 2006). The acids, esters, and lactones found were considered to be produced by the lipid metabolism in the flavor development of mango fruit during ripening.

Papaya

Papaya possesses a characteristic aroma, which is due to several volatile components such as alcohols, esters, aldehydes, and sulfur compounds (Chan et al. 1973). Fifty-one volatile compounds from intact "Hawaiian" papaya at different ripening stages were detected. Linalool, followed by linalool oxide A, linalool oxide B, ethyl acetate, phenylacetonitrile, and benzyl isothiocynate, was the major compound in the fully ripe fruits (Flath et al. 1990). Other work indicated the esters as the predominant compounds among the volatiles of papayas from Sri Lanka and Colombia (Heidlas et al. 1984; Macleod and Pieris 1984). In addition, methyl butanoate, ethyl butanoate, 3-methylbutanol, benzyl alcohol, α-terpineol, and butanol are found to be important volatiles in papaya fruit (Almora et al. 2004; Pino et al. 2003).

Pineapple

More than 280 volatile compounds have been found in pineapple fruit (Tokitomo et al. 2005). The major volatile compounds are identified as 4-methoxy-2,5-dimethyl-2(H)-furan-3-one, 2-propenyl hexanoate, sesquiterpene hydrocarbons, 1-(*E*,*Z*)-3,5-undecatriene, 1-(*E*,*Z*,*Z*)3,5,8-undecatetraene, 2-propenyl *n*-hexanoate ethyl, para-allyl phenol, γ-butyrolactone, γ-octalactone, acetoxyacetone, methyl esters of β-hydroxybutyric, and β-hydroxyhexanoic acids. Monoterpene alcohols (linalool, α-terpineol, and terpinen-4-ol) and sesquiterpenes were also identified (Berger et al. 1985; Flath and Forry 1970). In addition, the sulfur compounds such as methyl 3-(methylthio)-(*E*)-2-propenoate, methyl 3-(methylthio)-(*Z*)-2-propenoate, ethyl 3-(methylthio)-(*Z*)-2-propenoate, ethyl 3-(methylthio)-(*E*)-2-propenoate, methyl 5-hexenoate, methyl (*E*)-4-hexenaote, methyl 4-(methylthio)-butanoate, nonanol, and ethyl 4-methylthiobutanoate, were reported as impact-flavor compounds in fresh "Hawaiian" pineapple (Takeoka et al. 1991).

Plum

Approximately 75 volatile compounds have been identified in plum juices (Maarse 1991). Lactones from C_6 to C_{12} are major classes of volatile compounds in plums (Horvat 1992), but the key flavor compounds in fresh plum fruit are not yet identified.

CONCLUDING REMARKS

The diversity of varieties of fruit for today's human consumption has resulted from a long history of natural development, selection, and scientific breeding. Fruits play important roles in human nutrition and diet. However, they are perishable due to natural ripening, senescence, and pathological decay. Fruit quality attributes, such as texture, appearance, flavor, and nutrition, significantly change during ripening, but they have not been understood fully. Thus, the further development of modern technologies of breeding, production, and postharvest handling will enable consumers to enjoy fruits and their products without limitations of seasons and geographic locations.

Fruit flavor is an important aspect of quality. Many compounds are responsible for the fruit aromas that have strong penetration odors with low threshold values. Advances in identifying and quantifying volatile compounds by improved analysis techniques in various fruits have greatly increased our knowledge about fruit flavor (Brückner 2008; Song 2007; Song and Forney 2008; Tholl et al. 2006; Tzortzakis 2007). Advances in the biogenesis of volatile compounds in fresh fruits have also improved our current understanding; however, knowledge of the biochemical pathways and key regulating steps of the synthesis of these volatile compounds is still incomplete. A fuller understanding of the flavor chemistry and biology of volatile compounds of fruits is important to improve the flavor quality of fresh fruit that complies with the consumer needs for better quality. Furthermore, employing state-of-the-art genomic, proteomic, and microscopy tools to study fundamental metabolism, and combining these results with direct measurement of chemical and sensory properties (Baldwin 2002a; Bood and Zabetakis 2002; Raab et al. 2006; Song and Forney 2008) will lead to a better understanding of how to optimize and retain fruit flavor quality in the market places for the benefit of both consumers and fruit industry.

ACKNOWLEDGMENTS

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REFERENCES

Acree TE, Barnard J, Cunningham DG. 1984. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem* 14:273–86.

Almora K, Pino JA, Hernandez M, Duarte C, Gonzalez J, Roncal E. 2004. Evaluation of volatiles from ripening papaya. *Food Chem* 86:127–30.

Argenta LC, Fan XT, Mattheis JP. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. *J Agric Food Chem* 51:3858–64.

Aubert C, Gunata Z, Ambid C, Baumes R. 2003. Changes in physicochemical characteristics and volatile constituents of yellow- and white-fleshed nectarines during maturation and artificial ripening. *J Agric Food Chem* 51:3083–91.

Aubert C, Milhet C. 2007. Distribution of the volatile compounds in the different parts of a white-fleshed peach (*Prunus persica* L. Batsch). *Food Chem* 102:375–84.

Baldwin EA. 1993. Citrus fruit. In Seymour GB, Taylor JE, Tucker GA (eds.), *The Biochemistry of Fruit Ripening*. New York: Chapman and Hall, pp. 107–49.

Baldwin EA. 2002a. Commercialized biotechnology, food for thought: Introduction to the colloquium. *HortScience* 37:446–7.

Baldwin EA. 2002b. Fruit flavour, volatile metabolism and consumer perception. In Knee M (ed.), *Fruit Quality and Its Biological Basis*. Boca Raton, FL: Sheffield Academic Press and CRC Press, pp. 89–106.

Baldwin EA, Plotto A, Goodner K. 2007. Shelf-life versus flavor-life for fruits and vegetables: How to evaluate this complex trait. *Stewart Postharvest Rev* 1:3.

Bellincontro A, Nicoletti I, Valentini M, Tomas A, De Santis D, Corradini D, Mencarelli F. 2009. Integration of nondestructive techniques with destructive analyses to study postharvest water stress of winegrapes. *Am J Enol Vitic* 60:57–65.

Berger RG. 2007. *Flavours and Fragrances—Chemistry, Bioprocessing and Sustainability*. Berlin: Springer-Verlag.

Berger RG, Drawert F, Kollmannsberger H. 1986. Geruchsaktive spurenkomponenten des bananen aromas. *Chem Mikrobiol Technol Lebensm* 10:120–4.

Berger RG, Drawert F, Kollmannsberger H, Nitz S, Schraufstetter B. 1985. Volatiles in pineapple fruit and their sensory properties. *J Agric Food Chem* 32:232–5.

Berger RG, Drawert F, Nitz S. 1983. Sesquiterpene hydrocarbons in pineapple fruit. *J Agric Food Chem* 31:1237–9.

Berry RE, Shaw PE, Tatum JH, Wilson CW III. 1983. Citrus oil flavor and composition studies. *Food Technol* 37:88–91.

Bood KG, Zabetakis I. 2002. The biosynthesis of strawberry flavour (II) biosynthesis and molecular biology studies. *J Food Sci* 67:2–8.

Boudhrioua N, Giampaoli P, Bonazzi C. 2003. Changes in aromatic components of banana during ripening and air-drying. *Lebensm Wiss Technol* 36:633–42.

Brückner B. 2008. *Fruit and Vegetable Flavour: Recent Advances and Future Prospects*. Abington Hall, Cambridge, UK: Woodhead Publishing Limited.

Chan HT Jr., Flath RA, Forry RR, Cavaletto CG, Nakayama TOM, Brekke JE. 1973. Development of off-odors and off-flavors in papaya puree. *J Agric Food Chem* 21:566–70.

Chan HWS. 1987. *Autoxidation of Unsaturated Lipids*. London: Academic Press.

Cunningham AJ. 1985. Ventricular arrhythmia and adrenaline infiltration during gynecological surgery. *Br J Anaesth* 57:936–7.

Cunningham AJ, Barry P. 1986. Intraocular-pressure-physiology and implications for anesthetic management. *Can Anaesth Soc J* 33:195–208.

Derail C, Hofman T, Schieberle T. 1999. Differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure. *J Agric Food Chem* 47:4742–5.

Dirinck P, De Pooter H, Schamp N. 1989. Aroma development in ripening fruits. In Teranishi R, Buttery R (eds.), *Flavor Chemistry: Trends and Developments. ACS Symposium Series 388*. Washington, DC: American Chemical Society, pp. 23–34.

Dirinck PJ, De Pooter HL, Willaert GA, Schamp NM. 1981. Flavor quality of cultivated strawberries: The role of the sulfur compounds. *J Agric Food Chem* 29:316–21.

Dixon J, Hewett EW. 2000. Factors affecting apple aroma/flavour volatile concentration: A review. *N Z J Crop Hortic Sci* 28:155–73.

Drawert F, Heimann W, Emberger R, Tressl R. 1969. Über die Biogenese von Aromastoffen bei Pflanzen und Früchten. IV. Mitt Bildung der Aromamstoffe des Apfels im Verlauf des Wachstums und bei der Largerung. *Zeit Lebens Unters Forsch* 140:65–87.

Dull GG, Hulme AC. 1971. Quality. In Hulme AC (ed.), *The Biochemistry of Fruits and Their Products*. London: Academic Press, pp. 721–5.

Echeverria G, Fuenters MT, Graell J, Lopez ML. 2004. Relationships between volatile production, fruit quality and sensory evaluation of Fuji apples stored in different atmospheres by means of multivariate analysis. *J Sci Food Agric* 84:5–20.

Engel KH, Flath RA, Buttery RG, Mon TR, Ramming DW, Teranishi R. 1988. Investigation of volatile constituents in nectarines. 1. Analytical and sensory characterization of aroma components in some nectarine cultivars. *J Agric Food Chem* 36:549–53.

Euromonitor. 2008. International: Fresh foods: Euromonitor from trade source/national statistics. May 5, 2008.

Fellman JK, Miller TW, Wattinson DS, Matthesis JP. 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. *HortScience* 35:1026–33.

Flath RA, Forrey RR, Teranishi R. 1969. High resolution vapor analysis for fruit variety and fruit product comparisons. *J Food Sci* 34:382–5.

Flath RA, Forry RR. 1970. Volatile components of Smooth Cayenne pineapple. *J Agric Food Chem* 18:306–9.

Flath RA, Light DM, Jang EB, Mon TR, John JO. 1990. Headspace examination of volatile emissions from ripening papaya (*Carica papaya* L. Solo variety). *J Agric Food Chem* 38:1060–3.

Forney CF, Kalt W, Jordan MA. 2000. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *HortScience* 35:1022–6.

Goff S, Klee J. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311:815-9.

Gomes da Silav MDR, Chavees das Neves HJ. 1999. Complementary use of hyphenated purge-and-trap gas chromatography techniques and sensory analysis in the aroma profiling of strawberries (*Fragaria ananassa*). *J Agric Food Chem* 47:4568–73.

Hakala M, Lapvetelainen AT, Lallio HP. 2002. Volatile compounds of selected strawberry varieties analyzed by purge and trap headspace GC-MS. *J Agric Food Chem* 50: 1133–42.

Heidlas J, Lehr M, Idstein H, Schreier P. 1984. Free and bound terpene compounds in papaya (*Carica papaya* L.) fruit pulp. *J Agric Food Chem* 32:1020–1.

Horvat RJ, Chapman GW, Robertson JA, Meredith FI, Scorza R, Callahan AM, Morgens P. 1990. Comparison of the volatile compounds from several commercial peach cultivars. *J Agric Food Chem* 38:234–7.

Horvat RJ, Chapman GW Jr., Senter SD, Robertson JA, Okie WR, Norton JD. 1992. Comparison of the volatile compounds from several commercial plum cultivars. *J Sci Food Agric* 60:21–3.

Jayanty S, Song J, Rubinstein N, Chong A, Beaudry RM. 2002. Temporal relationship between ester biosynthesis and ripening events in bananas. *J Am Soc Hortic Sci* 127:998–1005.

Jennings WG. 1967. Peaches and pears. In Schultz HW, Day EA, Libbey LM (eds.), *Chemistry and Physiology of Flavors*. Westport, CT: AVI Publishing, p. 419.

Kader AA (ed.). 2002. *Postharvest Technology of Horticultural Crops*. Oakland, CA: University of California, Division of Agriculture and Natural Resources Publication 3311, p. 535.

Kahle K, Preston C, Richling E, Heckel F, Schreier P. 2005. On-line gas chromatography combustion/pyrolysis isotope ratio mass spectrometry (HRGC-C/P-IRMS) of major volatiles from pear fruit (*Pyrus communis*) and pear products. *Food Chem* 91:449–55.

Knee M. 2002. *Fruit Quality and Its Biological Basis*. Boca Raton, FL: Sheffield Academic Press.

Lavilla T, Puy J, Lopez ML, Recasens I, Vendrell P. 1999. Relationships between volatile production, fruit quality, and sensory evaluation in Granny Smith apples stored in different controlled-atmosphere treatments by means of multivariate analysis. *J Agric Food Chem* 47:3791–803.

López ML, Villatoro C, Fuentes T, Graell J, Lara I, Echeverrıa G. 2007. Volatile compounds, quality parameters and consumer acceptance of "Pink Lady" apples stored in different conditions. *Postharvest Biol Technol* 43:55–66.

Maarse H. 1991. *Volatile Compounds in Foods and Beverages*. New York: Dekker.

Maciel MI, Hansen TJ, Aldinger SB, Labows JN. 1986. Flavor chemistry of cashew apple juice. *J Agric Food Chem* 34:923–7.

MacLeod A, Snyder CH. 1985. Volatile components of two cultivars of mango from Florida. *J Agric Food Chem* 33:380–4.

Macleod AJ, Pieris NM. 1984. Volatile components of papaya (*Carica papaya* L.) with particular reference to glucosinolate products. *J Agric Food Chem* 31:1005–8.

Macleod AJ, Troconis NG. 1982. Volatile flavour components of mango fruit. *Phytochemistry* 21:2523–6.

Malundo TMM, Shewfelt RL, Ware GO, Baldwin EA. 2001. Sugars and acids influence flavor properties of mango. *J Am Soc Hortic Sci* 126:115–21.

Narain N, Hsieh TCY, Johnson CE. 1990. Dynamic headspace concentration and gas chromatography of volatile flavor components in peach. *J Food Sci* 55:1303–7.

Pino JA, Almora K, Marbot R. 2003. Volatile components of papaya (*Carica papaya* L., Marado variety) fruit. *Flav Frag J* 18:492–6.

Pino JA, Mesa J. 2006. Contribution of volatile compounds to mango (*Mangifera indica* L.) aroma. *Flav Frag J* 21:207–13.

Plotto A, McDaniel MR, Mattheis JP. 2000. Characterization of changes in "Gala" apple aroma during storage using Osme analysis, a gas chromatography-olfactometry techniques. *J Am Soc Hortic Sci* 125:714–22.

Raab T, Lopez-Raez JA, Klein D, Caballero JL, Moyano E, Schwab W, Munnoz-Blanco J. 2006. FaQR, required for the biosynthesis of the strawberry flavour compound 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, encodes an enone oxidoreductase. *Plant Cell* 18:1023–37.

Rapparini F, Predieri S. 2003. Pear fruit volatiles. In Janick J (ed.), *Horticultural Reviews*. Hoboken, NJ: John Wiley & Sons, pp. 237–324.

Reineccius G. 2006. *Flavor Chemistry and Technology*, 2nd Ed. Boca Raton, FL: CRC Press.

Rizzolo A, Cambiaghi P, Grassi M, Zerbini PE. 2005. Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of conference pears. *J Agric Food Chem* 53:9781–9.

Rizzolo A, Sodi C, Poleselllo A. 1991. Influence of ethylene removal on the volatile development in *Passa crassana* pears stored in a controlled atmosphere. *Food Chem* 42:275–85.

Rosilllo L, Salinas MR, Garijo J, Alonso GL. 1999. Study of volatiles in grapes by dynamic headspace analysis Application to the differentiation of some *Vitis vinifera* varieties. *J Chromatogr A* 847:155–9.

Salunkhe DK, Do JY. 1976. Biogenesis of aroma constituents of fruits and vegetables. *CRC Crit Rev Food Sci Nutr* 8:161–90.

Sanz C, Perez, AG, Richardson DG. 1994. Simultaneous HPLC determination of 2,5-dimethyl-4-hydroxy-3 (2H)-furanone and related flavor compounds in strawberries. *J Food Sci* 59:39–41.

Schieberle P, Ofner S, Grosch W. 1990. Evaluation of potent odorants in cucumbers (*Cucumis sativus*) and muskmelons (*Cucumis melo*) by aroma extract dilution analysis. *J Food Sci* 55:193–5.

Song J. 1994. *Einfuβ verschiedener erntzeitpunkte auf die fruchtreife under besonderer berücksichtigung der aromabildung bei äpfeln, tomaten und erdberren*. Stuttgart: Verlag Ulrich Grauer.

Song J. 2007. Flavour volatile production and regulation in apple fruit. *Stewart Postharvest Rev* 2:2.

Song J, Bangerth F. 2003. Fatty acids as precursors for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit. *Postharvest Biol Technol* 20:113–21.

Song J, Forney CF. 2008. Flavour volatile production and regulation in fruit. *Can J Plant Sci* 88:537–50.

Takeoka GR, Buttery RG, Teranishi R, Flath RA, Güntert M. 1991. Identification of additional pineapple volatiles. *J Agric Food Chem* 39:1848–51.

Tan T, Bangerth F. 2001. Are adenine and/or pyridine nucleotides involved in the volatile production of prematurely harvested or long term stored apple fruits? *Acta Hortic* 553: IV International Conference on Postharvest Science.

Tholl D, Boland W, Hansel A, Loreto F, Rose USR, Schnitzler JP. 2006. Practical approaches to plant volatile analysis. *Plant J* 45:540–60.

Tokitomo Y, Steinhaus M, Büttner A, Schieberle P. 2005. Odor-active constituents in fresh pineapple (*Ananas comosus* [L.] Merr.) by quantitative and sensory evaluation. *Biosci Biotechnol Biochem* 69:1323–30.

Tzortzakis NG. 2007. Maintaining postharvest quality of fresh produce with volatile compounds. *Innov Food Sci Emerg Technol* 8:111–6.

Visai C, Vanoli M. 1997 Volatile compound production during growth and ripening of peaches and nectarines. *Sci Hortic* 70:15–24.

Whitaker RJ, Evans DA. 1987. Plant biotechnology and the production of flavor compounds. *Food Technol* 46:86–101.

Wyllie SG, Leach DN, Wang YM, Shewfelt RL. 1995. Key aroma compounds in melon: Their development and cultivar dependence. In Rouseff RL, Leahy MM (eds.), *Fruit Flavors: Biogenesis, Characterization, and Authentication*. Washington, DC: American Chemical Society, pp. 248–257.

CHAPTER 2

Physiology and Biochemistry of Fruit Flavors

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PRIMARY AND SECONDARY METABOLISM CONNECTIONS

Metabolism in fruit involves the conversion of high-molecular-weight precursors to smaller compounds that help to obtain viable seeds and to attract seed-dispersing species. The flavor of fruit is generally determined from tens to hundreds of constituents, most of them generated during the ripening phase of the fruit growth and development process. Any study on the metabolic pathways leading to their synthesis must be considered in the context of this developmental process. Thus, it is known that the rapid growth phase in fruits acts as strong sinks that import massive amounts of photoassimilates from photosynthesizing organs. The translocation occurs in the phloem, with sucrose being the most translocated sugar, although in some species, other predominant compounds are polyalcohols, such as mannitol or sorbitol, and even oligosaccharides. These translocated compounds, which are the result of the primary metabolism, are the precursors of most of the metabolites that account for the fruit flavor, generally classified as secondary metabolites. Thus, the synthesis of these compounds is necessarily supported by the supply of the primary photoassimilates.

Flavor perception is often described as a combination of taste and smell. Some of these primary metabolites can be essential components of taste since they might be, depending on the species, main components of the harvested fruits, being recognized by sweet taste receptors. Accordingly, the first part of this chapter is focused on primary metabolism, as a source of tasteful compounds and as a support for the synthesis of secondary metabolites.

The sugar, or sugar alcohol, delivered to the fruit is converted to starch (mango, banana, kiwifruit), stored as reducing sugar (tomato, strawberry), or stored as sucrose (wild tomato, water melon, grape), and even might be converted to lipids (olive) (Fig. 2.1). The variability in the content of sucrose and hexoses is the result

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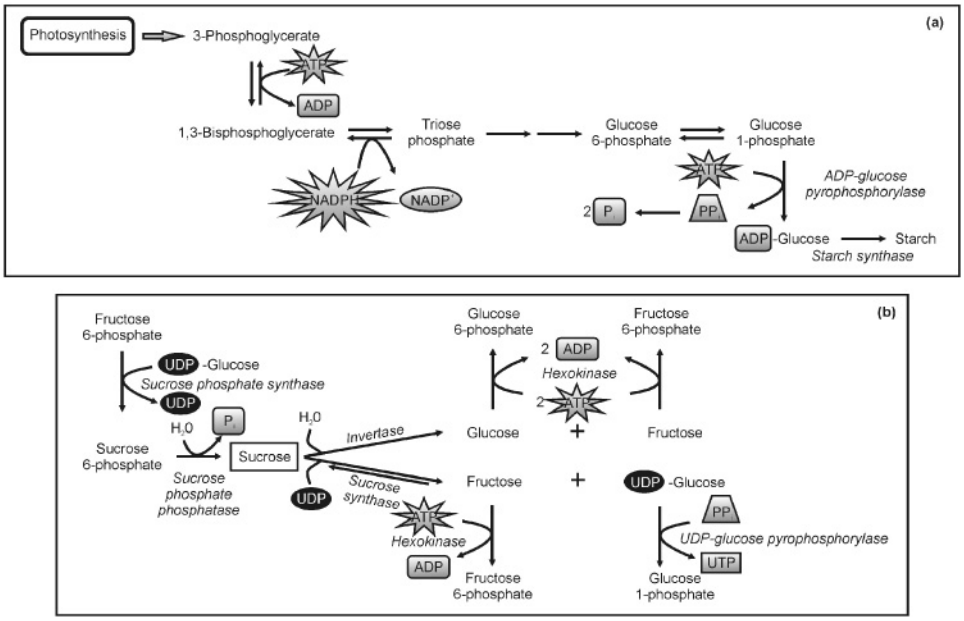

Figure 2.1. Schematic representation of the reactions involved in the synthesis and degradation of starch (A) in the chloroplast and sucrose (B) in the cytosol.

of the activities of the enzymes responsible for its degradation and synthesis, being invertase and sucrose synthase the most studied. In tomato, the involvement of apoplastic invertase in the sucrose/hexoses balance has been thoroughly studied, taking advantage of the fact that the wild species accumulates sucrose but the cultivated species accumulates hexoses (Klann et al. 1996). This allowed performing genetic and biochemical studies that provided evidence that the kinetic properties of the invertase from the domesticated cultivars accounted for the hexose accumulation in the fruits of these species (Fridman et al. 2004). In contrast, there is little evidence of a role of sucrose synthase in fruit metabolism (Carrari and Fernie 2006). Apoplastic invertase has been studied in the fruits of species other than tomato, like strawberry, not only by its critical role in determining the sucrose/hexoses ratio but also because this ratio determines the sink strength of the fruit and, indirectly, fruit size. In this fruit, the levels of sucrose and hexoses (glucose and fructose) increased during fruit ripening, and other sugars like xylose and galactose, and the polyol inositol decreased (Bood and Zabetakis 2002).

In addition to starch and sucrose, other intermediate metabolites are important as flavor components, either by themselves or as precursors of other secondary metabolites. Their content is dependent on the activity of main metabolic pathways like glycolysis, tricarboxylic acid cycle (TCA), and respiration. Most detailed studies have been performed in tomato and strawberry fruits. In this fruit, the main organic acids are the TCA intermediates citrate and malate, jointly with quinate, with other TCA intermediates like oxalate, succinate, isocitrate, fumarate, and aconitate being in lower amounts. In tomato fruits, the changes in primary metabolites have been more thoroughly studied. The study has been facilitated by the availability of high

throughput techniques allowing the analysis of tens/hundreds of metabolites at a single step. Thus, after analyzing more than 70 metabolites in this metabolomic approach (Roessner-Tunali et al. 2003), a large increase in hexoses as glucose and fructose was reported, whereas most of the TCA intermediates decreased in the red fruits as well as sucrose, hexose phosphates, and most of the sugar alcohols. In addition, an increase in the aromatic amino acids, as well as in aspartate, lysine, methionine, and cysteine, was also reported. This is especially important since some of them are precursors of flavor compounds. In general, studies on tomato fruit have revealed that in red fruits, with low starch content, glycolysis and respiration are the main fluxes of primary metabolites in the fruit (Carrari and Fernie 2006). Effectively, esters constitute an important fraction of flavor compounds in many fruits. A major source of alcohols for the esterification reactions is derived from pyruvate, the end product of glycolysis. The pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase complex, or via pyruvate decarboxylase and alcohol dehydrogenase (ADH).

Different metabolic pathways, which start from primary metabolism routes leading to the synthesis of flavor compounds in fruits, are now considered.

BIOSYNTHETIC PATHWAY OF VOLATILES RESULTING FROM LIPID DEGRADATION

During the last decade, important advancements have been achieved for the elucidation of biosynthesis pathways of fatty acid-derived aroma compounds. Generally, the flavor compounds from fatty acids are formed by enzymatically catalyzed degradation processes. The enzymatic oxidative degradation of fatty acid is preceded by the action of acyl hydrolase, liberating the free fatty acids from acylglycerols.

The most important degradation processes of fatty acids for the generation of volatiles are the lipoxygenase (LOX) reaction (in-chain oxidation), α- and β-oxidations. The LOX reaction generates hydroxyl, oxo, and epoxy fatty acids and is involved in the formation of polyfunctional fatty acids. The β-oxidation process leads to complete degradation of fatty acids with repetitive β-oxidations. However, α-oxidation appears to be restricted to long-chain fatty acids and to shorten them not beyond the C_{12} chain length. Intermediates and products of those reactions can be metabolized to form volatiles from several chemical classes such as straight-chain alcohol, aldehydes, carboxylic acids, lactones, esters, or ketones.

LOXs are essential components of the oxylipin pathway, converting unsaturated fatty acids into saturated and unsaturated volatile C6- and C9-aldehydes and alcohols in plants, which are found to affect the flavor of fruits and vegetables. The C6-aldehydes, alcohols, and their esters are named "green leaf volatiles" for the distinctive scent that is produced when leaves are crushed (Hatanaka 1993). They are used in industrial processes for the production of natural "green notes" and as food additives because of their "fresh green" odor. LOX is a nonheme, iron dioxygenase that adds molecular oxygen to unsaturated fatty acids containing one or more 1*Z*,4*Z*-pentadienoic moieties (Liavonchanka and Feussner 2006). In plants, there exist two types of LOXs, depending on the positional specificity of fatty acid oxygenation, which can be in C_9 (9-LOX) or C_{13} (13-LOX) of the hydrocarbon backbone, leading to the 9- or 13-hydroperoxy fatty acid, respectively (Fig. 2.2). The

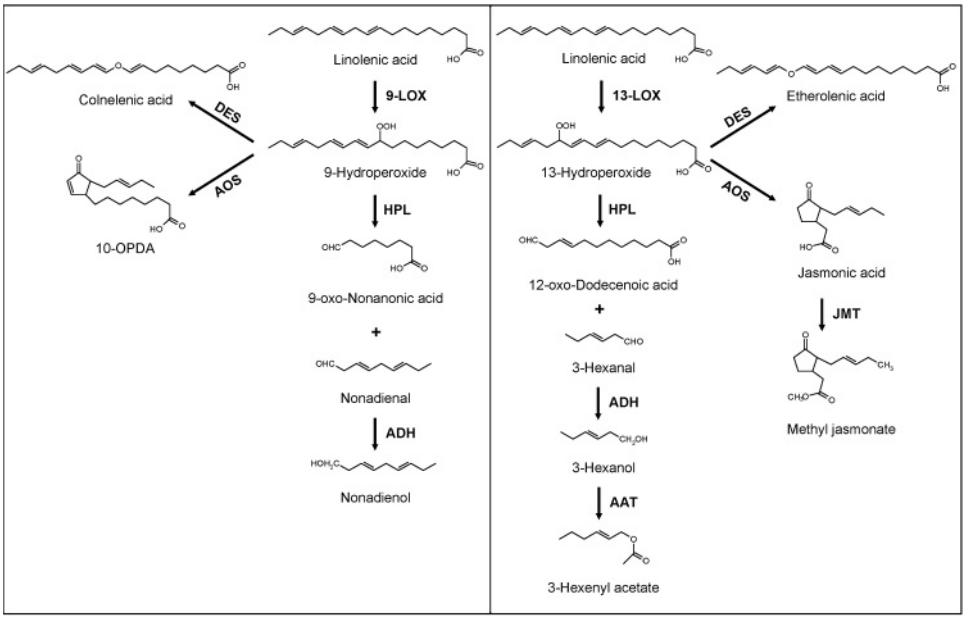

Figure 2.2. The 9-LOX and 13-LOX pathways for the metabolism of linolenic acid (modified from Howe and Schilmiller 2002). AAT, alcohol acyltransferase; ADH, alcohol dehydrogenase; AOS, allene oxide synthase; DES, divinil ether synthase; HPL, hydroperoxide lyase; JMT, jasmonate methyltransferase; LOX, lipoxygenase; 10-OPDA, 12.oxo-phytodienoic.

hydroperoxy derived from LOX is metabolized by several enzymes: allene oxide synthase (AOS), hydroperoxide lyase (HPL), divinil ether synthase (DES), epoxy alcohol synthase, peroxygenase, alkyl hydroperoxide reductase, and LOX itself (Blee 1998; Feussner et al. 2001; Grechkin 1998) (Fig. 2.2).

The HPL branch of the 13-LOX pathway directs the formation of C6-aldehydes and C_{12} ω-keto fatty acids. The best characterized metabolites of the HPL branch are the green leafy volatiles C6-aldehydes (*Z*)-3-hexenal, *n*-hexanal, and their respective derivatives such as (*Z*)-3-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, and the corresponding *E*-isomers (Matsui 2006). The C_{12} compound derived from linolenic acid is a precursor of traumatin, a mitogenic compound that is implicated in wound healing (Zimmerman and Coudron 1979). The short-chain C_6 derivatives, apart from being important volatile constituents of fruits, vegetables, and green leaves, also play main roles in defense against microbial pathogens and insects (Bate and Rothstein 1998; Vancanneyt et al. 2001). Metabolism of 13-hydroperoxides by DES gives rise to divinyl ether fatty acids, that is, etherolenic acid. This metabolic route has been demonstrated in both green leaves and nonphotosynthetic tissues, but its biological function is not known (Grechkin et al. 1995; Hamberg 1998). The AOS branch of the 13-LOX pathway leads from α-linolenic acid to the jasmonic family that includes jasmonic acid and its methyl ester (Howe and Schilmiller 2002). They are essential not only for stress-induced responses but also for developmental process (Feys et al. 1994; Ishiguro et al. 2001). In addition, methyl jasmonate is the main component of the scent of jasmine flowers and contributes to the precious flavors of *Rosmarinus*, *Gardenia*, *Artemisia*, and lemon oil.

Metabolism of 9-hydroperoxy fatty acids by AOS, HPL, and DES generates oxylipins structurally related to the oxylipins derived from the 13-LOX pathway. The 9-hydroperoxides are cleaved by HPL to form the volatile products 3*Z*-nonenal, 2*E*-nonenal, 3*Z*,6*Z*-nonadienal, and 2*E*,6*Z*-nonadienal, which are found in cucumber aroma (Fig. 2.2). 2*E*,6*Z*-nonadienal is of commercial importance because it is one of the most potent fragrance and flavor substance known (Schwab and Schreier 2002).

C6- and C9-aldehydes can be further metabolized by ADH to form the corresponding alcohols. *ADH* genes that participate in the production of aromas are expressed in a developmentally regulated manner, particularly during fruit ripening (Manriquez et al. 2006).

α- and β-Oxidation

Catabolism of straight-chain fatty acids by α- and β-oxidations is a major process for the production of flavor volatiles; however, the specific pathways in plants are not well understood. The β-oxidation results in the successive removal of C2 units (acetyl-CoA) from the parent fatty acid. The detailed mechanisms of conventional β-oxidation are well established (Goepfert and Poirier 2007). Saturated and linear carboxylic acids contribute to the aroma, which are formed during repeated β-oxidation cycles followed by the action of an acyl-CoA hydrolase. Aliphatic acids up to C_{10} are used to accentuate certain aroma characteristics. They play a significant role in flavors due to their sharp, buttery, and cheese-like odors, not only on their own, such as in dairy flavors, but also as substrates for other flavor biosynthesis.

A major group of fatty acid-derived flavor compounds are lactones or alkanolides, which are organoleptically important. They have generally γ-(4) or δ-(5) lactone structures and are linear chained, and a few are even macrocyclic. The γ-lactones are found primarily in plants and δ-lactones primarily in animal products. Sensory important lactones usually have 8–12 carbon, and some are very important volatiles in fruits, such as pineapples, apricots, and strawberry (Basear and Demirci 2007). In addition, due to their low odor threshold, they have a high flavor value in fruits. In plants, lactones are produced in a very low amount by catabolic processes involving the structurally related fatty acids. However, the fact that both the optical purity and the absolute configuration can vary for identical lactones isolated from different sources supports the idea of the presence of different biosynthetic pathways. On the other hand, all lactones originate from their corresponding hydroxyl carboxylic acids (4- or 5-hydroxy carboxylic acid), which are formed by either of these reactions (Albrecht et al. 1992; Haffner and Tressl 1996; Schöttler and Boland 1996):

- reduction of oxo acids by NAD-linked reductase,
- hydrolysis or epoxidation of unsaturated fatty acids,
- reduction of hydroperoxides,
- from naturally occurring hydroxyl fatty acids,
- cleavage of hydroxylated long-chain fatty acids.

In contrast to 4- and 5-hydroxy fatty acids, the 3-hydroxy acids, which are the regular intermediates of the β-oxidation, do not form lactones. However, they are

converted to methyl- or ethyl-3-hydroxyester in plants and contribute to the aroma of fruits such as pineapples, apples, and tamarillos (Torrado et al. 1995; Umano et al. 1992).

α-Oxidation is known to act only on free fatty acids of $C_{14–18}$ chain length, which are degraded to $C_{n–1}$ aldehydes together with varying amounts of C_n hydroxy acids and $C_{n–1}$ fatty acid (Hamberg et al. 1999). Alternatively, short- and medium-chain aldehydes and alcohols are formed by enzymatic reduction of the parent acyl-CoA (Flamini et al. 2007). Aldehydes and alcohols are volatile products; however, alcohols are less important as flavor compounds due to their high odor thresholds in comparison with their aldehyde homologues.

Aliphatic esters contribute to the aroma of nearly all fruits and many other foods. Some are also responsible for the smell of a particular flower; however, many of these esters possess a nonspecific fruity odor. As the number of carbon atoms increases, the odor changes to fatty soapy and even metallic. The straight-chain ester constituents are believed to be synthesized via β-oxidation of fatty acid, which may be then reduced to the corresponding alcohols before transesterification (Schwab and Schreier 2002). Alcohol acyltransferases (AATs) are responsible for the transfer of alcohol to acyl-CoA, resulting in the synthesis of a wide range of esters. Numerous *AAT* genes have been characterized in fruit (Aharoni et al. 2000; Beekwilder et al. 2004; El-Sharkawy et al. 2005).

Methylketones are assumed to be involved in the formation of important aroma secondary alcohols, that is, 2-pentanol and 2-heptanol (Strohalm et al. 2007), and even the odd-numbered methylketones from 5 to 11 carbons are highly potent flavor molecules that have been found in numerous plants. Recently, the first methylketone synthase, *MKS1*, has been isolated from tomato, which catalyze *in vitro* reactions in which C12, C14, and C16 β-ketoacyl-acyl carrier proteins (intermediates in fatty acid biosynthesis) were hydrolyzed and decarboxylated to give C11, C13, and C15 methylketones, respectively (Fridman et al. 2005).

BIOSYNTHESIS PATHWAY OF VOLATILE TERPENES

Terpenoids, are the largest and, perhaps, the most structurally varied family of plant secondary metabolites, consisting of more than 40,000 different molecules. Many terpenoids present a commercial interest because of their use as flavors and fragrances in foods and cosmetics, or because they are important for the quality of agricultural products, and also as raw material for the manufacture of vitamins and other chemicals. In addition, some of them have other potential applications such as herbicides, pesticides, antimicrobial agents, and dietary anticarcinogenics (Crowell 1999). All terpenoids are derived by repetitive fusion of branched five carbon units based on isopentane skeleton. Many of them are volatile, as hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), and even some diterpenes (C_{20}). Terpenoids are derived either from mevalonate (MVA) pathway, which is active in cytosol and starts from acetyl-CoA, or from methylerythritol-4-phosphate (MEP) pathway, which is active in the plastids and starts from pyruvate and glyceraldehyde-3-phosphate (Rodriguez-Concepcion and Boronat 2002). Monoterpenes and sesquiterpenes have been identified at varying levels in the flavor profiles of most soft fruits (Maarse 1991). In some species, they are very important for the characteristic flavor and aroma, that is, in citrus fruit aroma where the monoterpene *R*-limonene

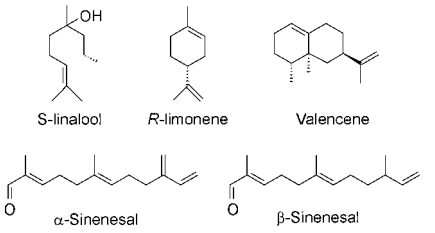

Figure 2.3. Some important plant-derived volatile terpenoids.

normally accounts for over 90% of its essential oils (Weiss 1997). The sesquiterpenes valencene α- and β-sinensals also play an important role in the flavor and aroma of orange fruit, although these are present in minor quantities (Maccarone et al. 1998; Vora et al. 1983; Weiss 1997). The monoterpene *S*-linalool has been described with an important role in strawberry aroma (Aharoni et al. 2004) and is also important for the flavor of other fruits such as tomato (Baldwin et al. 2000) (Fig. 2.3).

The pathway of volatile terpenoids biosynthesis can be summarized in three phases:

1. formation of the terpenoid building C_5 units, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP);
2. the sequential head-to-tail addition of IPP units to DMAPP (C_5 units) to form C_{10}, C_{15}, or C_{20} prenyl diphosphates; and
3. conversion of the resulting prenyl diphosphates to end products.

In the first phase, both the MVA and the MEP pathways lead to the formation of IPP and DMAPP, the basic terpenoid biosynthesis building block. The cytoplasmic MVA pathway is generally considered to supply the precursors (C_5) for sesquiterpene biosynthesis (Newman and Chappell 1999), while MEP pathway provides the C_5 unit for the production of monoterpenes and diterpenes (Lichtenthaler et al. 1997), although there is increasing evidence of the exchange of intermediates between compartments (Laule et al. 2003; Ohara et al. 2003; Schuhr et al. 2003). The terpenoids with C_5 units, hemiterpenes (half terpenes), are the smallest ones. The best known hemiterpene is isoprene itself, a volatile product released from photosynthetically active tissues (Croteau et al. 2000).

In the second phase, the biosynthesis of the terpene precursors, geranyl diphosphate (GDP), farnesyl diphosphate (FDP), and geranylgeranyl diphosphate (GGPP), precursors of monoterpenes, sesquiterpenes, and diterpenes, respectively, is catalyzed by prenyl transferases. These prenyl transferases are GDP synthase, FDP synthase, and GGPP synthase, respectively (Liang et al. 2002; Wang and Ohnuma 1999) (Fig. 2.4), which catalyze the addition of IPP units to prenyl diphosphate with allylic double bonds or the diphosphate moiety. Most of the prenyl transferases accept DMAPP as the initial substrate, but they also bind GDP or FDP depending on the particular prenyl tranferases (Tarshis et al. 1994, 1996; Withers and Keasling 2007).

The third phase of terpene volatile biosynthesis involves the conversion of the various prenyl diphosphates DMAPP (C_5), GDP (C_{10}), FDP (C_{15}), and GGPP (C_{20})

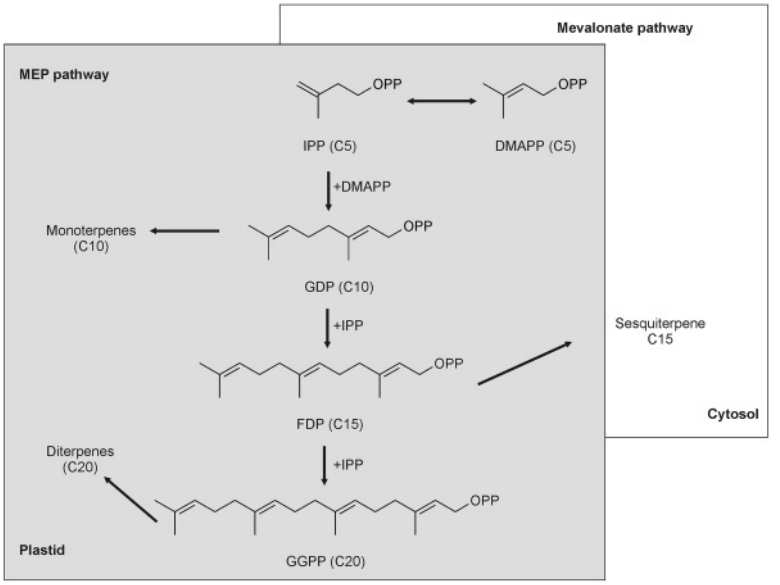

Figure 2.4. Terpenoid pathway for volatile in plants (modified from Aharoni et al. 2004). IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GDP, geranyl diphosphate; FDP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

to hemiterpenes, monoterpenes, sesquiterpenes, and diterpenes, respectively. These reactions, carried out by a large family of enzymes known as terpene synthases, produce the primary representatives of each skeletal type. The investigation of terpene synthases is a very active area of plant volatile research. Many of these genes have been isolated and characterized from various plant species (Bohlmann et al. 1998; Tholl 2006). One of the most exceptional properties is their tendency for making multiple products from a single substrate.

While many terpene volatiles are direct products of terpene synthases, many others are formed through transformation of the initial products by oxidation, dehydrogenation, acylation, and other reactions (Dudareva et al. 2004; Pichersky et al. 2006).

There are a group of terpenoid flavor volatile compounds present at relatively low concentrations but possess strong effects on the human appreciation. This very diverse group of compounds is presumably generated by an oxidative cleavage of the carotenoid (tetraterpenoids, C_{40}) molecule between the C_9 and C_{10} positions, yielding apocarotenoids (also called norisoprenes) with 13 carbon atoms. Although other apocarotenoids of 9–20 carbon atoms are present in nature, only C_{13} has been described an important role in some fruit flavors and in the scent of some flowers (Simkin et al. 2004a,b).

Recently, two carotenoid cleavage dioxygenase genes, *LeCCD1A* and *LeCCD1B*, whose expression was upregulated upon fruit development, were shown to cleave multiple cyclic carotenoids *in vitro* at the 9,10 (9′,10′) double bond, generating a variety of C_{13} apocarotenoids, geranylacetone, pseudoionone, and β-ionone (Simkin et al. 2004a) (Fig. 2.5).

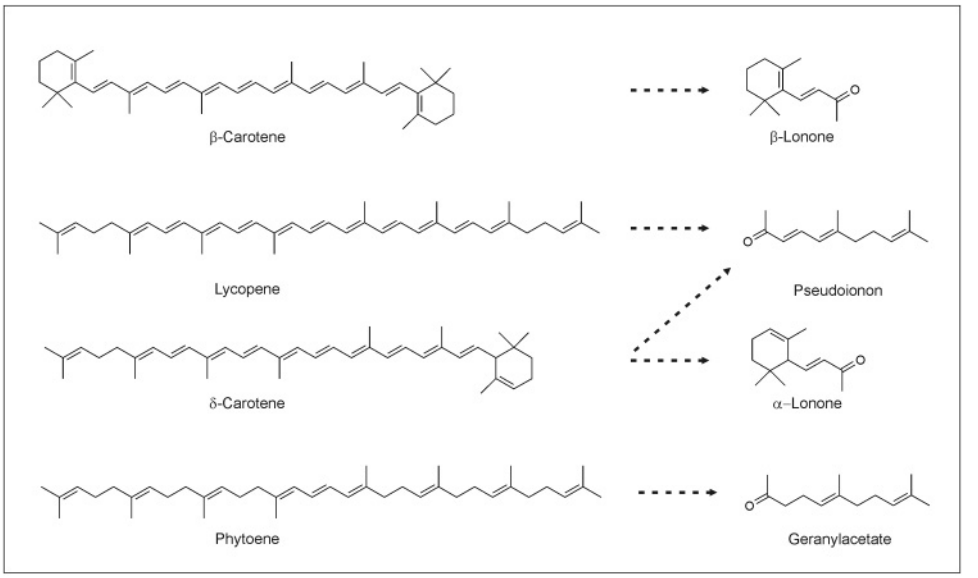

Figure 2.5. Scheme for the reactions catalyzed by LeCCD1 proteins. Carotenoid substrates (left) are oxidatively cleaved to yield the apocarotenoids derivatives (right). (modified from Simkin et al. 2004).

BIOSYNTHESIS PATHWAY OF VOLATILE AMINO ACIDS DERIVATIVES

Phenylpropanoid and benzenoid compounds constitute a large class of secondary metabolites in plants that are derived from phenylalanine via a complex series of branched pathways. The phenylpropanoid compounds are nonvolatile, but when reduced at the C9 position to aldehyde, alcohol, or alkene/alkane, or when they contain alkyl additions to the hydroxyl group of the phenyl ring or to the carboxyl group, these compounds are volatiles. They are common constituents of scent of many plant species (Dudareva and Pichersky 2006).

Phenylpropanoid-related compounds such as 2-phenylacetaldehyde and 2-phenylethylalcohol have an important contribution to tomato flavor (Buttery 1993). At low concentration, both compounds have pleasant fruity or floral odors and are also major contributors to scent in many flowers (Knudsen et al. 1993). However, at high levels, the pungent aroma of 2-phenylacetaldehyde is unpleasant (Tadmor et al. 2002). Formation of both compounds initiates from phenylalanine (Fig. 2.6). Despite the importance of both compounds to flavor and aroma, it is not clear how plants synthesize them. Deuterium-labeling phenylalanine studies in rose suggest that phenylacetaldehyde is not a precursor of phenylethanol, and the major flux goes through a different route, possibly through phenylpyruvate and phenyllactic acid (Watanabe et al. 2002). It has been recently described that a small family of L-amino acid decarboxylases (AADCs) from tomato catalyzed the conversion of L-phenylalanine to 2-phenylethylamine (Tieman et al. 2006). Overexpression of the corresponding genes in transgenic tomato plants led to the accumulation of significantly higher levels of 2-phenylacetaldehyde and 2-phenylethanol as well as the related compounds 2-phenylacetonitrile and 1-nitro-2-phenylethane.

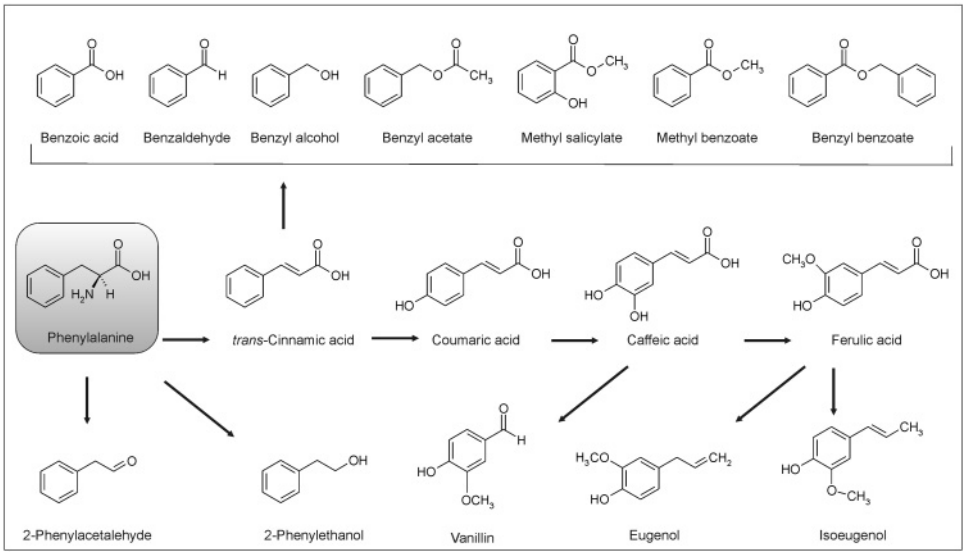

Figure 2.6. Schematic representation of the shikimate pathway that leads volatile benzenoid and phenylpropenes volatiles (modified from Verdonk et al. 2005).

Other benzenoids such as methyl salicylate and methyl benzoate are common components of floral scent, that is, vanillin, an important aroma compound, and have *trans*-cinnamic acid as the precursor (Fig. 2.6). In general, the first step in the biosynthesis of some benzenoid compounds is catalyzed by phenylalanine ammonia lyase (PAL) enzyme, which produces *trans*-cinnamic acid by deamination of phenylalanine. Then, the *trans*-cinnamic acid side chain is shortened by C2 units, in which several routes have been proposed (Schuurink et al. 2006). One route can be through the action of the β-oxidative CoA-dependent pathway, analogous to β-oxidation of fatty acids and proceeds through the formation of CoA-ester intermediates. Experiments with stable isotope-labeled precursor in tobacco leaves suggested that benzoic acid is produced from *trans*-cinnamic acid via this β-oxidative CoA-dependent pathway, first yielding benzoyl-CoA, which can be hydrolyzed by a thioesterase to free benzoic acid (Ribnicky et al. 1998). Another route can be through non-β-oxidative CoA-independent pathway that involves the hydration of the free *trans*-cinnamic acid to 3-hydroxy-phenylpropionic acid and side-chain degradation via a reverse aldol reaction with formation of benzaldehyde, which is then oxidized to benzoic acid by an $NADP^+$-dependent aldehyde dehydrogenase (Dudareva and Pichersky 2006). *In vivo* isotope labeling and metabolic flux analysis in petunia flowers have revealed that both pathways are involved in the formation of benzenoid compounds, and that benzyl benzoate is an intermediate between L-phenylalanine and benzoic acid (Boatright et al. 2004).

Other phenylpropenes, such as isoeugenol or eugenol and related compounds, are associated with pleasure aromas and flavors; however, the biochemical pathways for their synthesis have not been completely elucidated yet. Previous studies indicated that these are likely derived from molecules downstream of *trans*-cinnamic acid (Gang et al. 2001) (Fig. 2.6). Recently, it has been described

that phenylpropene-forming enzymes belong to a structural family of NADPH-dependent reductases that use coniferyl acetate and NADPH to form eugenol in sweet basil and isoeugenol in petunia (Koeduka et al. 2006).

Very little is known about enzymes and genes responsible for metabolic steps leading to phenylpropanoid and benzenoid volatiles; still, significant progress has been made in the discovery of common modifications. Compounds that are already somewhat volatile may also be modified, resulting in enhanced volatility or changed olfactory properties. The majority of these modifications involve reactions such as hydroxylation, acetylation, oxidation, and methylation of downstream products (Dudareva et al. 2004).

Other groups of flavor compounds are sulfur molecules. The sulfur-containing flavor compounds are synthesized from methionine and cysteine. In onion and garlic, a series of volatile sulfur compounds responsible for the typical flavors and odors of *Alliums* have been described. These metabolites are *S*-methyl cysteine sulfoxide (MCSO, methiin; present in most *Alliums*, some Brassicaceae), *S*-allyl cysteine sulfoxide (ACSO, alliin; characteristic of garlic), *S-trans*-prop-1-enyl cysteine sulfoxide (PECSO, isoalliin; characteristic of onion), and *S*-propyl cysteine sulfoxide (PCSO, propiin; in onion and related species), which are generated by the cleavage of relatively stable, odorless *S*-alk(en)yl cysteine sulfoxide flavor precursors by the enzymes allinase and lachrymatory-factor synthase. The biosynthetic pathway of flavor precursors involves alk(en)ylation of the cysteine in glutathione, followed by cleavage and oxidation to form the sulfoxides or (thio)alk(en)ylation of cysteine or *O*-acetylserine (Jones et al. 2004).

Little is known about the catabolism of methionine in plant cells. Several authors have reported that plants emit volatile sulfur-containing compounds such as methanethiol, dimethyl disulfide, or dimethyl sulfide as a consequence of the accumulation of free methionine (Boerjan et al. 1994; Hacham et al. 2002). The process responsible for the production of these volatiles is still unknown. Recently, in *Arabidopsis* cells, methionine γ-lyase enzyme that catalyzed the formation of methanethiol, α-ketobutyrate, and ammonia from the catabolism of methionine has been described (Rebeille et al. 2006).

Amino acids such as alanine, leucine, isoleucine, and valine are also involved in volatile synthesis. In tomatoes, valine is a reported precursor for 1-*N*-2-methylpropane, 3-methylbutylnitrile, 1-*N*-3-methylbutane, and 2-isobutylthiazole (Buttery and Ling 1993). Isoleucine is a precursor of 2-methylbutanol and 2-methylbutyric acid. Compounds derived from leucine, 3-methylbutanal, 3-methylbutanol, and 3-methylbutanoic acid are abundant in various fruits such as strawberry, tomato, and grape varieties (Aubert et al. 2005). In addition, amino acids, as well as their alcohol and acid derivatives, can be esterified to compounds with a large impact on fruit odor, such as 3-methylbutyl acetate and 3-methylbutyl butanoate in banana (Nogueira et al. 2003), by deamination, decarboxylation, several reductions, and esterification (Perez et al. 1992).

GLYCOSYLATION

Many secondary metabolites are subject to the final step of glycosylation; that is, they are conjugated to different positions to simple sugars or multiple sugars,

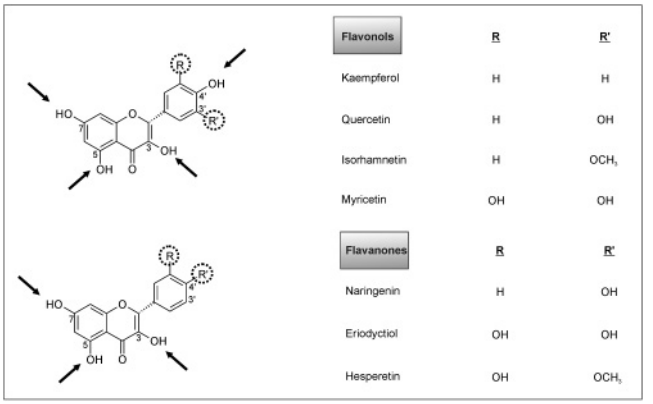

Figure 2.7. Chemical structures from more common flavonoids where the glycosylation sites are highlighted.

like di-, tri-, and oligosaccharides. For instance, for the flavonol quercetin, more than 300 glycosides have been identified. This metabolic change is especially important for lipophilic compounds, which as a consequence change their chemical properties. The result is that water solubility is increased, and commonly also stability.

The glycosylation reactions are catalyzed for a class of enzymes named as glycosyltransferases (GTs). There are many GT families, but those catalyzing the transfer of sugars to small lipophilic molecules are classified as GT1 family (Bowles et al. 2006). The sugar donor in the reaction is a nucleotide sugar, most commonly UDP-glucose, although other sugars and sugar derivatives, like galactose, rhamnose, xylose, and glucuronic acid, can be the substrate for the plant GT enzymes. Functional groups that might accept sugars include –OH, –COOH, $–NH_2$, –SH, and C–C. In addition, regioselectivity in the conjugation reaction, and the possibility of single/multiple reactions at different positions, enhances significantly the number of possible conjugates for a compound. Figure 2.7 as an example shows the multiple glycosylation sites for flavonoids.

Studies on plant GTs indicate that the glycosylation reactions take place in the cytosol; however, the conjugated products can be transported to other cell compartment, as its accessibility to some transport systems can result. For instance, anthocyanins, which are produced by glycosylation of flavonoids, are targeted for accumulation in the vacuole. In plant genomes, many GT-encoding genes have been identified. Moreover, *in vitro* studies of these enzymes have demonstrated that not only multiple GTs have the capacity to glycosylate the same substrate, but also that individual GT can glycosylate multiple substrates. This opens a wide field of study that is highly important in the metabolic pathways determining the organoleptic properties in the fruits of the plants. Thus, it is known that in grapefruit, the rhamnose linkage to the naringenin 7-O-glucoside determines the bitter flavor, but if the 2-OH position of the glucose is rhamnosylated, the resulting compound is bitter; when the 6-OH position is rhamnosylated, the compound is tasteless.

THE REGULATION OF METABOLIC PATHWAYS

The identification of enzymes and intermediates involved in a metabolic pathway is not sufficient to have a deep knowledge about its functioning. Regulation of the pathway, that is, knowledge of the metabolic fluxes under specific conditions, is also a priority to have a deep knowledge of metabolism. Understanding the regulation will require, besides the identification and quantification of the structural components (genes, enzymes, and intermediates), an estimation of the modifications and interactions that they are susceptible. Since these changes occur in the context of a developmental program in a plant organ, this means that there is a changing regulation pattern along time during the ripening of the fruit, and also that environmental factors, including pathogen interactions, can have an effect on the fluxes of the metabolic pathway.

The final objective is to know which are the fluxes in a metabolic pathway; therefore, enzymes and metabolites are relevant, as well as their modifications and interactions. Their joint functioning has been afforded by various theoretical approaches that basically provide a holistic view of the pathway under study. However, since enzymes are the products of gene expression, a gene regulatory model must also be considered in terms of structural components and their modifications and interactions (Sweetlove et al. 2008). All these elements can be contemplated in a network (Lange 2006), as shown in Figure 2.8.

Various metabolic models have been advanced to explain the flux through the reactions in the pathway, as well as to predict the values of the fluxes in response

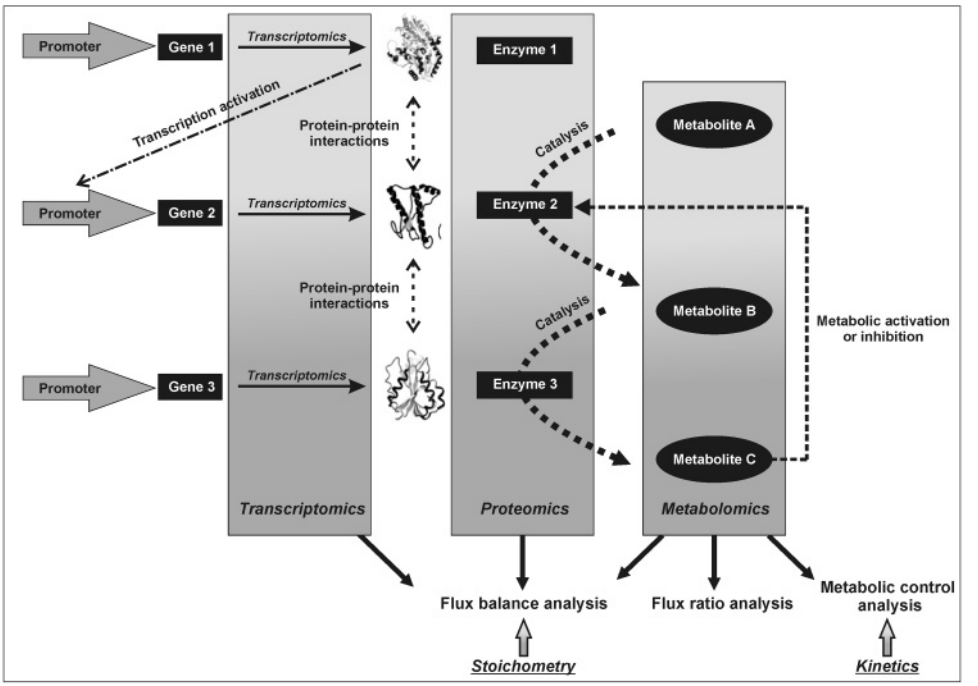

Figure 2.8. Outline of a hypothetical metabolic network indicating the possible control elements in a metabolic flux (adapted from Lange 2006).

to changes in two of the structural components, enzymes and metabolites. The most used model is known as the kinetic model (Sweetlove et al. 2008). The model assumption is that the kinetic properties of the enzymes catalyzing a set of reactions will determine the flux through the pathway. For instance, the method estimates the contribution of any enzyme to the overall flux of the pathway, and this is quantified in a value known as "flux control coefficient." It is a mechanistic model in the sense that, based on differential equations, it establishes the relationship between substrate and effector concentrations and reaction velocity. This gives a predictive value to the model and, consequently, the possibility to hypothesize about the changes in the pathway when one of the structural components changes. This might be important for the metabolic engineering of the future that must look for adapting the fruit properties and composition to the needs of different groups of consumers.

Basic principles of one of the kinetic models were formulated some time ago (Kacser and Burns 1973). Two main corollaries can be deduced from the model. First is that the control of the flux must be shared by several elements; this means that several steps in the pathway might be responsible for the changes in the flux. Actually, this is the more common situation, in contrast with early analysis of metabolic regulation that searched for the bottleneck step controlling the whole pathway. This model has been applied in tomato fruits in order to analyze the interconnections in sucrose/starch synthesis. The study concluded that the flux was shared by sucrose synthase, fructokinase, and ADP-glucose pyrophosphorylase (ADPGase) (Schaffer and Petreikov 1997) (Fig. 2.1). The second corollary is a consequence of another property of the model that establishes that once a pathway has been delimited, the summation of all the "flux control coefficients" remains constant. Consequently, a change in any of them directly causes a change in the other coefficients. As structural components, enzymes and metabolites might change for different cells, stages, and organisms; hence, the regulation of the pathway also changes. Thus, the same sucrose/starch transition previously studied in tomato fruits was also studied in potato tuber. It was concluded that only ADPGase exerted the control of the flux in this organ/species (Carrari and Fernie 2006).

The quality of this metabolic model rests on the quality enzyme kinetics data. This has been a shortcoming for its application to plant metabolism. Although the general corollaries above indicated have been valid to change the view on the regulation of the metabolic pathways, the "flux control coefficients" have been determined for only a small number of plant enzymes.

There is also another element that limits the application of this flux control model; it is the fact that it must be applied to a subset of reactions that constitute a pathway, that is, an artificial closed system. This is simplistic since metabolic barriers do not exist within the cell where frequent cross talk among pathways is known to exist. For example, going back to the same example of starch metabolism in tomato fruits, it has been found that changes in the NAD-malic enzyme, in the mitochondria and uridine monophosphate synthase, a distant reaction, had major effects on the accumulation of starch (Sweetlove et al. 2008).

The consequences of a change in the environmental, or in the developmental circumstances that commonly accompany the growth and ripening of the fruits, are recently being studied at the gene regulation level. The availability of oligo-based chips for many plant species has allowed us to know the gene expression at transcriptional level, "transcriptomics," in fruits during development and under different

growing conditions. In some cases, the observed metabolic changes have been explained by the changing patterns of gene expression, with the benefit of identifying the main gene(s) and enzyme(s) responsible for the metabolic output (Aharoni et al. 2000). However, the limited success in identifying the key regulatory elements in a pathway using transcriptomics has led to the use of other "omics" approaches, like proteomics and metabolomics. These new holistic approaches are fore viewed as important tools for a deep knowledge of the metabolism, and its regulation, at tissue, organ, and cellular levels.

REFERENCES

Aharoni A, Giri AP, Verstappen FW, Bertea CM, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bouwmeester HJ. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–31.

Aharoni A, Keizer LC, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen AM, De Vos RC, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *Plant Cell* 12:647–62.

Albrecht W, Schwarz M, Heidlas J, Tressl T. 1992. Studies on the biosynthesis of aliphatic lactones in *Sporobolomyces odorus*. Conversion of (S)- and (R,S)-13-hydroxy-(Z,E)-9,11-octadecadienoic acid into optically pure (R)-.delta.-decalactone. *J Org Chem* 57:1954–6.

Aubert C, Baumann S, Arguel H. 2005. Optimization of the analysis of flavor volatile compounds by liquid-liquid microextraction (LLME). Application to the aroma analysis of melons, peaches, grapes, strawberries, and tomatoes. *J Agric Food Chem* 53:8881–95.

Baldwin ES, Scott JW, Shewmarker CK, Schuch W. 2000. Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience* 35:1013–22.

Basear KHC, Demirci F. 2007. Chemistry of essential oil. In Berger RG (ed.), *Flavors and Fragrances*. Heidelberg: Springer, p. 43.

Bate NJ, Rothstein SJ. 1998. C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. *Plant J* 16:561–9.

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135:1865–78.

Blee E. 1998. Phytooxylipins and plant defense reactions. *Prog Lipid Res* 37:33–72.

Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N. 2004. Understanding in vivo benzenoid metabolism in petunia petal tissue. *Plant Physiol* 135:1993–2011.

Boerjan W, Bauw G, Van Montagu M, Inze D. 1994. Distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco. *Plant Cell* 6:1401–14.

Bohlmann J, Meyer-Gauen G, Croteau R. 1998. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. *Proc Natl Acad Sci U S A* 95:4126–33.

Bood KG, Zabetakis I. 2002. The biosynthesis of strawberry flavor (II): Biosynthetic and molecular biology studies. *J Food Sci* 67:2–8.

Bowles D, Lim E-K, Poppenberger B, Vaistij FE. 2006. Glycosyltransferases of lipophilic small molecules. *Annu Rev Plant Biol* 57:567–97.

Buttery RG. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree TE, Teranishsi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: American Chemical Society, pp. 259–286.

Buttery RG, Ling LC. 1993. Volatiles of tomato fruit and plant parts: Relationship and biogenesis. In Teranishsi R, Buttery RG, Sugisawa H (eds.), *Bioactive Volatile Compounds from Plants*. Washington, DC: ACS Books, p. 11.

Carrari F, Fernie AR. 2006. Metabolic regulation underlying tomato fruit development. *J Exp Bot* 57:1883–97.

Croteau R, Kutchan TM, Lewis NG. 2000. Natural products (secondary metabolites). In Buchanan B, Gruissem W, Jones R (eds.), *Biochemistry and Molecular Biology of Plants*. Rockville, MD: American Society of Plant Physiology, p. 68.

Crowell PL. 1999. Prevention and therapy of cancer by dietary monoterpenes. *J Nutr* 129:775S–78S.

Dudareva N, Pichersky E. 2006. Floral scent metabolic pathways: Their regulation and evolution. In Dudareva N, Pichersky E (eds.), *Biology of Floral Scent*. Boca Raton, FL: CRC Press, p. 23.

Dudareva N, Pichersky E, Gershenzon J. 2004. Biochemistry of plant volatiles. *Plant Physiol* 135:1893–902.

El-Sharkawy I, Manriquez D, Flores FB, Regad F, Bouzayen M, Latche A, Pech JC. 2005. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. *Plant Mol Biol* 59:345–62.

Feussner I, Kuhn H, Wasternack C. 2001. Lipoxygenase-dependent degradation of storage lipids. *Trends Plant Sci* 6:268–73.

Feys B, Benedetti CE, Penfold CN, Turner JG. 1994. Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell* 6:751–9.

Flamini G, Tebano M, Cioni PL. 2007. Volatiles emission patterns of different plant organs and pollen of *Citrus limon*. *Anal Chim Acta* 589:120–4.

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D. 2004. Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* 305:1786–9.

Fridman E, Wang J, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E. 2005. Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. *Plant Cell* 17:1252–67.

Gang DR, Wang J, Dudareva N, Nam KH, Simon JE, Lewinsohn E, Pichersky E. 2001. An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol* 125:539–55.

Goepfert S, Poirier Y. 2007. Beta-oxidation in fatty acid degradation and beyond. *Curr Opin Plant Biol* 10:245–51.

Grechkin A. 1998. Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog Lipid Res* 37:317–52.

Grechkin AN, Fazliev FN, Mukhtarova LS. 1995. The lipoxygenase pathway in garlic (*Allium sativum* L.) bulbs: Detection of the novel divinyl ether oxylipins. *FEBS Lett* 371:159–62.

Hacham Y, Avraham T, Amir R. 2002. The N-terminal region of *Arabidopsis cystathionine* gamma-synthase plays an important regulatory role in methionine metabolism. *Plant Physiol* 128:454–62.

Haffner T, Tressl R. 1996. Biosynthesis of (R)-γ-decanolactone in the yeast *Sporobolomyces odorus*. *J Agric Food Chem* 44:1218–23.

Hamberg M. 1998. A pathway for biosynthesis of divinyl ether fatty acids in green leaves. *Lipids* 33:1061–71.

Hamberg M, Sanz A, Castresana C. 1999. Alpha-oxidation of fatty acids in higher plants. Identification of a pathogen-inducible oxygenase (piox) as an alpha-dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid. *J Biol Chem* 274:24503–13.

Hatanaka A. 1993. The biogeneration of green odour by green leaves. *Phytochemistry* 34:1201–18.

Howe GA, Schilmiller AL. 2002. Oxylipin metabolism in response to stress. *Curr Opin Plant Biol* 5:230–6.

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K. 2001. The defective in anther dehiscience gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. *Plant Cell* 13:2191–209.

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA. 2004. Biosynthesis of the flavor precursors of onion and garlic. *J Exp Bot* 55:1903–18.

Kacser H, Burns JA. 1973. The control of flux. *Symp Soc Exp Biol* 27:65–104.

Klann EM, Hall B, Bennett AB. 1996. Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiol* 112:1321–30.

Knudsen JT, Tollsten L, Bergstron LG. 1993. Floral scents a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33:253–80.

Koeduka T, Fridman E, Gang DR, Vassao DG, Jackson BL, Kish CM, Orlova I, Spassova SM, Lewis NG, Noel JP, Baiga TJ, Dudareva N, Pichersky E. 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Plant Biol* 103:10128–33.

Lange BM. 2006. Integrative analysis of metabolic networks: From peaks to flux models? *Curr Opin Plant Biol* 9:220–6.

Laule O, Furholz A, Chang HS, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange M. 2003. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 100:6866–71.

Liang PH, Ko TP, Wang AH. 2002. Structure, mechanism and function of prenyltransferases. *Eur J Biochem* 269:3339–54.

Liavonchanka A, Feussner I. 2006. Lipoxygenases: Occurrence, functions and catalysis. *J Plant Physiol* 163:348–57.

Lichtenthaler HK, Schwender J, Disch A, Rohmer M. 1997. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. *FEBS Lett* 400:271–4.

Maarse H. 1991. *Volatile Compounds in Food and Beverages*, 1st Ed. New York: CRC.

Maccarone E, Campisi S, Fallico B, Rapisarda P, Sgarlata R. 1998. Flavor components of Italian orange juices. *J Agric Food Chem* 46:2293–8.

Manriquez D, El-Sharkawy I, Flores FB, El-Yahyaoui F, Regad F, Bouzayen M, Latche A, Pech JC. 2006. Two highly divergent alcohol dehydrogenases of melon exhibit fruit ripening-specific expression and distinct biochemical characteristics. *Plant Mol Biol* 61:675–85.

Matsui K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Curr Opin Plant Biol* 9:274–80.

Newman JD, Chappell J. 1999. Isoprenoid biosynthesis in plants: Carbon partitioning within the cytoplasmic pathway. *Crit Rev Biochem Mol Biol* 34:95–106.

Nogueira JM, Fernandes PJ, Nascimento AM. 2003. Composition of volatiles of banana cultivars from Madeira Island. *Phytochem Anal* 14:87–90.

Ohara K, Ujihara T, Endo T, Sato F, Yazaki K. 2003. Limonene production in tobacco with Perilla limonene synthase cDNA. *J Exp Bot* 54:2635–42.

Perez AG, Rios JJ, Sanz C, Olias JM. 1992. Aroma components and free amino acids in strawberry variety chandler during ripening. *J Agric Food Chem* 40:2232–5.

Pichersky E, Noel JP, Dudareva N. 2006. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* 311:808–11.

Rebeille F, Jabrin S, Bligny R, Loizeau K, Gambonnet B, Van Wilder V, Douce R, Ravanel S. 2006. Methionine catabolism in *Arabidopsis* cells is initiated by a gamma-cleavage process and leads to S-methylcysteine and isoleucine syntheses. *Proc Natl Acad Sci U S A* 103:15687–92.

Ribnicky DM, Shulaev VV, Raskin II. 1998. Intermediates of salicylic acid biosynthesis in tobacco. *Plant Physiol* 118:565–72.

Rodriguez-Concepcion M, Boronat A. 2002. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiol* 130:1079–89.

Roessner-Tunali U, Hegemann B, Lytovchenko A, Carrari F, Bruedigam C, Granot D, Fernie AR. 2003. Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiol* 133:84–99.

Schaffer AA, Petreikov M. 1997. Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiol* 113:739–46.

Schöttler M, Boland W. 1996. Biosynthesis of dodecano-4-lactone in ripening fruits: Crucial role of an epoxide-hidrolase in enantioselective generation of aroma components of the nectarine (*Prunus persica* var *nucipersica*) and strawberry (*Fragaria ananassa*). *Helv Chim Acta* 79:1488–96.

Schuhr CA, Radykewicz T, Sagner S, Latzel C, Zenk MH, Arigoni D, Bacher A, Rohdich F, Eisenreich W. 2003. Quantitative assessment of crosstalk between the two isoprenoid biosynthesis pathways in plants by NMR spectroscopy. *Phytochem Rev* 2:3–16.

Schuurink RC, Haring MA, Clark DG. 2006. Regulation of volatile benzenoid biosynthesis in petunia flowers. *Trends Plant Sci* 11:20–5.

Schwab W, Schreier P. 2002. Enzymatic formation of flavor volatiles from lipids. In Kuo TM, Gardner HW (eds.), *Lipid Biotechnology*. New York: Marcel Dekker, p. 25.

Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ. 2004a. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles beta-ionone, pseudoionone, and geranylacetone. *Plant J* 40:882–92.

Simkin AJ, Underwood BA, Auldridge M, Loucas HM, Shibuya K, Schmelz E, Clark DG, Klee HJ. 2004b. Circadian regulation of the PhCCD1 carotenoid cleavage dioxygenase controls emission of beta-ionone, a fragrance volatile of petunia flowers. *Plant Physiol* 136:3504–14.

Strohalm H, Dregus M, Wahl A, Engel KH. 2007. Enantioselective analysis of secondary alcohols and their esters in purple and yellow passion fruits. *J Agric Food Chem* 55:10339–44.

Sweetlove LJ, Fell D, Fenie AR. 2008. Getting to grips with the plant metabolic network. *Biochem J* 409:27–41.

Tadmor Y, Fridman E, Gur A, Larkov O, Lastochkin E, Ravid U, Zamir D, Lewinsohn E. 2002. Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. *J Agric Food Chem* 50:2005–9.

Tarshis LC, Proteau PJ, Kellogg BA, Sacchettini JC, Poulter CD. 1996. Regulation of product chain length by isoprenyl diphosphate synthases. *Proc Natl Acad Sci U S A* 93:15018–23.

Tarshis LC, Yan M, Poulter CD, Sacchettini JC. 1994. Crystal structure of recombinant farnesyl diphosphate synthase at 2.6-A resolution. *Biochemistry* 33:10871–7.

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr Opin Plant Biol* 9:297–304.

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ. 2006. Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci U S A* 103:8287–92.

Torrado A, Suárez A, Duque C, Krajewski D, Neugebauer W, Schreier P. 1995. Volatile constituents from tamarillo (*Cyphomandra betacea* Sendtn.) fruit. *Flav Frag J* 10:349–54.

Umano K, Hagi Y, Nakahara K, Shoji A, Shibamoto T. 1992. Volatile constituents of green and ripened pineapple (*Ananas comosus* [L.] Merr.). *J Agric Food Chem* 40:599–603.

Vancanneyt G, Sanz C, Farmaki T, Paneque M, Ortego F, Castanera P, Sanchez-Serrano JJ. 2001. Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. *Proc Natl Acad Sci U S A* 98:8139–44.

Verdonk JC, Haring MA, van Tunen AJ, Schuurink RC. 2005. ODORANT1 regulates fragrance biosynthesis in petunia flowers. *Plant Cell* 17:1612–24.

Vora JD, Matthews RF, Crandall PG, Cook R. 1983. Preparation and chemical composition of orange oil concentrates. *J Food Sci* 48:1197–9.

Wang K, Ohnuma S. 1999. Chain-length determination mechanism of isoprenyl diphosphate synthases and implications for molecular evolution. *Trends Biochem Sci* 24:445–51.

Watanabe S, Hayashi K, Yagi K, Asai T, MacTavish H, Picone J, Turnbull C, Watanabe N. 2002. Biogenesis of 2-phenylethanol in rose flowers: Incorporation of [2H8]L-phenylalanine into 2-phenylethanol and its beta-D-glucopyranoside during the flower opening of Rosa "Hoh-Jun" and *Rosa damascena* Mill. *Biosci Biotechnol Biochem* 66:943–7.

Weiss EA. 1997. *Essential Oil Crops*. Wallingford, UK: CAB International, Springer.

Withers ST, Keasling JD. 2007. Biosynthesis and engineering of isoprenoid small molecules. *Appl Microbiol Biotechnol* 73:980–90.

Zimmerman DC, Coudron CA. 1979. Identification of traumatin, a wound hormone, as 12-oxo-trans-10-dodecenoic acid. *Plant Physiol* 63:536–41.

CHAPTER 3

Sensory Evaluation of Fruit and Vegetable Flavors

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INTRODUCTION

The organoleptic quality of fruit and vegetables plays an important role in consumers' satisfaction, and it can influence their choice at the moment of purchase and modify the degree of pleasure they experience when consuming these products. The sensory characteristics are the primary reason consumers purchase a particular type of fruit (Wismer et al. 2005). These characteristics (appearance, aroma, taste, and texture) contribute differently to the acceptability of various fruits and vegetables.

For the last decades, the genetic improvement of plants, carried out by conventional techniques or by molecular engineering, was mainly focused to increase yield, to get more plague- or illness-resistant genotypes, or to improve their agronomical characteristics or even their shelf life (Ranalli and Cubero 1997). During this time, a number of fruits and vegetables with better agronomical characteristics and especially with a longer shelf life have appeared on the market, but, in general, their sensorial properties were clearly different from the products coming from traditional varieties and obtained by traditional growing techniques. Fruits and vegetables in the market are frequently found that, though having adequate color and texture, show a lack of flavor or even a different flavor from that expected by the typical consumer. This is because in the past improvement programs, sensory quality control was considered at most as a secondary activity, usually based on the opinion and expertise of a reduced number of judges. Additionally, these judges were mainly focused on detecting the genotypes showing any important defect in color, flavor, or texture. At present, it is widely recognized that the success in the market of any fruit or vegetable is not dependent only on the absence of perceivable defects but also on the fulfillment of the consumers' requirements and, furthermore, on the degree of satisfaction produced upon their consumption (Hampson et al. 2000; Wismer et al. 2005). To date, any genetic improvement program will certainly include

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sensory techniques and consumer tests in order to identify the more promising products as to their future success in the market (Harker et al. 2003; Jaeger and Harker 2005).

For most of the plant products and especially for fruits, flavor is considered as the key factor for their acceptance, but its measurement and control present serious problems. The first question is: What is flavor? There is no universally accepted definition. Some authors identify flavor with the volatile content, due to the unquestionable influence of volatiles' presence and concentration. Others consider flavor to be the global sensation produced in humans during consumption of the particular foodstuff. More often accepted and used is to consider flavor as the combination of the sensations perceived simultaneously by the senses of taste and olfaction. Both of these are chemical senses; for example, they respond to stimuli that are essentially molecules capable of activating the different receptors located in the sensitive cells in the mouth and nose (Durán and Costell 1999). Perhaps, one of the best definitions of flavor is that proposed by Heymann and others (1993): "Flavor is the biological response to chemical compounds (the physical stimuli) by the senses, interpreted by the brain in the context of human experience." Relative to the physical stimuli, Kader (2008) stated that fruit and vegetable flavor depends upon taste, which is mainly related to sugar and organic acid content and other compounds such as tannins, polyacetylenes, or phenolic acids, and aroma, related to the odor-active volatile compounds (esters, alcohols, aldehydes, and ketones). In the matter of flavor perception, the establishment of relationships between the physical stimuli and the physiological reaction of humans and between the latter and the sensation experienced by people upon consuming is not an easy task. This is why it is very difficult to make predictions as to the possible perceptible differences between products differing in composition or structure as a result of genetic manipulation, preharvest, harvest, or postharvest factors (Bartoszewski et al. 2003; Edelenbos et al. 2006). Even more difficult will be to predict the degree of acceptance by the consumer.

The study of food flavor requires a multidisciplinary approach combining information on the following: concentration of both volatile and nonvolatile compounds with possible influence on flavor, structure and other physical characteristics of the food matrix, physicochemical mechanisms governing release of tasting and odoring compounds, adequate sensory techniques to ascertain how flavor is perceived, and how this perception affects the final acceptance of the product.

In the context of fruit and vegetable breeding programs, sensory analysis plays an important role as a selection tool (Causse et al. 2001; Hampson et al. 2000; Jaeger and Harker 2005; Rouseff et al. 1994). Sensory analysis provides, in fact, the adequate methodology to investigate, for example, how different tomato genotypes (Sinesio et al. 2007) or different apple varieties affect flavor (Gómez et al. 1998) or how flavor varies depending on genetic modifications (Bartoszewski et al. 2003; Causse et al. 2001), on breeding or storage conditions (Auerswald et al. 1999; Maul et al. 2000), or on the ripeness degree on harvesting (Cascales et al. 2005). It should be noted here that the application of sensory analysis to the evaluation of fruits and vegetables flavor is not straightforward. It requires a wide knowledge of the different experimental methods and of the various possible statistical treatments to be used in order to choose the most adequate ones. Extensive information is available

at present on sensory methodology, on the experimental conditions to carry out the various sensory tests (Lawless and Heymann 1998; Meilgaard et al. 1999), and on the experimental designs and the statistical treatment applicable to the different types of data (Gacula 1993; MacFie and Thomson 1994; Meullenet et al. 2007; O'Mahony 1985). This information constitutes the base of the development and setup of efficient systems to measure and control fruit and vegetable flavor as required by the particular characteristics of any product (Costell 2002; Hampson et al. 2000; Muñoz et al. 1992).

This chapter will deal with some of the more relevant aspects of the mechanisms involved in the release and perception of flavor as well as with the description of the sensory techniques applicable to the sensory analysis of the flavor of fruit and vegetables.

FLAVOR RELEASE AND PERCEPTION

Both the release and the perception of flavor are complex processes involving different physicochemical and physiological phenomena (Overbosch et al. 1991; Taylor 2002; Taylor and Roberts 2004). The flavor perception process begins when the flavoring and odoring molecules are released from the food matrix and travel to either the taste or the odor receptors located in the mouth and nose, respectively. The nonvolatile compounds are transported in the saliva to the taste receptors in the mouth, while the volatile ones are carried in the air to the odor receptors located in the nose. Then, a process of transduction of the signals detected by the corresponding receptors through the brain occurs, where the flavor sensation experienced by man is generated.

It is evident that the origin of such a sensation is linked to the presence in the food of those components capable of stimulating the senses of taste and odor, when in certain concentrations. A great deal of information on the volatile content of different fruits and vegetables is available to date. Important advances have been made during the last decades on the identification of the components of these products having clear effects on their flavor (Maarse 1991). In general, it can be said that among the hundreds of identifiable compounds for each species, only a few may be odor active. For example, in fresh tomato, only 30 of the more than 400 identified volatiles may contribute to its flavor (Azondanlou et al. 2003). Identification of the compounds responsible for the flavor of any product can be approached in different ways. For the last years, the gas chromatography olfactometry has been used to obtain direct information on the relationship between composition and sensory responses. Different methods for determining relative aroma potency of compounds, such as Charm analysis, aroma extract dilution analysis, or detection frequency method, have also been established (Acree and Barnard 1994; Linssen et al. 1993; van Ruth and O'Connor 2001). In contrast to the behavior of volatile compounds, the stimuli responsible for taste are nonvolatile and water-soluble chemical compounds, which, on leaving the food matrix, incorporate into the aqueous phase (saliva) where they get in contact with the human receptors. In fruits and vegetables, the concentration and type of sugars and organic acids and especially the balance between them decisively contribute to the product flavor. Other compounds like isocumarins and phenolics acids may also contribute with a bitter flavor (Kreutzmann

et al. 2008) and proanthocyanidins and tannins with astringency. Different high-performance liquid chromatography methods can be used for analyzing this type of compounds.

All the above-cited methods provide very useful information on the compounds that could potentially contribute to the flavor, but flavor is not the result of the sum of both volatile and nonvolatile compounds of a certain fruit or vegetable. In fact, it depends on the interactions between those compounds and possibly with other ones and also on the chemical composition and the physical characteristics of the food matrix. All these factors will certainly condition both the release and the perception during consumption of the taste and odor stimuli contacting the human receptors. It is well-known that the characteristics and the structure of the matrix exert a decisive influence on the effective concentration of the chemical stimuli and on their rate of transport to the receptors. The theoretical bases of the transport phenomena governing the release of chemical stimuli out of the food matrix, such as phase partition, mass transport, and diffusion are well defined. The methods used to monitor diffusion and mass transport of volatile compounds have been recently described by Cayot and others (2008). However, the study of these processes in the actual mouth conditions during mastication is far more complex. The food surface area in contact with saliva and the concentration of both volatile and nonvolatile stimuli present in the aqueous and air phases undergo continuous changes mainly due to the continuous movements of the mouth components during mastication and deglutition. This type of study gets even more complicated, especially in the case of solid foods, due to the individual differences in respiration rates, in salivation, and in mastication patterns, all of them affecting the transport of the stimuli to the receptors. Several theoretical models have been proposed to predict the effect of the food matrix characteristics on stimuli delivery, but, as commented by Taylor (2002), none of them are totally validated.

Basic knowledge of the effects of the different factors involved in the release of stimuli from the food matrix is needed to understand the first phase of the flavor perception process, related with the transport of chemical stimuli to the receptors. In the second phase of the flavor perception process, beside the type and concentration of stimuli reaching the receptors, other factors like individual physiological characteristics, interactions between sensory modalities, and psychological and cognitive interactions have to be taken into consideration. As stated by Keast and others (2004), "the flavor of the food/beverage is the result of complex stimulus-response interactions between a food matrix and human sensory, perceptual and cognitive processes." The above-described phenomena would partially explain the difficulties found when trying to establish a good correlation between a chemical or instrumental measurement and a set of sensory data. From a practical point of view, the analysis of the relations between concentration of some volatile and nonvolatile components, and the perceptible intensity of different sensory attributes can be useful to better understand the correspondence between both types of variables. Colaric and others (2005) evaluated and compared the chemical and sensory attributes of fruit of various peach and nectarine cultivars to determine which chemical compounds correlate best with aroma and taste perceived sensorially. They concluded that sugars/acid ratio, and levels of citric and shikimic acid have significant impacts on perception of sweetness; total organic acids, sucrose, sorbitol, and malic acid influence aroma and malic/acid ratio; total sugars, sucrose, and malic acid have

an important influence on taste. A similar approach was used by Tandon and others (2003) to develop prediction models for sensory descriptors of fresh tomato flavor based on volatile and nonvolatile measurements. According to their results, both volatile and nonvolatile components influenced the aromatic attributes perceived, but the volatile components provided more consistent relationships.

Another important question is that in most cases, the instrumental data on chemical compounds concentrations in fruits and vegetables are obtained after submitting the original products to treatments like grinding, homogenization, or extraction to improve precision of the results. These treatments undoubtedly decrease the representative value of the data with respect to the actual conditions of the presence of such compounds in the analyzed item. The effect of the nature of the matrix on flavor release and perception was illustrated by Tandon and others (2000) on studying the influence of the medium of evaluation on odor thresholds of volatile compounds in fresh tomato. They determined the threshold values of 15 volatile compounds previously identified as important contributors to the aroma of fresh tomatoes in deionized water; in a mixture of ethanol, methanol, and water; and in a deodorized tomato homogenate. They observed that different media produced different thresholds and thus different odor units. Odor thresholds were lower in deionized water for all compounds and higher in the deodorized tomato for most compounds. They concluded that in assessing the importance of a volatile compound, it is necessary to first identify the best medium for testing. Logically, the threshold values determined in the juice or the puree of the corresponding product will be closer to those perceived by men than those measured in plain water. The important question to solve here is how similar, both qualitatively and quantitatively, is the information obtained in a certain liquid medium to that obtained from the process of mastication and deglutition of a solid product. Up to now, only the application of sensory techniques can afford information on this point and on the effects that interactions among the different chemical, physical, sensory, and cognitive factors have on fruits and vegetables flavor.

SENSORY MEASUREMENT OF FLAVOR

A good number of sensory tests of variable complexity are available for application to the sensory analysis of fruits and vegetables. Their detailed discussion is out of scope of this chapter. They are extensively treated in many general texts, like those cited above in the Introduction. Good examples are the books of Lawless and Heymann (1998) or of Meilgaard and others (1999). Besides, the International Organization for Standardization (ISO) has edited various standards, many of them of methodological character, on sensory analysis, which can be obtained from www.iso.org under section 67.240. The use of the sensory methodology in the study of fruits and vegetables flavor like for any type of foodstuff permits the obtention of information on three basic questions:

1. Are there any perceptible differences in flavor between samples?
2. If so, in which attribute and in which magnitude are they different?
3. Are there differences in preference or in acceptance of the samples by the consumer?

Answer to any of these questions require a different type of sensory test. The election of the most adequate test depends on the specific objective seeked and on the available time and particular experimental conditions. The other fundamental point for a correct sensory analysis of the product flavor is the composition and characteristics of the panel designed to perform the selected test. The quality of the data obtained with any measuring instrument mainly depends on the characteristics of the equipment used, its working efficiency, and its calibration. Similarly, quality of the sensory data will depend on the methods used to select and train the panelists and on the correct evaluation of their capacity to perform the desired task (ISO 1993, 2006, 2008).

Difference Tests

The main objective of these tests is to determine if there are perceptible differences between two samples, but it may also be used to determine the sensorial similarity between them. The best known and used are the triangle (ISO 2004a) and the duo-trio (ISO 2004b) tests. These tests are applicable when the nature of the difference is unknown and they require that the products to be compared are fairly homogeneous. The information supplied by discriminant tests in evaluating perceptible differences in flavor of fruits and vegetables has exploratory character. They can then be useful, for example, to get a preliminary information on whether certain preharvest or postharvest treatments or modifications applied to improve yield or agronomical characteristics of a particular cultivar also produce alteration of the original product flavor. Evidently, if the result is negative, for example, no perceptible differences are found between samples, it can be concluded that the variables considered do not affect the product flavor. But if the result is positive, for example, perceptible differences are detected, the next step will be to identify and quantify those differences and find out whether they have influence on the final response of the consumer. The difference tests are easy to perform, consume little time, and do not require a panel of trained judges. In contrast, their practical implementation may present some inconvenience, especially in two situations: when the samples to be compared are not integrated by homogeneous products and when the number of samples is high. Harker and others (2005) analyzed the problems posed by the natural heterogeneity of fruits and vegetables when trying to establish whether there are significant differences between two products using the triangle test. They concluded that the biological variability associated with fruit and vegetable will often overwhelm attempts to identify statistically significant differences. In case the number of samples to compare is high, a preliminary information on the possible detection of perceptible differences can be obtained by a similarity analysis (Schiffman et al. 1981). In this type of analysis, the assessors evaluate the total perceptible difference in each pair of samples. Differences within a great number of samples can be detected simultaneously in several directions and an approximation to their relative magnitude can be obtained with this method. Experimentally, it is easy to carry out; only its duration may be long if the total number of samples to compare is high. The main drawback is that the data thus obtained require a specific multivariant statistical treatment: multidimensional scaling (Schiffman and Beeker 1986). Considering the great variety of factors conforming the fruit and vegetable flavor and the difficulties inherent to the description and quantification of all the

sensory attributes involved, the above-described method may be useful to comprehensibly ascertain the possible existence of perceptible differences in flavor among a number of samples.

Another possible method to detect sensory differences is the R-index test. It is a shortcut signal detection measure that is applicable to the measurement of slight differences between food stimuli. This index provides the existing probability that a judge could distinguish a sensory signal from a background noise; the greater the degree of difference, the higher the probability of distinguishing between them (O'Mahony, 1983). This test can be carried out by comparing each sample with a control sample, and identifying whether the first sample is "the control sample," "perhaps the control sample," "perhaps not the control sample," and "not the control sample." The R-index measure can also be applied to multiple difference testing, using rating or ranking data (Ishii et al. 1992). The statistical significance of each R-index value can be determined using the tables reported by Bi and O'Mahony (1995). Recently, the R-index has been successfully used to determine the influence of wash-water temperature and chlorine concentration on the sensory properties of cut iceberg lettuce (Delaquis et al. 2004) and to detect the cooked flavor in pasteurized guava beverages (Argaiz et al. 2005).

Descriptive Tests

Several sensory techniques are available to obtain both qualitative and quantitative information on the perceptible differences in food flavor, the most popular being the so-called profiles. Sensory profiles are very attractive both in research and in industrial quality control because they allow, at least theoretically, to get a great deal of information in an apparently easy manner. The profile is based on the idea that the sensation produced in humans by consumption of foods is defined by a number of identifiable attributes and on that the perceptible differences between samples are due to the different intensity of each attribute in each one of them. This technique has been shown to be useful to describe and quantify the perceptible differences among different fruit cultivars like, for example, those of peaches and nectarines (Colaric et al. 2005) or of tomatoes (Sinesio et al. 2007); to study the influence of the degree of ripeness on some flavor and texture attributes of a certain peach variety (Cascales et al. 2005); or to investigate the sensory attributes that influence consumer perception of apple freshness (Peneau et al. 2007). It has also been applied to identify the tomato genoma regions related to certain sensory attributes of the fruit (Causse et al. 2001) and to carry out the final selection among several apple cultivars based on their sensory attributes (Hampson et al. 2000). The experimental running of sensory profiles is not an easy task. The actual value and usefulness of the data obtained mainly depend on (1) the selection of terms describing clear and unequivocably the perceived stimuli, (2) the ability of the judges on identifying each pair descriptor-sensation and to quantify the intensity of each attribute, (3) the experimental conditions of the test, and (4) the correct statistical analysis of the data obtained. On the basis of these four points, several techniques have been developed in time affording successive improvements. From the pioneer flavor profile method proposed in 1950 by Cairncross and Sjöström to the Quantitative Descriptive Analysis and Spectrum™ method or more recently to the Free Choice Profile (Meilgaard et al. 1999; Murray et al. 2001), and the Dynamic

Profile (DeRovira 1996) or the Flash Profile (Dairou and Sieffermann 2002), a long list of advances have occurred. However, most of these techniques imply the use of trained and experienced assessors, who normally tend to generate complex and scientifically orientated terms. The Free Choice Profile is a sensory methodology developed to avoid these disadvantages. It differs from conventional profiling in the development of an individual list of terms by each assessor to describe the samples rather than a common scorecard; but it is similar in that the assessors must be able to detect the differences between samples, to verbally describe the perceived attributes, and to quantify them (Oreskovich et al. 1991). The assessors only have to be objective, to be capable of using line scales, and to use their developed vocabulary consistently (Piggott et al. 1990). One of the advantages of this method is that it allows one to gather information about cognitive perception directly from consumers (Russell and Cox 2003). A more structured approach of Free Choice Profile has been developed incorporating the Repertory Grid Method as a previous step. This method is particularly suited to vocabulary development and can therefore solve the difficulties in generating sufficient and suitable descriptors, a problem which usually arises when working with consumers (Gómez et al. 1998; González-Tomás and Costell 2006; Jaeger et al. 2005).

At present, analysis of the data obtained with descriptive tests provides different types of information as a function of the specific objectives of the corresponding study. A graphic representation of the results gives quick and easy information on the perceived differences within a group of samples. Furthermore, the statistical significance of the differences in intensity of the evaluated attributes can be ascertained by univariate analysis of variance (O'Mahony 1985). The differences between samples considering jointly the variability of all analyzed attributes can be treated by applying principal components analysis or multivariate analysis of variance (Bieber and Smith 1986).

Preference and Hedonic Tests

Consumer response to a product is mainly defined by (1) a cognitive component, coming from the knowledge and opinions about a product; (2) an affective component, responsible for positive or negative feelings toward a product; and (3) a behavioral component, involving intentions or actions, defining how willing a consumer is to do something in certain situations. The cognitive component is related to the information that a person has about an object, the affective component summarizes the general feelings of a person about a product, and the component relative to an action or intention (behavioral) reflects the intentions of a person about his or her future behavior. The evaluation of preference or of degree of acceptance of a food product by consumers is carried out by using qualitative methods and those whose objective is to investigate consumer attitudes, opinions, and expectations (Barrios and Costell 2004; Harker et al. 2003), but the most frequently used are the conventional quantitative methods. Among the three classical methods (paired preference, ranking preference tests, and hedonic scale), the paired comparison is preferred because it offers a clear approach and a clear and unequivocal data analysis (ISO 2005). The analysis of the information obtained with ranking tests is initially based on establishing the possible statistical significance of the differences between

ranking sums using the Friedman analysis of variance, and the data obtained with the hedonic scale is based on the significance of the average values of the scores assigned to samples, as determined by applying the parametric analysis of variance (O'Mahony 1985). Validity of the results obtained with the latter methods depends on the homogeneity and unidirectionality of the preference or acceptance criteria of the participating consumers. In general, variability of data is high and nullifies the desired homogeneity, which may lead to false conclusions. When the individual responses come from consumers with different preference criteria, the average values obtained from the whole population tested do not reflect the actual situation. Average results are not correctly interpreted if the individual differences are ignored.

To study the individual differences, the average values from the whole group of consumers must be substituted by the analysis of the average values provided by subgroups, created by some classical segmentation criteria like sex, age, and frequency of consumption. Another possibility is to establish the subgroups of consumers as a function of their preferences. Several techniques can be used for the formation of the subgroups: grouping those consumers preferring the same products by applying cluster analysis to the acceptance data or considering the influence of the product sensory attributes variation on its acceptance. Another alternative method to study the structure of this type of data is to construct internal preference maps, a variant of the principal components analysis, in which the samples are represented by points and the consumers by vectors (Greenhoff and MacFie 1994).

The subgroups of consumers coinciding in the preferred samples are located as a function of the position of vectors in the map. The latter approach differs from the previous ones described in that the basic information considered is the integrated reactions of individual consumers, originated by the simultaneous perception of the different stimuli. In this way, the subgroups are formed by consumers showing homogeneous preference criteria. In the following step, from the analysis of the relationships between the dimensions of preference and the values assigned to the different sensory attributes integrating the samples profile, information can be obtained on the relative influence of each attribute on the acceptance criteria of each consumer subgroup (Costell et al. 2000; Greenhoff and MacFie 1994).

Jaeger and others (2003) used the internal preference map to investigate the preference criteria of consumers applied to eight kiwi genotypes and concluded that the consumer population studied showed different responses to the different genotypes. Particularly, two of the genotypes were acceptable to one of the consumer subgroup and not to another one. Carbonell and others (2008) correlated the intensity data of 14 sensory attributes evaluated by a trained panel on apples from different varieties with acceptability data from a panel of 99 consumers. They projected the sensory attributes as vectors on the space obtained from the consumer data by the internal preference map. The results obtained showed that a subgroup of consumers preferred crispy, hard, and acid apples, whereas the other group preferred sweet and aromatic apples. The practical usefulness of this segmentation criterion is based on the fact that within a group of consumers, three or four different preference tendencies can be identified.

CONCLUSIONS

Sensory analysis constitutes an indispensable tool to obtain information on those aspects of food quality to which any other analytic technique can be applied. In the particular case of fruits and vegetables, flavor sensory analysis is needed to know in which extent the changes in composition or structure affect the perceived flavor. Another interesting point is the possibility of analyzing the relationship between the sensorially perceived changes and the consumer response. Providing consumers with fruits and vegetables that meet their requirements about flavor will undoubtedly increase consumption.

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REFERENCES

Acree TE, Barnard J. 1994. Gas chromatography-olfactometry and charm analysis. In Maarse H, van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier, pp. 220–1.

Argaiz A, Pérez-Vega O, López-Malo A. 2005. Sensory detection of cooked flavor development during pasteurization of a guava beverage using R-index. *J Food Sci* 70:S149–52.

Auerswald H, Schwartz D, Kornelson C, Krumbein A, Brückner B. 1999. Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. *Sci Hortic* 82:227–42.

Azondanlou R, Darbellay C, Luisier JL, Villettaz JC, Amado R. 2003. Development of a model for quality assessment of tomatoes and apricots. *Lebensm Wiss Technol* 36:223–33.

Barrios EX, Costell E. 2004. Review: Use of methods of research into consumers' opinions and attitudes in food research. *Food Sci Technol Int* 10:359–71.

Bartoszewski G, Niedziela A, Szwacka M, Nienirowicz-Szczytt K. 2003. Modification of tomato taste in transgenic plants carrying a thaumatin gene from *Thaumatococcus daniellii* Benth. *Plant Breed* 122:347–51.

Bi J, O'Mahony M. 1995. Table for testing the significance of the R-index. *J Sens Stud* 10:341–7.

Bieber SL, Smith DV. 1986. Multivariate analysis of sensory data: Comparison of methods. *Chem Senses* 11:19–47.

Carbonell L, Izquierdo L, Carbonell I, Costell E. 2008. Segmentation of food consumers according to their correlations with sensory attributes projected on preferente spaces. *Food Qual Prefer* 19:71–8.

Cascales AI, Costell E, Romojaro F. 2005. Effects of the degree of maturity on the chemical composition, physical characteristics and sensory attributes of the peach (*Prunus persica*) cv. Caterin. *Food Sci Technol Int* 11:345–52.

Causse M, Saliba-Colombani V, Lesscheave I, Buret M. 2001. Genetic analysis of organoleptic quality in fresh market tomato. 2. Mapping QTLs for sensory attributes. *Theor Appl Genet* 102:273–83.

Cayot N, Dury-Brun C, Karbowiak T, Savary G, Voilley A. 2008. Measurement transport phenomena of volatile compounds: A review. *Food Res Int* 31:349–62.

Colaric M, Veberic R, Stampar F, Hudina M. 2005. Evaluation of peach and nectarine fruit quality and correlation between sensory and chemical attributes. *J Sci Food Agric* 85:2611–16.

Costell E. 2002. A comparison of sensory methods in quality control. *Food Qual Prefer* 13:345–53.

Costell E, Pastor MV, Izquierdo L, Duran L. 2000. Relationships between acceptability and sensory attributes of peach nectars using internal preference mapping. *Eur Food Res Technol* 211:199–204.

Dairou V, Sieffermann M. 2002. A comparison of 14 jams characterized by conventional profile and a quick original method, the flash profile. *J Food Sci* 67:826–34.

Delaquis PJ, Fukumoto LR, Toivonen PMA, Cliff MA. 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biol Technol* 31:81–91.

DeRovira D. 1996. The dynamic profile method. *Food Technol* 50:55–60.

Durán L, Costell E. 1999. Percepción del gusto. Aspectos fisicoquímicos y psicofísicos. *Food Sci Technol Int* 5:299–309.

Edelenbos M, Thybo AK, Christensen LP. 2006. Flavour quality of organic tomatoes grown in different systems. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Advances and Trends*. Amsterdam: Elsevier, pp. 301–4.

Gacula MC. 1993. *Design and Analysis of Sensory Optimization*. Trumbull, CT: Food & Nutrition Press.

Gómez C, Fiorenza F, Izquierdo L, Costell E. 1998. Perception of mealiness in apples: A comparison of consumers and trained assessors. *Z Lebensm Unters Forsch A* 207:304–10.

González-Tomás L, Costell E. 2006. Sensory evaluation of vanilla dairy desserts by repertory grid method and free-choice profile. *J Sens Stud* 21:20–33.

Greenhoff K, MacFie HJH. 1994. Preference mapping in practice. In MacFie HJH, Thomson DMH (eds.), *Measurements of Food Preferences*. London: Blackie Academic & Professional, pp. 137–66.

Hampson CR, Quamme HA, Hall JW, MacDonald RA, King MC, Cliff MA. 2000. Sensory evaluation as a selection tool in apple breeding. *Euphytica* 111:79–90.

Harker FR, Gumson FA, Jaeger SR. 2003. The case for fruit quality: An interpretative review of consumer attitudes and preferences from apples. *Postharvest Biol Technol* 28:333–47.

Harker FR, Norquay C, Amos R, Jackman R, Gunson A, Williams M. 2005. The use and misuse of discrimination test for assessing the sensory properties of fruit and vegetables. *Postharvest Biol Technol* 38:195–201.

Heymann H, Holt DL, Cliff MA. 1993. Measurement of flavor by sensory descriptive techniques. In Ho CT, Manley CH (eds.), *Flavor Measurement*. New York: Marcel Dekker, pp. 113–31.

International Organization for Standardization (ISO). 1993. Sensory analysis. General guidance for the selection, training and monitoring of assessors—Part 1: Selected assessors. No 8586, Geneva, Switzerland.

International Organization for Standardization (ISO). 2004a. Sensory analysis. Methodology. Triangle test. No. 2140, Geneva, Switzerland.

International Organization for Standardization (ISO). 2004b. Sensory analysis. Methodology. Duo-trio test. No. 10399, Geneva, Switzerland.

International Organization for Standardization (ISO). 2005. Sensory analysis. Methodology. Paired comparison test. No. 5495, Geneva, Switzerland.

International Organization for Standardization (ISO). 2006. Sensory analysis. Methodology. Initiation and training of assessors in the detection and recognition of odours. No. 5496, Geneva, Switzerland.

International Organization for Standardization (ISO). 2008. Sensory analysis. General guidance for the selection, training and monitoring of assessors. Part 2: Expert sensory assessors. No. 8586-1, Geneva, Switzerland.

Ishii R, Vié A, O'Mahony M. 1992. Sensory difference testing: Ranking R-indices are greater than rating R-indices. *J Sens Stud* 7:57–61.

Jaeger SR, Harker FR. 2005. Consumer evaluation of novel kiwifruit: Willingness-to-pay. *J Sci Food Agric* 85:2519–26.

Jaeger SR, Rossiter KL, Lau K. 2005. Consumer perceptions of novel fruit and familiar fruit: A repertory grid application. *J Sci Food Agric* 85: 480–8.

Jaeger SR, Rossiter KL, Wismer WV, Harker FR. 2003. Consumer driven product development in the kiwi industry. *Food Qual Prefer* 14:187–98.

Kader AA. 2008. Perspective: Flavor quality of fruits and vegetables. *J Sci Food Agric.* doi: 10.1002/jfsa.3293.

Keast RSJ, Dalton PH, Breslin PAS. 2004. Flavor interactions at the sensory level. In Taylor AJ, Roberts DD (eds.), *Flavor Perception*. Ames, IA: Blackwell Academic and Professional, pp. 228–249.

Kreutzmann S, Christensen LP, Edelembos M. 2008. Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. *Lebensm Wiss Technol* 41:193–205.

Lawless HT, Heymann H. 1998. *Sensory Evaluation of Foods: Principles and Practices*. New York: Chapman and Hall.

Linssen JPH, Janssens JLGM, Roozen JP, Posthumus MA. 1993. Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in laminated packages. *Food Chem* 8:1–7.

Maarse H. 1991. *Volatile Compounds in Foods and Beverages*. New York: Marcel Dekker.

MacFie HJH, Thomson DMH. 1994. *Measurement of Food Preferences*. London: Blackie Academic & Professional.

Maul F, Sargent SA, Sims CA, Baldwin EA, Balaban MO, Huber DJ. 2000. Tomato flavor and aroma quality as affected by storage temperature. *J Food Sci* 65(7):1228–37.

Meilgaard M, Civille GV, Carr BT. 1999. *Sensory Evaluation Techniques*, 3rd Ed. Boca Raton, FL: CRC Press.

Meullenet JF, Xiong R, Findlay CJ. 2007. *Multivariate and Probabilistic Analyses of Sensory Science Problems*. Ames, IA: Blackwell Academic and Professional.

Muñoz AM, Civille GV, Carr BT. 1992. *Sensory Evaluation in Quality Control*. New York: Van Nostrand Reinhold.

Murray JM, Delahunty CM, Baxter IA. 2001. Descriptive sensory analysis: Past, present and future. *Food Res Int* 34:461–71.

O'Mahony M. 1983. Adapting short cut signal detection measures to the problem of multiple difference testing: R-index. In Williams AA, Atkin RK (eds.), *Sensory Quality in Foods and Beverages Definition, Measurement and Control*. Chichester, UK: Ellis Horwood, pp. 69–81.

O'Mahony M. 1985. *Sensory Evaluation of Food: Statistical Methods and Procedures*. New York: Marcel and Dekker.

Oreskovich DC, Klein BP, Sutherland JW. 1991. Procrustes analysis and its application to free choice and other sensory profiling. In Lawless HT, Klein BP (eds.), *Sensory Science Theory and Application in Foods*. New York: Marcel Dekker, pp. 353–94.

Overbosch P, Afterof WGM, Haring PGM. 1991. Flavour release in the mouth. *Food Rev Int* 7:137–84.

Peneau S, Brockhoff PB, Hoehn E, Escher F, Nuessli J. 2007. Relating consumer evaluation of apple freshness to sensory and physico-chemical measurements. *J Sens Stud* 22:313–35.

Piggott JR, Sheen MR, Apostolidou SG. 1990. Consumers' perceptions of whiskies and other alcoholic beverages. *Food Qual Prefer* 2:177–85.

Ranalli P, Cubero JI. 1997. Bases for genetic improvement of grain legumes. *Field Crops Res* 53:69–82.

Rouseff R, Gemitter F, Grosser J. 1994. Citrus breeding and flavour. In Piggott JR, Paterson A (eds.), *Understanding Natural Flavours*. London: Blackie Academic & Professional, Chapman & Hall, pp. 113–27.

Russell CG, Cox DN. 2003. A computerised adaptation of the repertory grid methodology as a useful tool to elicit older consumers' perceptions of foods. *Food Qual Prefer* 14:681–91.

Sinesio F, Moneta E, Peparajo M. 2007. Sensory characteristics of traditional field grown tomato genotypes in Southern Italy. *J Food Qual* 30:878–95.

Schiffman SS, Beeker TG. 1986. Multidimensional scaling and its interpretation. In Piggott JR (ed.), *Statistical Procedures in Food Research*. London: Elsevier Applied Science, pp. 255–92.

Schiffman SS, Reynolds ML, Young FW. 1981. *Introduction to Multidimensional Scaling. Theory, Methods and Applications*. NewYork: Academic Press.

Tandon KS, Baldwin EA, Scott JW, Shewfelt RL. 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. *J Food Sci* 68:2366–71.

Tandon KS, Baldwin EA, Shewfelt RL. 2000. Aroma perception of individual volatile compounds in fresh tomatoes (*Licopersicon esculentum*, Mill.) as affected by the medium of evaluation. *Postharvest Biol Technol* 20:261–8.

Taylor AJ. 2002. Release and transport of flavor in vivo: Physicochemical, physiological, and perceptual considerations. *Compr Rev Food Sci Saf* 1:45–57.

Taylor AJ, Roberts DD. 2004. *Flavor Perception*. Ames, IA: Blackwell Academic and Professional.

van Ruth SM, O'Connor CH. 2001. Evaluation of three gas chromatography-olfactometry methods: Comparison of odour intensity-concentration relationships of eight volatile compounds with sensory headspace data. *Food Chem* 74:341–7.

Wismer WV, Harker FR, Gunson FA, Rossiter KL, Lau K, Seal AG, Lowe RG, Beatson R. 2005. Identifying flavour targets for fruit breeding: A kiwifruit example. *Euphytica* 141:93–104.

CHAPTER 4

Fermentation and Fruit Flavor Production

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For thousands of years, microbial processes have played the decisive but unrecognized role of producing more desirable foods and beverages for mankind such as bread, cheese, beer, wine, and soy sauce. It was in 1923 when the first scientific review on microbial flavors appeared (Omelianski 1923).

Nowadays, biotechnological production of flavor compounds is a mature discipline in the chemical industry, with an estimated 100 molecules in the market produced by enzymatic or microbial processes (Gatfield 1999). The predominant driving force was and still is the fact that flavor compounds produced from natural raw materials by microbial or enzymatic methods can be labeled "natural" in accordance with European and U.S. legislations, thereby satisfying the unbroken consumer trend toward all "bio" or "natural" products in the food sector (Schrader 2007).

Among the natural flavor molecules with microorganisms are some real bulk products, such as amino acids and organic acids manufactured on the million-ton scale, but the majority of the target compounds are produced for highly specific applications and thus are rather niche products with market volumes below 1 ton/year. Here, industry avoids costly research and development effort to establish more sophisticated processes owing to the limited market volume of these products. Nevertheless, some natural flavors that have a broader application are produced in amounts of around one to several tons per year, such as vanillin, 2-phenylethanol, and 4-decanolide. These flavor compounds have an increasing market owing the steadily improved bioprocesses.

The biotechnological approach implies additional advantages. Flavors are bioactive compounds, and the known effects of chirality on odor perception suggest the use of biocatalysts. Further advantages associated with the biotechnological principle are independence from agriculture and possible shortages caused by local conditions of production (climate, diseases, pesticides, fertilizers, trade restrictions, sociopolitical instabilities), and ability for scaled-up and industrial-scale production using engineered pathways, upregulated metabolisms, and gentle product recovery to create an inexhaustible source of homogenous, well-defined product and

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

responsible care of natural resources in developing countries (Krings and Berger 1998).

Essential oils of higher plants, fruit juices, vegetable extracts, and very few products of animal origin (amber, musk, zibet) were, for a long time, the sole sources of natural flavors. Biotechnological options comprise single-step biotransformations, bioconversions, and de novo synthesis with microorganisms, plant cells, and biocatalysis (Krings and Berger 1998).

1. *De Novo Synthesis*. Fermentation process, also known as de novo synthesis, implicates the production of flavor compounds using simple culture media, without the addition of any special carbon source. This method uses the entire metabolic arsenal from the microorganism and, in general, produces a mix of several flavor compounds, which are important for the formation of the "bouquet" of the product (Berger 1995). Although for a multitude of microorganisms, the metabolic potential for de novo flavor biosynthesis is immense and a wide variety of valuable products can be detected in microbial culture media or their headspaces, the concentrations found in nature are usually very low for commercial applications. Exceptions to the rule can be found, where the flavor compounds are derived from primary metabolism as in the case for some nonvolatile compounds (Schrader 2007).

   Whole cells catabolize carbohydrates, fats, and proteins, and further convert the breakdown products to more complex flavor molecules, a property that is traditionally used during the production of fermented foods with their amazing number of flavor chemicals compounds (Engels and Visser 1994, Imhof and Bosset 1994, Jeon 1994, Hamada et al. 1991, Maarse 1991, and Pinches 1994 as cited in Krings and Berger 1998). Starter cultures produce primary metabolites in considerable amounts but only traces of more complex aroma chemicals. For example, very efficient lactic acid producers contribute to dairy flavors. Small amounts of chemically quite different volatile flavors, such as short-chain alcohols, aldehydes, ketones, methyl ketones, and acids, as well as pyrazines, lactones, and thiols, are formed concurrently (Cogan 1995 and Imhof and Bosset 1994 as cited in Krings and Berger 1998). Rapid and continuous lactic acid formation should now be taken for granted, and more attention should be paid to starter cultures with enhanced flavor potential. However, an immediate improvement is often prevented by a lack of metabolic knowledge (Krings and Berger 1998).
2. The second bioprocess for flavor production is biotransformation. Biotransformation is the conversion of a compound into the product using living plant cells, enzymes, or microorganisms. According to Berger (1995), biotransformation is defined as a reaction capable of catalyzing the transformation of the substrate in one single step. On the other hand, bioconversion happens with two or more biochemical steps, although there are controversies around these concepts being different in the literature. At any rate, bioconversion or biotransformation is a concept that refers to the synthesis of one or more compounds of flavor through the addition of precursors in the culture media. This strategy to obtain a determinate product works by the action of constitutive or inducible enzymes, sometimes in just one-step reactions (Berger 1995).

The biocatalytic conversion of a structurally related precursor molecule is often a superior strategy, which allows the accumulation of a desired flavor product to be significantly enhanced. As a prerequisite for this strategy, the precursor must be present in nature, and its isolation in sufficient amounts from the natural source must be easily feasible in an economically viable fashion. Inexpensive, readily available, and renewable natural precursors, such as fatty or amino acids, can be converted to more highly valued flavors.

3. The third process that can be employed to obtain bioflavors is biocatalysis. Biocatalysis competes best with chemical catalysis in the following types of reactions:
   - resolution of chirality,
   - functionalization of chemically inert carbons,
   - selective modifications of one functional group in multifunctional molecules, and
   - resolution of racemates.

   Since techniques like enzyme immobilization, among others, were developed to increase the stability of microbial enzymatic process, the role that enzymes would have on the flavor industry was clear. Biocatalysis has a wide range of applications on aroma production. The enzymes can be used directly on the food as additives, as well as to provide or free aromas of the product and to avoid undesirable aromas caused by some compounds (Macedo 1997).

   The enzymes present in the process of aroma production in food can be endogenous (belong to the food) or enzymes from microbial sources. For example, lipases from *Rhizopus* sp. can be used as biocatalyst for the synthesis of esthers derived from fat acids and short-chain alcohols, both known as important aroma compounds (Macedo 1997).
4. *Production of Flavors by Plant Cell and Tissue Culture*. The first experiments with plant tissue culture were developed more than 100 years ago (by Haberland in 1902), but the utilization of it to the aroma production began only in the 1970s (Hrazdina 2006).

   This method has advantages when compared with the production of aromatic compounds by traditional agriculture. Plant cultivation depends on the season, while cell plant cultivation provides a system that works all year and is not dependent on the climate, season, place, and so on. Cells can be induced to produce the interesting metabolite to be obtained directly on the culture media or in the cells (Murashige and Skoog 1962). Even so, the process of aroma production from plant cell cultivation has its limitations. Some species are quite difficult to grow *in vitro* (Berger 1995).

There are several published works on the last decade on bioflavor production, using at least those processes described above. The scope of this chapter is to give an overview of the studies for the production of fruits aroma through de novo synthesis and biotransformation.

In Figure 4.1, microbial routes from natural raw materials to and between natural compounds are depicted.

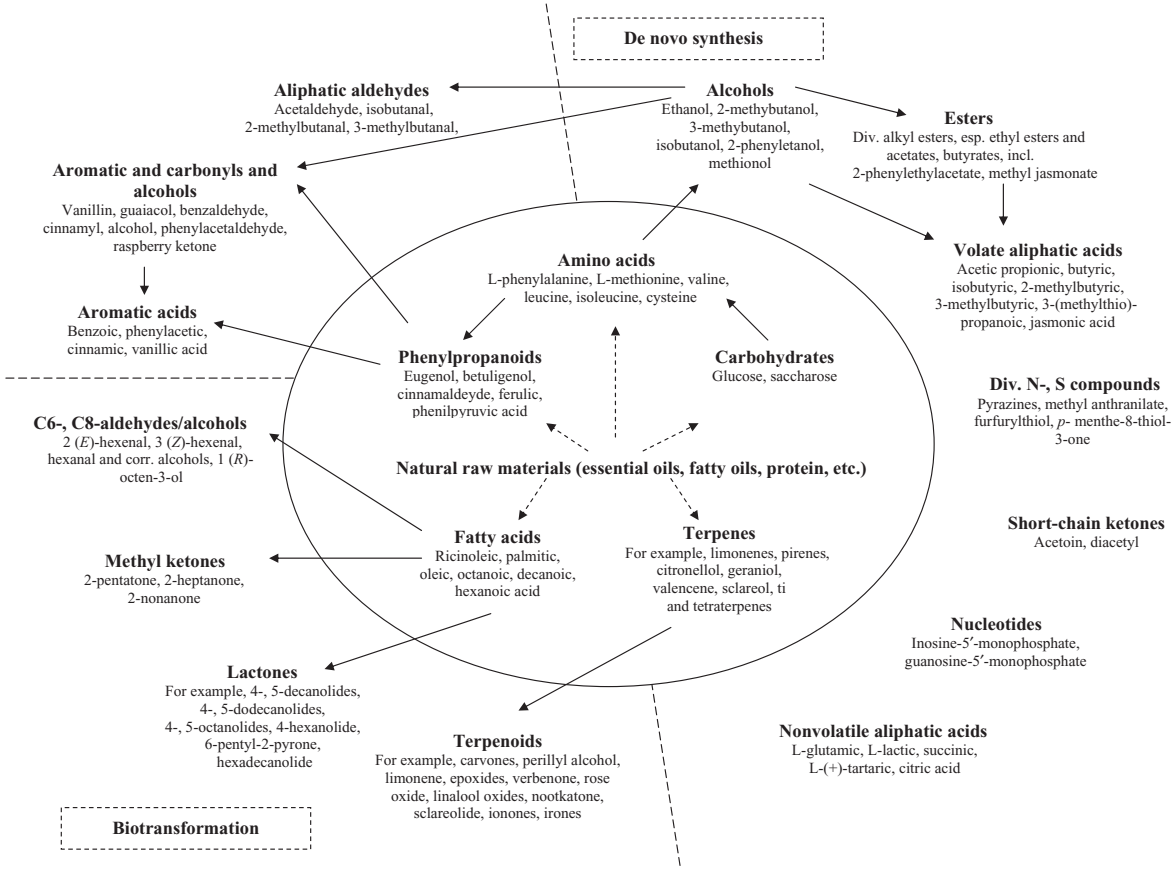

Figure 4.1. Microbial routes from natural raw materials to and between natural flavor compounds (solid arrows). Natural raw materials are depicted within the ellipse. Raw material fractions are derived from their natural sources by conventional means, such as extraction and hydrolysis (dotted arrows). De novo indicates flavor compounds that arise from microbial cultures by de novo biosynthesis (e.g., on glucose or other carbon sources) and not by biotransformation of an externally added precursor. It should be noted that there are much more flavor compounds accessible by biocatalysis using free enzymes that are not described in this chapter, especially flavor esters by esterification of natural alcohols (e.g., aliphatic or terpene alcohols) with natural acids by free lipases. For the sake of completeness, the C6-aldehydes are also shown although only the formation of the corresponding alcohols involves microbial cells as catalysts. The list of flavor compounds shown is not intended to be all embracing but focuses on the examples discussed in this chapter.

BIOTRANSFORMATION PROCESS

Recently, various flavor compounds, such as alcohols, aldehydes, esters, lactones, and terpenes, produced by microorganisms by biotransformation, including bacteria, yeasts, and fungi, have been isolated (Table 4.1).

Lactones are essential materials in processing foods and are increasingly used in the food industry. This has led to numerous patents being taken out, and nowadays, the biotechnologically produced lactone family is mainly represented by γ-decalactone (DECA), but also to a smaller extent by γ-dodecalactone and γ-octalactone. These compounds exist in small quantities in some fruits, exuding the characteristic sweet smell as that of peach, damson plum, and coconut.

δ-Decalactone is an aroma compound of industrial interest that can be produced biotechnologically by some microorganisms.

Nago and others (1993) studied the degradative pathway of 5-alkanolides (alkano-δ-lactones) and 2-deceno-δ-lactone (massoialactone) by *Fusarium solani* PM-1, a massoialactone-producing fungus. Alkano-δ-lactones were shown to be degraded first to a one-carbon-atom-less methyl ketone, 4-hydroxy-2-alkanones, after hydroxylation.

Esaki and others (1994) isolated 160 bacterial strains from the soil, which are tolerant to high concentrations of oleic acid, and examined their ability to transform oleic acid to new fatty acid derivatives. One of the isolated strains, *Alcaligenes* sp. 5–18, produced several compounds from oleic acid: 3-hydroxyoleic acid, 3-hydroxyhexadecenoic acid, octadecadienoic acid, hexadecadienoic acid, and tetradecadienoic acid. These compounds are intermediates in the β-oxidation pathway of oleic acid, and their accumulation is probably due to defective β-oxidation of oleic acid in the microorganism. Neither hydroxy nor enoic derivatives of fatty acids with a carbon chain length shorter than 14 were produced.

Sporobolomyces odorus produces DECA and *cis*-6-dodecen-4-olide, substances which exhibit odors characteristic of peaches and lamb meat (mutton), respectively. The growth and production of DECA by *S. odorus* in broth to which fatty acids or oils had been added were investigated. The addition of decanoic acid or dodecanoic acid to the culture reduced the growth and production of DECA by *S. odorus*. Without affecting the growth, the presence of Miglyol oil reduced the yield, whereas the addition of castor oil enhanced the production of DECA by *S. odorus*. The greatest enhancement, with a yield of 8.62 mg/L of DECA, about six times that in the control broth, was noted when 3% castor oil was added after 24 h of cultivation. On the other hand, the fatty acids and oils tested either reduced or had no effect on the production of *cis*-6-dodecen-4-olide by *S. odorus* (Lee and Chou 1994).

Researchers followed these experiments with the production of DECA by *S. odorus* in a 5-L fermentor with a working volume of 2 L in culture medium modified YNB containing 0.06% ricinoleic acid with the addition of an appropriate amount of castor oil hydrolysate. A maximum yield of DECA of 54.6 mg/L was noted after 120 h of cultivation. A significant increase in the maximum yield of DECA was noted in fed-batch culture of *S. odorus*. The maximum yield of DECA increased with the number of castor oil hydrolysate feedings. Fed-batch cultivation of *S. odorus* with additions of castor oil hydrolysate on the third, fourth, and fifth day of cultivation resulted in a maximum yield of 208 mg/L DECA, which was noted after 7 days of cultivation.

TABLE 4.1. Fruit-Type Flavors Production by Biotransformation

Compounds	Odor Assessment	Microorganisms	References
γ-Octalactone	Fruits, coconut	—	Maga (1976)
γ-Nonalactone	Peach	—	Maga (1976)
γ-Decalactone	Peach, damson plum	—	Maga (1976, 1995)
γ-Undecalactone	Damson plum	—	Maga (1976)
Δ-Decalactone	Peach, coconut	—	Maga (1976)
Δ-Decalactone	Peach	—	Dufossé and others (1994)
2-Deceno-δ-lactone	Coconut	—	Dufossé and others (1994)
6-Pentil-α-pirone	Coconut	—	Collins and Halim (1972)
Massoialactone	Fruits	*Fusarium solani*	Nago and others (1993)
3-Hydroxyoleic acid, 3-hydroxyhexadecenoic acid, octadecadienoic acid, hexadecadienoic acid, and tetradecadienoic acid	Fruits	*Alcaligenes* sp.	Esaki and others (1994)
γ-Decalactone and *cis*-6-dodecen-4-olide	Peaches	*Sporobolomyces odorus*	Lee and Chou (1994), Shi and others (1995), Lin and others (1996)
γ-decalactone	Fruits	*Sporidiobolus salmonicolor*, *Sporidiobolus ruinenii*	Feron and others (1996)
γ-decalactone	Fruits	*Sporidiobolus*: *S. salmonicolor*, *S. ruinenii*, *S. johnsonii*, and *S. pararoseus*	Dufossé and others (1998)
Methyl anthranilate	Orange blossom, wood strawberry	*Trametes* sp., *Polyporus* sp.	Lomascolo and others (1999)
Acetophenone	Orange blossom	*Polyporus* sp.	
p-Methyl-acetophenone	Fruity	*Polyporus* sp.	
p-Methyl-benzyl alcohol	Fruity	*Polyporus* sp.	
Methyl-benzoato	Fruity	*Polyporus* sp.	
Ethyl-benzoato	Fruity	*Polyporus* sp.	
Methyl-cinnamate	Fruity	*Polyporus* sp.	
Raspberry ketone	Raspberry	*Nidularia* sp.	
6-Pentyl-α-pyrone	Coconut-like	*Trichoderma harzianum*	Sarhy-Bagnon and others (2000), Galindo and others (2004), Rocha-Valadez and others (2006)
γ-Decalactone	Fruits	*Yarrowia lipolytica*	Waché and others (2002)
γ-Octalactone and γ-decalactone	Fruits	*Piptoporus soloniensis*	Okamoto and others (2002)
Lactone	Fruits	*Yarrowia lipolytica*	Groguenin and others (2004)
γ-Decalactone	Fruits	*Geotrichum* sp. and *G. fragrans*	Neto and others (2004)
γ-Decalactone	Fruits	*Yarrowia lipolytica*	Aguedo and others (2005)

Lin and others (1996) studied the influence of sodium salts of palmitic, stearic, oleic, linoleic, and ricinoleic addition during the fermentation, in various concentrations, in a culture medium to DECA production by *S. odorus*. The growth behavior of *S. odorus* in a medium containing 0.06% palmitic, stearic, or oleic acid was observed to be similar to that in a medium lacking these acids. However, the growth of *S. odorus* was partially inhibited in the presence of linoleic acid or ricinoleic acid. Addition of palmitic, stearic, oleic, or linoleic acid to the medium reduced the yields of DECA as determined after 168 h of cultivation. The concentration of ricinoleic acid, as well as the time of addition, was also observed to affect the amount of DECA produced by fungi. When 0.06% ricinoleic acid was added to the medium at the beginning of cultivation, a maximum yield of approximately 135.4 mg/L DECA was obtained after 216 h of cultivation.

The levels of lactone production in the presence of high concentrations of ricinoleic acid methyl ester differed in the two *Sporidiobolus* species. During the bioconversion of ricinoleic acid to DECA under controlled pH conditions, *Sporidiobolus salmonicolor* produced only the lactone form, while *Sporidiobolus ruinenii* produced both the lactone form and a precursor (Feron et al. 1996).

Dufossé and others (1998) studied the bioconversion of ricinoleic acid methyl ester to DECA with four species of *Sporidiobolus*: *S. salmonicolor*, *S. ruinenii*, *Sporidiobolus johnsonii*, and *Sporidiobolus pararoseus*. When cells reached the stationary phase, the desired volume of methyl ricinoleate was added to the medium to initiate the bioconversion process. With 4.1 g/L of ricinoleic acid methyl ester, only *S. salmonicolor* and *S. ruinenii* were able to produce DECA (12 and 40 g/L, respectively). During four successive batch cultivations in a 7-L bioreactor, 5.5 g/L of DECA was produced with *S. ruinenii* in each 10-day run.

Sarhy-Bagnon and others (2000) compared the production of 6-pentyl-α-pyrone (6-PP), a compound that has a strong coconut-like aroma, by *Trichoderma harzianum* in liquid and in solid-state cultivation (LC and SSC). The same liquid medium was used to impregnate sugarcane pith bagasse, used as support in SSC. The maximum concentration of 6-PP produced by *T. harzianum* in SSC was 2.8 mg (g dry cell mass)$^{-1}$ equivalent to 0.9 g/L of impregnation medium, which is 17 times higher than that obtained in LC. The glucose consumed to yield 6-PP in SSC was 52 mg (g glucose)$^{-1}$, eight times higher than that found in LC.

Production of DECA by *Yarrowia lipolytica* was investigated in conditions of growth using methyl ricinoleate as the only carbon source. The production of DECA was studied using a genetic engineering of the strain and wild type. Cells were cultured at 27°C in 500-mL Erlenmeyer flasks containing 200 mL medium and agitated at 140 rpm for the comparison of the various strains. The wild type produced DECA rapidly in the first 12 h, reaching concentrations of 71 mg/L, and then reconsumed it. The genetic engineered strain produced DECA slowly but steadily during 4 days to a concentration of 150 mg/L DECA (Waché et al. 2002).

A wild strain of brown-rot basidiomycete *Piptoporus soloniensis* produced a sweet flavor similar to tropical fruits in liquid cultures. The major and minor compounds were identified to be DECA and γ-octanolactone by gas chromatography (GC)–mass spectrometry analysis, respectively. The growth and production of DECA by *P. soloniensis* in broth to which fatty acids had been added were investigated. The addition of 12-hydroxystearic acid and ricinoleic acid to the culture

markedly enhanced the production of DECA. On the other hand, the addition of myristic acid, palmitic acid, stearic acid, and oleic acid to the culture resulted in a higher production of γ-octanolactone. The addition of hexanoic acid, octanoic acid, decanoic acid, lauric acid, linoleic acid, and linolenic acid to the culture reduced the growth of *P. soloniensis* and the production of DECA and γ-octanolactone.

The yeast *Y. lipolytica* possesses five acyl-CoA oxidases (Aox1p to 5), the enzyme catalyzing the first reaction of β-oxidation. It was observed that Aox4p exhibits a slight activity on a broad spectrum of substrates and that it is involved in lactone degradation. Its growth was only slightly altered, and it produced 10 times more lactone than the wild type in 48 h (Groguenin et al. 2004).

The production of DECA by *Geotrichum* species in broth, to which enzymatically hydrolyzed castor oil had been added, was investigated.

The study of different microbial lipases, aimed at producing the best hydrolyzed oil for use as a precursor for the production of DECA, was also studied. The greatest enhancement of DECA production was noted, with a yield of 600 mg/L, when 5% of hydrolyzed castor bean oil was added to the medium and fermented by *Geotrichum fragrans* for 96 h.

Two strains of a *Geotrichum* sp. and *G. fragrans* were used in bioconversion studies using castor bean oil hydrolyzed by *Alcaligenes* sp. lipase. These strains were previously studied for lipase and flavor production in the authors' laboratory (Macedo 1997; Pastore et al. 1994; Sousa 1996). These microorganisms produce decalactone, which exhibits odors characteristic of fruit and chocolate derivatives.

Bioconversion by strains of a *Geotrichum* sp. and *G. fragrans* was compared with respect to the yield of DECA. This study concluded that both strains are good potential producers of DECA. In spite of the good results obtained using both strains, *G. fragrans* showed the best yields of DECA under the conditions tested.

The results showed that the addition of ricinoleic acid or hydrolyzed castor bean oil to the culture medium enhanced DECA production. Hydrolyzed castor oil and its fatty acid derivatives proved to be effective precursors of DECA by either *Geotrichum* sp. or *G. fragrans*. The best conditions tested were a medium containing 20 g/L of glucose, 1% of autolyzed yeast, and 5% of hydrolyzed castor oil, with incubation at 30°C for 96 h, producing 600 mg/L of DECA by *G. fragrans* (Neto et al. 2004).

6-PP is a coconut-like aroma compound of interest in the food industry and can be produced by fermentation. Galindo and others (2004) studied the production of 6-PP by *T. harzianum*. The fungus grew as loose aggregates, being smaller at the highest agitation rate. In baffled flasks, loose aggregates were only observed at the lowest agitation speed (100 rpm). At higher speeds, nearly 90% of the total biomass was attached on the surface of the flask's wall. The remaining biomass (suspended in the liquid) was in the form of macroscopic pellets. 6-PP titer in baffled flasks cultures was maximum (96 mg/L) at 100 rpm and decreased at higher agitation rates (Galindo et al. 2004).

Y. lipolytica converts methyl ricinoleate to δ-decalactone, a high-value fruity aroma compound. The yeast was grown in 600-mL stainless steel high-pressure bioreactor and 2-L bioreactors containing a culture medium composed of 10 g/L methyl ricinoleate, 6.7 g/L yeast nitrogen base, 2.5 g/L NH_4Cl, and 1 g/L Tween 80. The fermentation in the pressurized reactor was carried out at 400 rpm and aeration rate of 0.9 vvm, while agitation rates of 300 and 600 rpm and aeration rates of

0.3–1.8 vvm were used in bioreactors. The highest amount of 3-hydroxy-*c*-decalactone produced by the yeast was 263 mg/L (Aguedo et al. 2005).

Rocha-Valadez and others (2006) studied an extractive fermentation process for 6-PP production by *T. harzianum*, which was scaled up from 500-mL shake flasks to 10-L stirred tank bioreactors, keeping the volumetric power drawn constant. The researchers obtained 230 mg/L of 6-PP.

DE NOVO SYNTHESIS FOR FRUITY FLAVOR PRODUCTION (TABLE 4.2)

A yeast strain, isolated and selected by Marques (1998) was identified as *Pichia membranaefaciens*. This yeast was tested under several conditions, such as variation of the culture medium composition, pH, temperature, and incubation time, with the objective of producing fruit flavors, specially ethyl acetate, a compound that has a similar aroma of banana. For this purpose, the following carbon sources were

TABLE 4.2. Fruit-Type Flavors Production by De Novo Synthesis

Compounds	Odor Assessment	Microorganisms	References
Ethyl acetate, propyl acetate, isobutyl acetate, isoamyl alcohol, isoamyl acetate, citronellol, geraniol, and nerol	Peach, banana, pear, or citrus	*Ceratocystis moniliformis*	Bluemke and others (2001)
Ethyl esters	Fruity	*Geotrichum candidum* ATCC 62217	Daigle and others (1999)
Acetone, ethyl acetate, ethanol, isobutanol, isopentyl alcohol (amyl alcohol), acetoin (3-hydroxy-2-butanone), acetic acid, isobutyric acid, butyric acid, 2-methyl-butanoic acid, benzyl alcohol, and 2-phenyl ethanol	Fruity and sweet	Non-identified microorganisms	Uenojo and Pastore (2006)
Ethyl acetate, ethanol, and acetaldehyde	Peach, pineapple, banana, and citrus	*Ceratocystis fimbriata*	Uenojo and Pastore (2006)
Ethyl acetate, ethanol, and acetaldehyde	Fruity	*Kluyveromyces marxianus*	Medeiros and others (2000)
Ethyl acetate, isobutanol, 1-butanol, ethyl propionate, iso-amyl alcohol, *n*-amyl alcohol, iso-amyl acetate, *n*-butyl acetate, ethyl caproate, phenylethyl alcohol	Banana and fruity	*K. marxianus*	Marques (1998)

selected on the tests with the best results: glucose, fructose, and mannose; and as nitrogen sources, autolyzed yeast and yeast extract. The main volatile compounds produced by *P. membranaefaciens* on the selected media were ethyl acetate, isobutanol, 1-butanol, ethyl propionate, iso-amyl alcohol, *n*-amyl alcohol, iso-amyl acetate, *n*-butyl acetate, ethyl caproate, and phenylethyl alcohol. These compounds were analyzed by GC and identified by GC–mass spectrometry. The production of volatile compounds by *P. membranaefaciens* was influenced mainly by the culture medium composition and incubation time and temperature. The maximum yields of each compound were registered under several fermentation conditions. However, for most of the volatile compounds formed, the incubation presenting the greatest yield of the volatile compounds was 72 h of incubation at 30°C.

The synthesis of ethyl acetate begins already in the active phase. The different compounds reach an optimum each at a distinct moment. The results showed that the culture medium composition, the fermentation conditions, and the choice of the strain determine the nature and the proportion of each volatile compound (Marques 1998). The author concluded that there are interesting possibilities in using *P. membranaefaciens* for the biosynthesis of ethyl acetate.

Daigle and others (1999) studied eight commercial yeast strains based on their aromatic potential to valorize bread by-products. The *Geotrichum candidum* ATCC 62217 was selected among cheese-ripening strains and other *G. candidum*-type strains and formed high concentrations of fruity aroma in a medium prepared with waste bread (35% solids). High concentrations of specific ethyl esters were produced after fermentation for 48–72 h, which corresponded to the stationary phase of growth and was related to the assimilation of organic acids. Aeration of the growth medium was essential, and better results were obtained at 30°C compared with 20°C.

Several microorganisms, including bacteria and fungi, are currently known for their ability to synthesize different aroma compounds. Attempts to use these microorganisms in submerged fermentation (SMF) resulted in low productivity of aroma compounds, which hampered the industrial application of these processes. Solid-state fermentation (SSF) could be of high potential for this purpose. One approach in this regard could be to use tropical agro-industrial residues such as cassava bagasse, sugarcane bagasse, coffee husk, and coffee pulp. Production of aroma compounds in SSF using naturally occurring substrates could offer potential benefits in production of food and fruity aroma compounds for human consumption at low cost (Pandey et al. 2000).

One major difficulty in this regard, however, remains the isolation and recovery of compounds produced, especially if the compounds have low boiling points. A few attempts have been made in this regard by trapping such compounds in suitable inert materials such as resins by adsorption. However, much remains to be done in this area. Fungi from the genus *Ceratocystis* produce a large range of fruit-like or flower-like aromas (peach, pineapple, banana, citrus, and rose), depending on the strain and the culture conditions. Among the genus, *Ceratocystis fimbriata* has a great potential for ester synthesis. It grows rapidly, has a good ability to sporulate, and produces a wide variety of aromas. The study evaluated the potential of several agro-industrial residues such as cassava bagasse, apple pomace, amaranth, and soybean using a strain of *C. fimbriata*. All media supported fungal growth. While amaranth medium produced pineapple aroma, media with other substrates produced strong fruity aroma (Pandey et al. 2000).

Aroma production was growth-dependent, and maximum intensity was detected a few hours before or after the maximum respirometric activity. Production of strong pineapple aroma was also reported when SSF was carried out using coffee husk as substrate by this strain. Medeiros and others (2000) cultivated a strain of *Kluyveromyces marxianus* in SSF using different solid substrates such as cassava bagasse, giant palm bran, apple pomace, sugarcane bagasse, and sunflower seeds. The feasibility of using cassava bagasse and giant palm bran as substrates to produce fruity aroma was confirmed, although the former proved to be superior. Esters are the source of the aromas (Pandey et al. 2000).

Pyrazines, especially alkylpyrazines, are heterocyclic compounds found in a wide variety of foods, which possess a nutty and roasty flavor. These compounds are used as food additive for flavoring. The production of 2,5-dimethylpyrazine (2,5-DMP) and tetramethylprazine (TTMP) was studied using *Bacillus natto* and *Bacillus subtilis*, respectively, on soybeans in SSF. Results demonstrated the suitability of SSF for the production of these compounds (Pandey et al. 2000).

A very interesting example of these studies cited above is fruit aroma production in SSF, using *K. marxianus*. Five agro-industrial residues were evaluated as substrate for cultivating a strain of *K. marxianus*. The results proved the feasibility of using cassava bagasse and giant palm bran (*Opuntia ficus indica*) as substrates to produce fruity aroma compounds by the yeast culture. In order to test the influence of the process parameters on the culture to produce volatile compounds, two statistical experiments were performed. The parameters studied were initial substrate pH, addition of glucose, cultivation temperature, and initial substrate moisture and inoculum size. Using a 2^5 factorial design, the addition of glucose and initial pH of the substrate was found to be statistically significant for aroma compound production on palm bran. Although this experimental design showed that the addition of glucose did not have a significant role with cassava bagasse, the 2^2 factorial design revealed that glucose addition was significant at higher concentrations. Headspace analysis of the culture by GC showed the production of 9 and 11 compounds from palm bran and cassava bagasse, respectively, which included alcohols, esters, and aldehyde. In both cases, two compounds remained unidentified, and ethyl acetate, ethanol, and acetaldehyde were the major compounds produced. Esters produced were responsible for the fruity aroma in both cases. With palm bran, ethanol was the compound produced at the highest concentration, and with cassava bagasse (both supplemented with 10% glucose), ethyl acetate was produced at the highest concentration, accumulating 418 and 1395 μmol/L/headspace/g substrate in 72 h, respectively (Medeiros et al. 2000).

The same research group developed another study, in which the ability of two different strains of *C. fimbriata* for fruity aroma production by SSF was tested on coffee pulp and coffee husk complemented with glucose as substrates. Headspace analysis of the culture by GC showed that 12 compounds were produced with coffee husk. Maximum total volatile (TV) concentration was reached after 72 h of culture with coffee husk as substrate (28 μmol/L/g). Ethyl acetate, ethanol, and acetaldehyde were the major compounds produced, representing 84.7%, 7.6%, and 2.0% of TV, respectively. A pretreatment with heat (100°C/40 min) of substrates did not improve TV production. Respirometry analysis was used to determine the growth of the culture by measuring carbon dioxide produced. Results showed that the CO_2 production follows the aroma production. This result shows the great potential of

using coffee pulp and coffee husk as substrates to microbial aroma production by SSF (Medeiros et al. 2000).

Filamentous fungi are an important source for flavoring compounds. In particular, the genus *Ceratocystis* produces a wide range of complex aromas, that is, peach, banana, pear, rose or citrus, depending on the strain and environmental conditions. Aroma compounds were produced in culture broth concentrations below 100 ppm (w/w); the following substances were identified: ethyl acetate, propyl acetate, isobutyl acetate, isoamyl alcohol, isoamyl acetate), citronellol, geraniol, and nerol (Bluemke et al. 2001).

Several microorganisms were isolated from coffee seeds and leaves, soil taken from plantations, and wastewater from the coffee seeds washing process due to their capacity to produce pectinolytic enzymes in clear halos around colonies by plate assay. From 104 strains, 18 strains were inoculated in a medium containing pectin as carbon source and were fermented at 30°C and 100 rpm for 96 h to determine pectinolytic activity of polygalacturonase (PG) and pectin lyase (PMGL). The strains 2, 9, 20, 39, 70, 74, and 99 showed activity units of PG higher than 80 μmol galacturonic acid/mL/min, and strains 17, 18, 31, 37, 73, 74, and 125 showed activity units of PMGL higher than 1000 ηmol unsaturated products/mL/min. The microorganisms 13, 70, 73, 74, 125, and 144 showed good descriptors of flavor perceived and the most intensities of flavor according to nontrained panel listing. The microorganisms 70, 73, 74, and 144 were selected to perform fermentation in a medium containing coffee husk and grape bagasse at 25°C and 100 rpm for 120 h, because these microorganisms are able to produce flavor by pectin degradation. In both media, the flavor compounds showed descriptors as fruity, sweet, floral, fermented, acidic, and solvent-flavored.. The compounds were separated and identified by GC and mass spectrometry. Acetone, ethyl acetate, ethanol, isobutanol, isopentyl alcohol (amyl alcohol), acetoin (3-hydroxy-2-butanone), acetic acid, isobutyric acid, butyric acid, 2-methyl-butanoic acid, benzyl alcohol, and 2-phenyl ethanol were produced in different combinations and different concentrations by the four microorganisms (Uenojo and Pastore 2006).

CONCLUSIONS

The production of flavors and aroma molecules by fermentation processes has become more or less routine. But until the regulation mechanisms of biosynthetic pathways are thoroughly understood, increased levels of desired metabolites will be achieved only randomly by bioprocesses. Nevertheless, the few examples presented here have demonstrated that fermentation processes have been quite successful for the production of flavors and have great potential.

REFERENCES

Aguedo M, Gomes N, Garcia EE, Waché Y, Mota M, Teixeira JA. 2005. Decalactone production by *Yarrowia lipolytica* under increased O_2 transfer rates. *Biotechnol Lett* 27:1617–21.

Berger RG. 1995. *Aroma Biotechnology*, 1st Ed. New York: Springer-Verlag.

Cogan TM. 1995. Flavour production by dairy starter cultures. *J Appl Bacteriol* 79:49–64.

Collins RP, Halim AF. 1972. Characterization of the major aroma constituent of the fungus *Trichoderma viride*. *J Agric Food Chem* 20(2):437–8.

Daigle P, Gelenos P, Leblanc D, Morin A. 1999. Production of aroma compounds by *Geotrichum candidum* on waste bread crumb. *Food Microbiol* 16:517–22.

Dufossé L, Feron G, Mauvais G, Bonnarme P, Durand A, Spinnler HE. 1998. Production of γ-decalactone and 4-hydroxy-decanoic acid in the genus *Sporidiobolus*. *J Ferment Bioeng* 86(2):169–73.

Dufossé L, Latrasse A, Spinnler HE. 1994. Importance dês lactones dans les arômes alimentaires: structure, distribution, propriétés sensorielles et biosynthèse. *Sci Aliments* 14:17–50.

Engels WJM, Visser S. 1994. Isolation and comparative characterization of components that contribute to the flavour of different types of cheese. *Netherlands Milk and Dairy Journal* 48:127–40.

Esaki N, Ito S, Blank W, Soda K. 1994. Biotransformation of oleic acid by *Alcaligenes* sp. 5–18, a bacterium tolerant to high concentrations of oleic acid. *J Ferment Bioeng* 77(2): 148–151.

Feron G, Dufosse L, Pierard E, Bonnarme P, Quere JL, Spinnler H. 1996. Production, identification, and toxicity of (gamma)-decalactone and 4-hydroxydecanoic acid from *Sporidiobolus* spp. *Appl Environ Microbiol* 62(8):2826–31.

Galindo E, Flores C, Larralde-Corona P, Corkidi-Blanco G, Rocha-Valadez JA, Serrano-Carreón L. 2004. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* cultured in unbaffled and baffled shake flasks. *Biochem Eng J* 18(1):1–8.

Gatfield IL. 1999. Biotechnological production of natural flavor materials. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer/Plenum, pp. 211–27.

Groguenin A, Waché Y, Garcia EE, Aguedo M, Husson F, LeDall MT, Nicaud JM, Belin JM. 2004. Genetic engineering of the β-oxidation pathway in the yeast *Yarrowia lipolytica* to increase the production of aroma compounds. *J Mol Catal B Enzym* 28:75–9.

Hamada T, Sugishita M, Fukushuma Y, Fukase T, Motai H. 1991. Continuous production of soy sauce by a bioreactor system. *Process Biochem* 26:39–45.

Hrazdina G. 2006. Aroma production by tissue cultures. *J Agric Food Chem* 54:1116–23.

Imhof R, Bosset JO. 1994. Quantitative GC-MS analysis of volatile flavour compounds in pasteurized milk and fermented milk products applying a standard addition method. *Lebensmittel-Wissenschaft und-Technologie* 27:265–69.

Jeon IJ. 1994. Flavour chemistry of dairy lipids: review of free fatty acids. *ACS Symp Series* 558:196–207.

Krings U, Berger RG. 1998. Biotechnological production of flavors and fragrances. *Appl Microbiol Biotechnol* 49:1–8.

Lee SL, Chou CC. 1994. Growth and production of γ-decalactone and *cis*-6-dodecen-4-olide by *Sporobolomyces odorus* in the presence of fatty acids and oils. *J Ferment Bioeng* 78(1):114–6.

Lin SJ, Lee SL, Chou CC. 1996. Effects of various fatty acid components of castor oil on the growth and production of γ-decalactone by *Sporobolomyces odorus*. *J Ferment Bioeng* 82(1):42–5.

Lomascolo A, Stentelaire C, Asther M, Lesage-Meessen L. 1999. Basidiomycetes as new biotechnological tools to generate natural aromatic flavours for the food industry. *Trends Biotechnol* 17:282–89.

Maarse H. 1991. Introduction. In Maarse H (ed.), *Volatile Compounds in Foods and Beverages*. New York: Dekker, pp. 1–39.

Macedo GA. 1997. Síntese de ésteres de aroma por lipases microbianas em meio livre de solvente orgânico. Doctor of Science Thesis [Portuguese], Univ. Estadual de Campinas, Brazil.

Maga JA. 1976. Lactones in foods. *Crit Rev Food Sci Nutr* 10:1–56.

Maga JA. 1995. *Food Additives Toxicology*. New York: Marcel Dekker.

Marques DB. 1998. Produção e caracterização de aroma de frutas por *Pichia membranaefaciens*. Master of Science Thesis [Portuguese], Univ. Estadual de Campinas, Brazil.

Medeiros ABP, Pandey A, Freitas RJS, Christen P, Soccol CR. 2000. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. *Biochem Eng J* 6: 33–9.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15(3):473–97.

Nago H, Matsumoto M, Nakai S. 1993. Degradative pathway of 2-deceno-δ-lactone by the lactone-producing fungus, *Fusarium solani*. *Biosci Biotechnol Biochem* 57(12):2111–5.

Neto RS, Pastore GM, Macedo GA. 2004. Biocatalysis and biotransformation producing γ-decalactone. *J Food Sci* 9:677–80.

Omelianski VL. 1923. Aroma producing micro-organisms. *J Bacteriol* 8:393.

Pandey A, Soccol CR, Mitchell D. 2000. New developments in solid state fermentation: I—Bioprocesses and products. *Process Biochem* 35:1153–69.

Pastore GM, Sato HH, Yang TS, Park YK, Min DB. 1994. Production of fruity aroma by newly isolated yeast. *Biotechnol Lett* 16(4):389–92.

Pinches M. 1994. Cheese: developing the flavour. *Dairy Ind Int* 59:37.

Rocha-Valadez JA, Estrada M, Galindo E, Serrano-Carreón L. 2006. From shake flasks to stirred fermentors: Scale-up of an extractive fermentation process for 6-pentyl-α-pyrone production by *Trichoderma harzianum* using volumetric power input. *Process Biochem* 41(6):1347–52.

Sarhy-Bagnon V, Lozano P, Saucedo-Castañeda G, Roussos S. 2000. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* in liquid and solid state cultures. *Process Biochem* 36(1–2):103–9.

Schrader J. 2007. Microbial flavor production. In Berger RG (ed.), *Flavors and Fragrances*. Berlin: Springer, p. 507.

Sousa RN. 1996. Produção, caracterização bioquímica e aplicações da lipase de *Alcaligenes* sp. Master of Science Thesis, Univ. Estadual de Campinas.

Uenojo M, Pastore MG. 2006. Isolamento e seleção de microrganismos pectinolíticos a partir de resíduos provenientes de agroindústrias para a produção de aromas frutais. *Ciênc Tecnol Aliment* 26(3):509–15.

Waché Y, Aguedo M, LeDall MT, Nicaud JM, Belin J. 2002. Optimization of *Yarrowia lipolytica*'s β-oxidation pathway for γ-decalactone production. *J Mol Catal B Enzym* 19–20:347–351.

CHAPTER 5

Environmental Effects on Flavor Changes

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INTRODUCTION

The effect of environmental factors on plant development, fruit set and growth, yield, and some quality attributes of fruits has been studied for many years, but the influence of these factors in flavor components, particularly aroma volatiles, started to be investigated more actively only in recent years when new and more efficient analytic procedures for volatile organic compounds became available. Techniques such as solid phase microextraction, a fast solvent-free extraction technique (Yang and Peppard 1994) facilitating the isolation of a large number of aroma volatiles from fruit samples, are now widely used. The study of environmental factors on flavor quality is relevant for the industry of fresh produce since fruit flavor plays an important role in consumer satisfaction and influences subsequent consumer purchases. In many markets, the demand for well-flavored fruits is growing, and the number of consumers who are willing to pay more for these high-quality fruits is increasing (Carbonell-Barrachina et al. 2006; Maul et al. 2000; Mitcham 1996).

Sugars and organic acids, frequently evaluated through soluble solid content (SSC) (or °Brix) and titratable acidity (TA), along with a large number of aroma compounds, are considered primary components of the flavor of fruits. These constituents, present in fruit tissues in very variable concentrations from parts per hundred to parts per trillion, individually elicit sensory responses recognized as flavor once they are integrated in the brain. These flavor constituents and their precursors accumulate during fruit growth and subsequently change during ripening and/or senescence. Both the accumulation and transformation of fruit flavor components are largely determined by cultivar genotype and maturity stage. They are also influenced by rootstock genotype and multiple and varied climatic and management factors (Mattheis and Fellman 1999). Once the fruit is separated from the plant, it is deprived from its source of carbohydrates, water, and nutrients, and its

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quality can be maintained by providing an appropriate postharvest environment but not improved. Therefore, preharvest factors, especially those that can be managed, require special attention from growers to optimize their impact on postharvest quality and flavor (Crisosto and Mitchell 2002; Hewett 2006).

The purpose of this chapter is to provide information about the main environmental factors influencing qualitative and quantitative changes of primary flavor components with emphasis on aroma compounds. When possible the sensory impact as a result of these changes will be mentioned. The literature cited herein is not intended to be an exhaustive review of scientific papers published on the topic but rather an illustration of how environmental factors influence the flavor of fresh fruits.

CLIMATE

Cultivated plants are adapted to grow in many climates, and they can be classified according to the zones where maximum productivity is achieved in tropical, subtropical, and temperate crops (Hewett 2006). Within each category, individual crops have optimum temperatures at which maximum yields and quality are obtained. Some horticultural crops such as tomatoes and strawberries can also be grown under greenhouse or plastic covers to ameliorate unfavorable growing conditions and to obtain high yields, a more uniform quality and "off-season" products for specific markets.

Temperature

Temperature is a critical factor for plant growth and development and strongly influences fruit quality attributes such as color, shape, size, and rind thickness (Arpaia et al. 2004; Reuther 1973; Westwood 1978). Temperature also has an effect on fruit flavor components such as SSC, TA, and aroma compounds.

A study about the effect of different growing day/night temperatures (25°C/25°C, 25°C/15°C, 25°C/10°C, and 25°C/5°C) on flavor components of "Camarosa" strawberries (Sanz et al. 2002) showed no significant differences in the content of glucose and fructose of berries grown at any of the selected day/night temperatures, but sucrose accumulation was the highest at 25°C/15°C and the production of aroma compounds was enhanced at 25°C/10°C day/night temperatures.

A good example of the effect of climate and specifically temperature on fruit flavor is found in winegrapes. A survey of worldwide viticultural areas would seem to indicate a relationship among climate, the flavor components of grapes, and the quality of wines. Wines made from the same variety often have, apart from their typical "varietal character," flavor characteristics identified with the region ("appellation of origin") (Jackson and Lombard 1993). Aroma compounds have a considerable effect on wine quality (Bravdo 2001; Jackson and Lombard 1993; Schultz 2002). Many odorous compounds are present in fruit, must, and wines as free, glycosidically bound and hydroxylated compounds. A group of aromatic substances present in several varieties of *Vitis vinifera* such as, Sauvignon Blanc and Cabernet Sauvignon are 2-methoxy-3-alkyl pyrazines (MP) (Jackson and Lombard 1993). These aroma

compounds are responsible for "roasted, nutty, and sweet" notes (when the alkyl chain is short) and for "vegetative, herbaceous, or green-like" notes (when the alkyl chain is long) (Dharmadhikari 1994). Variations in MP contents due to differences in climatic conditions, region of origin, and vintage are difficult to isolate, but it is generally accepted that wines from colder regions or vintages tend to exhibit a more pronounced "vegetative–herbaceous" aroma and contain higher amounts of MPs (Sala et al. 2004). This effect might be due to an increase of vine vigor and canopy shading that results from a better water availability and greater soil fertility generally found in colder regions.

Inadequate climatic conditions can lead to alterations of plant growth and development, abnormal vegetative or blossom growth, chilling or freeze injuries, and other physiological disorders. Unpredictable freeze injury in the field is a problem for some commercial crops such as apples and citrus. For growers and packinghouses faced with this problem, it is important to have a procedure that allows them to assess the extent of fruit damage before visual symptoms become evident. Extreme temperatures can promote the production of different volatiles in plant tissues (Kimmerer and Kozlowski 1982; Song et al. 2001). An increase in ethanol and ethyl acetate in two apple cultivars subjected to freezing was reported by Forney and others (2000a), and according to Obenland and others (2003), off-odor has been perceived as an early symptom of freeze injury in navel orange. In a study conducted to ascertain whether volatile compounds emitted from navel orange (*Citrus sinensis* L. Osbeck) could serve as early indicators of freezing, it was found that acidity and taste were more sensitive indicators of freezing than peel injury or internal drying (Obenland et al. 2003). It was also found that the emission of ethanol, ethyl butanoate, methyl hexanoate, and ethyl octanoate increased rapidly (4–6 h) after freezing and remained high after a 3-week period of storage, thus indicating that one or more of these volatiles can be viable markers of freeze-damaged navel oranges.

Light

The duration and intensity of light affect the quality of fruits (Pantastico 1975; Westwood 1978). Among fruit quality factors, numerous reports indicate that light influences the content of flavor components. This effect can be related to the rate of carbon fixed and translocated to the fruit, or to the fruit temperature associated to light exposure and its effect on fruit metabolism (Watson et al. 2002). In a series of three papers, Sites and Reitz (1949, 1950, and 1950 cited by Pantastico 1975) reported that "Valencia" oranges exposed to the sun had less weight, thinner rinds, and higher levels of SSC and TA than those that were shaded or located inside the canopy. Reflecting foils underneath the canopy of vineyards have been evaluated to reflect solar radiation back into the fruiting zone. The initial purpose of this foil was to improve color formation in red varieties. However, "Riesling" grapes (a white variety) responded to this treatment with a significant improvement in flavor development over several years without substantial differences in sugar levels (Schultz 2002).

For many crops such as apples and grapes, pruning practices to modify the amount of incident light on canopy and fruits are means to optimize productivity and produce good quality fruits (Hewett 2006). Miller and others (1998) observed that the position of "Delicious" apples in the canopy had an influence on acetate

ester production. Fruits with a western or southern exposure had higher ester production than those with northern or eastern exposure. Acetate esters, mainly butyl acetate, 2-methylbutyl acetate, and hexyl acetate, are major contributors to the characteristic apple-like aroma and flavor in most apple cultivars. Sunlight is an important factor contributing to the red color of apples. Fellman and others (2000) working with "Delicious" strains with different capacities for accumulation of anthocyanin pigments in peel tissue, found that the higher-coloring strains had lower level of acetate esters. They also found a positive relationship between shading and ester biosynthesis reinforcing the suggested relationship between color and synthesis of acetate esters.

Different pruning techniques may cause significant differences in the level of 3-isobutyl-2-methoxypyrazine, a relevant MP aroma compound of some winegrape cultivars (*V. vinifera* L.). This effect might be explained by the influence of the pruning system on cluster exposure to sunlight (Sala et al. 2004). For instance, leaf removal of Sauvignon Blanc vines decreased the concentration of MPs of grapes and the "herbaceous" character of the resulting wine due to an increase in fruit exposure to sunlight (Sala et al. 2004). Leaf removal around bunches prior to veraison (fruit color change at physiological maturity) is a common practice in viticultural areas, especially in cool and moist regions (Jackson and Lombard 1993). Severe leaf removal seems to be more effective at reducing the vegetal character of Sauvignon Blanc wine, and earlier treatments would be more effective than later treatments.

It has been shown that both the duration and the timing of shading are important factors influencing flavor quality (Garriz et al. 1998; Marini et al. 1991; Pattern and Proebsting 1986). The effect of shading on flavor components of strawberries cv. Elsanta grown in peat bags in a glasshouse was evaluated by Watson and others (2002). Different levels of shading (0%, 25%, and 47%) were applied for 2 weeks, starting at 7 days before the first fruit ripened. Fruits from the 47% shading treatment showed a significant reduction in sucrose concentration, glucose/fructose ratio, and levels of the aroma compounds: hexenal, hexanal, ethyl methyl butyrate, and methyl butyrate, compared with the unshaded treatment. It was also observed that a relatively short period of low light had a significant effect on the flavor components of berries. This apparent sensitivity of strawberry flavor to the light environment could explain, according to the authors, the poor taste sometimes associated with autumn-grown crops, as well as variations in flavor between fruits grown in the field and in a glasshouse, which can reduce light levels by 30%.

Season and Locations

Flavor differences among produce harvested at different dates and grown in different seasons and locations within a single crop have been demonstrated by several researchers. Watson and others (2002) reported differences in sugars and some "character-impact" aroma volatiles among "Elsanta" strawberries grown in a greenhouse and harvested at different dates through the season.

Klieber and Muchui (2002) failed to observed differences in the flavor quality of bananas (*Musa acuminata* Coll. Cavendish cv. Williams) harvested through winter, spring, and summer evaluated by sensory testing, even though SSC was higher in spring (23.6%) compared with winter (22.6%) and summer (21.9%). However, no

differences were found in aroma compounds evaluated by instrumental analysis in fruits harvested at different times of the year.

Seasonal variations in flavor, evaluated by sensory analysis, were partially related to variations in sugar and acid accumulation in grapefruit (*Citrus* × *paradisi* Macf.), tangerine (*Citrus reticulata*), and tangelo (*Citrus* × *tangelo*) (Harding and Fisher 1945, Harding and Sunday 1949, and Harding et al. 1959 as cited by Mattheis and Fellman 1999). Also, a seasonal effect on aroma compounds was reported by Lopez and others (1998) for "Golden Delicious" and "Granny Smith" apples in a 2-year study. The aroma volatiles were different in class and quantity and gave the characteristic sensorial perception to each fruit variety. In "Golden Delicious," ethyl propionate and butyl acetate gave a fruity aroma in the 1993 season, while ethyl acetate, ethyl propionate, and propyl acetate were the predominant compounds in the 1994 season. In "Granny Smith," the aromatic profile did not show the predominance of any compound in 1993, while in 1994, it was fruitier due to the higher content of ethyl propionate and propyl acetate.

Working with "Golden Delicious" apples from two locations in Italy, Zerbini and others, cited by Yahia (1994), found that after storage, fruits grown on the plains had higher soluble solids, reducing sugars, and sugar/acid ratio than those from the mountain area. It was concluded that warmer weather favored fruit quality, but might also increase the incidence of physiological disorders during storage.

Aroma-active volatiles of 130 blackcurrant thermal evaporative concentrates prepared from berries obtained from three seasons and different geographic origins, as well as three different frozen concentrates, were evaluated by Boccorh and others (1999). It was found that variation was dominated by seasonal effects and within a single season, geographic origin was more important than the use of fresh or frozen berries.

CULTURAL PRACTICES

Mineral Nutrition

Balanced and timely availability of mineral nutrients is important for optimum plant performance, good quality, and adequate postharvest life of fruits. Multiple chemical constituents of fruits can be affected by mineral nutrition, and some physiological disorders occurring in storage can be caused by deficiencies, excesses, and/or imbalances of various nutrients. Minerals can be applied by conventional methods (soil or foliar applications) or through irrigation systems such as fertigation, a relatively new method that has the advantage of increasing the uptake of mineral nutrients by plants compared with conventional fertilization. For many crops, much effort has been expended to develop protocols for estimating critical threshold levels of macroelements including nitrogen (N), potassium (K), and phosphorus (P), as well as microelements such as calcium (Ca), magnesium (Mg), boron (B), and zinc (Zn). Also, much experimental work has been conducted to establish appropriate levels of fertilizers and the time when a maximum benefit can be obtained (Hewett 2006). Unfortunately, fertilization recommendations have been established mainly to obtain high crop productivities and to optimize some quality characteristics of fruits, but not to improve their flavor.

Nutrient balance of crops is important for normal production of flavor and aroma volatiles by fruits. Several reports dealing with the influence of fertilizer treatments on fruit flavor ratings and aroma production by apples (*Malus sylvestris* Mill., *Malus pumila* Mill., *Malus domestica* Borkh.) were reviewed by Mattheis and Fellman (1999). Also, Fellman and others (2000) examined how nitrogen status affected apple aroma with emphasis on the production of three esters (butyl acetate, 2-methylbutyl acetate, and hexyl acetate), which are considered major contributors to the characteristic apple-like aroma and flavor in most cultivars.

Nitrogen has been the most studied element in relation to strawberry fruit quality (Nestby 2002). Proper nitrogen applications can increase levels of flavor components of berries. Nestby (1998) reported an increase in the concentration of glucose, fructose, and sucrose in "Korona" and "Bounty" strawberry fruits when nitrogen was applied as nitrate in fertigation treatments at levels up to 124 kg/ha, starting 4 weeks before harvest and ending after 2 weeks into the harvest period. Hennion and others (cited by Nestby 2002) evaluated the effect of various nitrogen fertilization treatments applied by drip irrigation in spring and found no differences in physical and chemical characteristics of fruits, but sensory analyses indicated an improvement in aroma and flavor in fruits treated with the highest nitrogen fertilization rate. There are few reports about the direct effect of other elements on the flavor components of strawberries. Boron and molybdenum have shown to be important for the content of vitamin C and sugars in strawberry fruits (Nestby 1998).

Wright and Harris (1985) applied three levels of N–K (100–150, 200–300, and 300–450 lb/ac) on tomato plants and observed an increase in TA and SSC with higher levels of fertilizers. Also, concentrations of the volatiles hexenal, 2-hexanone, benzaldehyde, phenylacetaldehyde, α-ionone, and 6-methyl-5-hepten-2-one increased with increasing N–K levels. However, flavor scores indicated detrimental effects on tomato flavor with high N–K levels. The effect of six levels of N on the flavor components of cherry tomatoes was studied by Yu-Tao and others (2007). Levels of sugars, TA, SSC, and some volatiles (1-penten-3-one, hexanal, *cis*-3-hexenal, 2-methyl-4-pentenal, *trans*-2-hexenal, and 6-methyl-5-hepten-2-one) increased with higher N applications. However, no sensory evaluation was conducted to observe the impact of these chemical changes on flavor perception. Phosphorus supplementations (above the recommended levels) through soil or foliar spray were tested on tomato quality during a 3-year field study (Oke et al. 2005). No significant increase in yield by the effect of soil and foliar P supplementation was observed. Also, compositional changes (pH, TA, and aroma volatiles) were not statistically significant among P treatments. Thus, the authors concluded that P supplementation may not affect quality characteristics of tomato fruits.

Lin and others (2004) evaluated the application of potassium at insufficient, suitable, and excessive levels (120, 240, and 360 mg/L of K, respectively) in nutrient solution on the fruit quality of "Tiantian No. 1" muskmelon (*Cucumis melo* var. reticulatus Naud.) in a soilless medium culture under greenhouse conditions. They found that potassium level at 240 mg/L improved the fruit quality as indicated by a significant increase in the concentrations of total sugars, SSC, glutamic acid, aspartic acid, alanine, and the volatile esters *n*-amyl acetate and 2-butoxyethyl acetate. No significant differences in fruit appearance or size were observed among treatments, but unfortunately, no sensory evaluations were conducted to observe the effect of K levels on flavor perception.

In a study with three fertigation levels (50, 100, and 150 kg/ha N using a 7-3-7 NPK liquid fertilizer) applied on a Cabernet Sauvignon vineyard, the highest NPK fertigation level enhanced the content of hexanal and 2-hexenal, two aldehydes contributing to the herbaceous character of the must and wine (Bravdo 2001). The lower fertigation levels also enhanced the content of hexanoic acid, hexyl acetate, and 1-heptanol in the must. However, no significant effects of the fertigation levels on wine quality were detected by the sensory evaluation panel.

Phosphorous fertilization of a "Cabernet Sauvignon" vineyard increased the content of monoterpenes in wine and the aroma quality score obtained by sensory evaluation (Bravdo 2001). Volatile monoterpenes are found as free or bound compounds in grapes, must, and wines, and contribute to the distinctive flavor and aroma of several winegrape varieties (e.g., from "Cabernet Sauvignon" to the white varieties Muscats, Sauvignon Blanc, Riesling, and Gewürztraminer) (Jackson and Lombard 1993) and their respective wines.

Irrigation

This cultural practice plays an important role in plant growth and development and is an essential factor to obtain high-quality fruits. Growers generally apply water, based on the evapotranspiration (ET) demand, to minimize moisture stress and to allow optimal plant growth and fruit yields. However, in some crops, it is possible to decrease water usage and improve crop quality without compromising sustainable plant growth (Hewett 2006).

Water is presently a limited natural resource. Since irrigation practices in agriculture and horticulture use 75% of the world's total fresh water resources, and excessive irrigation favors the leaching of biocides and the contamination of groundwater, some countries have even passed legislations to reduce the amount of water for crop production (van Hooijdonk et al. 2007). Therefore, strategies to reduce water usage in agriculture are currently important. Two water saving strategies are deficit irrigation (DI) and partial rootzone drying (PRD). In DI, the entire rootzone is irrigated with less water than in the prevailing ET, while in PRD, the water is applied to one-half of the rootzone during each irrigation time and the other half is left to dry to a predetermined level of soil moisture (van Hooijdonk et al. 2007). In response to PRD, roots—the primary sensors for soil dryness—produce chemical signals that are transported to the shoots where they promote a reduction in leaf stomatal conductance and transpiration (Davies and Zhang 1991; Stoll 2000; van Hooijdonk et al. 2007). In addition to preserving water and reducing the leaching of biocides into groundwater, water saving strategies can improve fruit quality (Behboudian and Mills 1997). Some reports indicate that water stress can be used as a management tool to manipulate flavor components of fruits.

Modise and others (2006) conducted a greenhouse study to investigate the production of some character-impact volatiles of "Elsanta" strawberries in response to three levels of water stress at two specific growth stages: at flowering and at fruiting, 10 days after anthesis. They found that severe water stress at fruiting significantly increased the level of ethyl and hexyl acetates, ethyl butyrate, methyl hexanoate, and methyl propyl acetate produced by ripe fruits.

Veit-Köhler and others (1999) investigated the effect of a small reduction in water supply (without visible symptoms of water stress) on flavor components of tomato. The tomato plants cv. Vanessa were grown in soil, and with the onset of fruit development, water supply was varied (70% and 50% water capacity). Results indicated that under conditions of lower water supply, the quality of tomatoes was higher as indicated by the higher levels of sugars, titratable acids, vitamin C, and aroma volatiles, particularly C6-aldehydes (hexanal, (*Z*)-3-hexenal and (*E*)-2-hexenal). Fruit growth and yield were similar in both treatments.

Crisosto and others (1997) evaluated the influence of various irrigation regimes (normal irrigation = 100% ET, over-irrigation = 150% ET, and DI = 50% ET) applied 4 weeks before harvest on peach quality during two seasons. Yield, flesh firmness, color, acidity, and pH at harvest were not affected by any irrigation regime, but SSC was the highest (13.3%) for fruit from the 50% ET treatment. Although fruits from this treatment were smaller, they probably would be preferred by consumers over fruit from the other treatments since peaches with 11% SSC or higher are highly acceptable to consumers and may have a higher retail value (Parker et al. 1991).

In a field experiment conducted by Behboudian and others (1998) with apple trees cv. Braeburn subjected to late-season DI, the authors observed an increase of aroma production by fruits from these stressed trees. In a second study (Mpelasoka et al. 2000), early deficit irrigation (EDI) and late deficit irrigation (LDI) were evaluated on the same apple cultivar, and it was found that SSC and total sugar concentration increased after a 12-week storage at 0°C and were higher in EDI and LDI than in control fruits before and after storage. However, no increase in aroma production by water-stressed fruits was found in this second study. According to the authors, this inconsistency could be due to different degrees of water deficit developed and to differences in fruit maturity at assessment. As it was expected, gross yield per tree decreased by the effect of DI treatments (77.9, 67.0, and 58.3 kg for control, EDI, and LDI treatments, respectively) and so did the corresponding mean fruit weights (199.0, 167.3, and 167.9 g, for control, EDI, and LDI treatments, respectively). In a third study (Bussakorn et al. 2002), the effects of DI applied throughout the season and the effect of two crop load treatments (commercial and light crop load) on volatiles and other flavor components of "Braeburn" apples were evaluated. Crop load was included because the negative effect of DI in reducing fruit size could be counteracted by a lighter crop load. Results indicated no interaction between irrigation and crop load on any individual quality attributes. Control and DI fruit had similar maturity at harvest, but DI fruit became more advanced in maturity during storage at 20°C and after cold storage. Apples from DI trees showed an enhanced production of aroma volatiles not only during ripening but also after cold storage, and SSC was higher once again in DI fruits. Quality enhancement in DI fruits was related, in part, to the advancement in ripening.

In a field study with "Pacific Rose" apple trees conducted by van Hooijdonk and others (2007), different irrigation treatments were evaluated: commercially irrigated (CI) control (soil maintained near field capacity throughout the season), PRD (half of the irrigation volume of CI was applied to only one side of the rootzone), and no irrigation ([NI] water was withheld for the duration of the experiment). Results indicated that gross yield per tree did not significantly differ among treatments. PRD treatment saved water by 50% and improved some fruit quality attributes including

the increase of some individual impact aroma compounds (e.g., 2-methyl-butyl acetate), a decrease of weight loss and a slower loss of firmness during storage at 0 ± 0.5°C. The authors indicated that further sensory analyses are required to determine if the observed compositional changes are reflected in fruit flavor.

A 5-year study was conducted by Reynolds and others (2005) to investigate the effect of different irrigation and fertigation treatments (of various durations and 80 kg N/ha as urea), on vine performance, fruit composition, and water relations of "Concord" and "Niagara" (*Vitis labruscana*) grapes used by the juice industry. Plant transpiration rate and soil moisture data of this study suggested that water stress was present in 3 of the 5 years of the study. Irrigation and fertigation led to an enhanced berry set, a larger berry size, an increase in vine size, and small increases in yield. In most seasons, yield increases were accompanied by small decreases in SSC (1.5–3.0°Brix) and levels of methyl anthranilate (an undesirable compound in winegrapes, but one of the main volatile esters found in *V. labruscana* grapes bound for juice production). However, all soluble solid concentrations were above the minimum levels accepted by local processors. Total volatile esters did not differ substantially among the treatments, and no major differences or trends in TA and pH were noticed between treatments. The timing of the fertilizer application did not appear to play a major role in any of these attributes.

According to Bravdo (2001), the irrigation of winegrapes was not recommended in some European regions due to a traditional belief that it reduces wine quality. In a study reported by the same author, no significant effect of the level of drip irrigation on the *Cabernet Sauvignon* wine quality was detected by sensory evaluation, and the gas chromatography–mass spectrometry (GC-MS) analyses of 47 volatiles in the must and wine showed only a significant increase of 3-methyl butyl octanoic acid and ethyl decanoate by lowering the drip irrigation rate. This and other studies have demonstrated that high-quality wines can be obtained under different irrigation regimes. Therefore, irrigation, particularly drip irrigation, applied during periods of insufficient precipitation is now a common cultural practice in many temperate wine production regions of the world, and it is an efficient mean of controlling the vegetative and reproductive stages of vines (Bravdo 2001).

Data regarding the effect of water stress on wine quality are also reported in the scientific literature. A decrease in shoot growth rate and leaf area in response to PRD was observed in a study (Stoll 2000) to evaluate the effects of PRD on grapevine physiology and fruit quality (*V. vinifera* L. cvs. Cabernet Sauvignon, Chardonnay, Shiraz, and Riesling). As a result, light penetration inside the canopy improved and so did fruit quality without an adverse effect on yield. Sensory evaluation indicated that high-quality wines from PRD-treated vineyards can be achieved. However, according to Bravdo (2001), reports about the effect of water stress on wine quality are not always consistent.

Orchard Management and Other Cultural Practices

Fruit thinning is a cultural practice that reduces crop load and improves the leaf/fruit ratio leading to an increase in fruit size. Excessive crop loads not only have negative effects on fruit size, but may also have adverse effects on fruit color, sugars, and other flavor components, as well as on fruit condition and storage life (Forshey

1986). A beneficial relationship between low crop load and flavor components of "Jonagored" apples was reported by Poll and others (1996). Fruits grown with the lowest fruit/leaf ratio had the highest SSC, TA, and production of butyl acetate and hexyl acetate, two volatiles contributing to the typical aroma of apples. Schultz (2002), who worked with the winegrape cultivar Riesling, found that early cluster thinning (approximately 3 weeks after bloom) significantly increased the content of secondary metabolites in berries and improved sensorial characteristics of the resulting wines. However, the authors mentioned that the success of this cultural practice strongly depends on the level of crop load, and in some cases, early thinning can lead to more compact clusters and larger berries with an increased risk of *Botrytis* growth.

In addition to fruit thinning, pruning and training systems of vines can also alter the vegetative to reproductive growth ratio or crop load (fruit weight per kilogram pruning weight or per square centimeter leaf area) and thereby the fruit and wine quality (Bravdo 2001). "Training refers to the structures used to support the vine, the methods used to affix the vine to that structure, and the configuration and geometry so produced" (Jackson and Lombard 1993). Cabernet Sauvignon wines from trained vines with vertically positioned shoots contained higher levels of butyl acetate, ethyl butanoate, and hexyl acetate as well as a lower concentration of ethyl 2-hexanoate. Among the 60 volatiles detected by GC-MS, these four esters, responsible for fruity aromatic notes, were the only ones significantly affected by the training system (Bravdo 2001).

Among orchard establishment factors, planting density can have a substantial effect on the ripening and flavor development of fruits. In an experiment with cv. Riesling on 5C rootstock planted at a row distance of 2 m and with different spacings within each row, only minor effects on yield were observed. However, large differences in amino acid concentration and small differences in sugar levels were recorded in grapes harvested from high-density plantings. These compositional changes improved the fermentation process with positive effects on the fruity character of the resulting wine (Schultz 2002).

Bagging of fruits during development is a cultural practice that primarily protects fruits against microorganisms, insects, and physical damage. However, it can have other benefits such as a fast maturation process and an increase in the level of aroma compounds. In mangoes (*Mangifera indica* L.), Hofman and others (1997) found that bagging accelerated fruit maturation, but it reduced the average temperature under the skin from 45.5 to 41°C. Apparently, the interception of radiation by bags decreased temperature stress favoring the fruit maturation process. No difference in consumer preference was found for bagged and non-bagged mango fruit. Hui-Juan and others (2005) conducted a 2-year-study to examine the influence of bagging on aroma volatiles and skin coloration of "Hakuho" peaches (*Prunus persica*). Fruits were covered with orange paper bags before pit hardening, and with single and triple parchment paper bags and orange paper bags (sunlight transmission: 80%, 50%, and 15%, respectively), 15 days before harvest. Results indicated that bagging caused earlier fruit ripening in the 2002 season but not in the 2003 one. SSC and TA were not affected by bagging treatments, and the red skin color increased with the increase in sunlight transmission. Aroma volatile content was the lowest in non-bagged fruit exposed to full sunlight, and different bagging treatments resulted in

significantly different levels of γ- and δ-decalactone (important volatiles contributing to peach aroma) between skin and flesh. According to the authors, a higher temperature by exposure to sunlight may have been the factor contributing to a lower synthesis of aroma compounds and/or to their faster biotransformation. Similarly, Miller and others (1998) showed that "Delicious" apples managed for maximum coloration (exposed to full sun) had low acetate esters, which are important contributors to the characteristic aroma of apples.

In commercial strawberry production, plastic mulches are frequently used in raised-bed culture to conserve water, control weeds, keep fruit clean, and produce ripe berries earlier in the season. In the study conducted by Kasperbauer and others (2001), the effect of a specially formulated red plastic mulch on the size and flavor components of strawberries (*Fragaria* × *ananassa* Duch) was compared with the standard black plastic mulch. Results indicated that fruits grown over the new red plastic mulch were about 20% larger and showed higher sugar to organic acid ratios and levels of aroma compounds. The authors hypothesized, based on previous studies, that the more far-red and red light (higher far-red/red ratio) reflected from the red mulch induced biochemical changes during growth and ripening through phytochrome-mediated regulation pathways with a subsequent improvement in berry size and augmented levels of flavor components.

The effect of elevated carbon dioxide on the flavor components of field-grown strawberries (*Fragaria* × *ananassa* Duch) was studied by Wang and Bunce (2004). High CO_2 concentrations (ambient + 300, and ambient + 600 μmol CO_2/mol) resulted in higher contents of fruit dry matter, fructose, glucose, and total sugar, as well as low levels of citric and malic acids. Also, high CO_2 growing conditions significantly enhanced the fruit content of ethyl hexanoate, ethyl butanoate, methyl hexanoate, methyl butanonate, hexyl acetate, hexyl hexanoate, furaneol, linalool, and methyl octanoate, which contribute to the characteristic aroma of strawberries. The highest levels of these aroma compounds were found in the strawberries grown under 600 μmol CO_2/mol.

Oil washes have been used for over a century to suppress insect and mite pests in orchards. Traditionally, the Washington State apple industry of the United States has relied on petroleum-based horticultural oils. However, soybean oil is also effective as an insecticide and is more environmentally friendly. Müller (2005) studied the effect of soybean oil applications on volatile aroma production of "Golden Delicious" apples (*Malus* × *domestica* Borkh.). Soybean oil emulsion was applied midseason, 21 days before harvest and 3 days before harvest. Fruits were harvested at commercial maturity and stored for up to 6 months at 0.5°C in air or under 2 kPa O_2 and 0.2 kPa CO_2 controlled atmosphere (CA) storage. When soybean oil emulsion was applied 21 days before harvest, apples showed consistently higher volatile production, mainly due to the presence in higher concentrations of the aldehydes hexanal and 2-hexenal. The loss of alcohols (mainly butanol, 2-methylbutanol) due to prolonged CA storage was diminished by oil-treated fruit, and more alcohols (butanol, 2-methyl butanol) were regenerated after 7 days of shelf life. The author concluded that soybean oil applied during the growing season of "Golden Delicious" apples can improve the retention and regeneration capacity of important aroma compounds. In addition, soybean oil delayed weight loss of apples in storage by improving fruit cuticular structure.

ORGANIC FRUITS

"Organic" is a system of managing crops that emphasizes natural pest control methods and soil inputs (Bruhn 2002). During the 1990s, organic farming became one of the fastest growing segments of agriculture in the United States and Europe (Reganold et al. 2001). Similarly, integrated farming, a system that combines organic and conventional techniques, has been successfully adopted on a wide scale in Europe. According to Theuer (2006), it would be interesting to investigate the difference in flavor between products cultivated by conventional systems and those grown by organic production systems since 43% of consumers of organic food give "better taste" as the main reason for purchasing organic fruits and vegetables. Also, it has been found that organic products have higher levels of some phenolic compounds (Benbrook 2005) that can interact with flavor perception.

As reported by the Food and Agriculture Organization of the United Nations (FAO 2000), in many studies, no clear differences in sensorial characteristics have been observed between organic and conventional products. However, some differences in flavor characteristics have been reported for strawberries, tomatoes, and apples. Organic strawberries cultivated in California, although smaller in size, had better quality attributes (sweeter, better appearance) and were preferred by consumers compared with conventionally grown berries (Theuer 2006). Organic and traditional grown tomatoes, harvested at the breaker stage and ripened at 20°C, were evaluated in two seasons for quality attributes (McCollum et al. 2005). Sensory evaluation indicated that panelists were able to perceive differences between conventional and organic tomatoes by smell or taste. Some panelists indicated that organic tomatoes were preferred because of their better taste, flavor, texture, and juiciness. In contrast, conventional tomatoes were described as "not as ripe," "dry," and having "less aroma."

In regard to apples, in a 5-year study conducted by Reganold and others (2001), the quality of "Golden Delicious" fruits grown by organic, conventional, and integrated production systems was compared. The ratios of SSC to TA were most often highest in organic apples. These results were supported by sensory evaluations using untrained panelists who found that organic apples were sweeter after 6 months of storage than conventional fruits and less tart at harvest and after 6 months of storage than those grown under conventional and integrated systems. However, apples from integrated systems had better flavor after 6 months of storage. In a similar study with "Galaxy Gala" apples, the quality of fruits grown by organic, conventional, and integrated production systems was assessed at harvest and after storage at 0–1°C for 3 months in air and for 3–6 months in a CA containing 1.5–2% O_2 (Peck et al. 2006). No differences in SSC, TA, or the SSC to TA ratios were detected among apples from the three production systems, but organic apples had 6–10 N (Newton) higher flesh firmness than conventional ones, and 4–7 N higher than integrated apples, for similar-sized fruits. Consumer panels tended to rate organic and integrated apples to have equal or better overall acceptability and texture than conventional apples, but they were unable to distinguish the higher concentration of flavor volatiles found in conventional apples. In contrast, Róth and others (2006) found, by comparing quality attributes and storage behavior in air and under ultralow oxygen conditions of apples grown in integrated versus organic orchards, that the

effect of storage condition on firmness and flavor components of apples was much greater than the production system effect.

CULTIVAR AND ROOTSTOCK GENOTYPE

Previous studies clearly demonstrate the strong impact that environmental factors have on the individual flavor component of fruits. However, as mentioned earlier, the content and delicate balance of flavor compounds that characterize each fruit depends primarily on the genetics of each species. For instance, in the case of strawberries, Shaw (1988) found a significant genotypic variation in the content of sucrose, glucose, and fructose in his breeding population. He also found that TA and organic acid content are genetically determined (Shaw 1988, 1990). Similarly, in a comparative study, Pelayo-Zaldivar and others (2005) found that cultivar variation was greater than harvest date variation in the primary flavor components of three strawberry cultivars, and these results were supported by sensory evaluations. Furthermore, qualitative and quantitative differences in strawberry aroma compounds among cultivars and between commercial cultivars and wild plants (native clones of *Fragaria chiloensis* and *Fragaria virginiana*) have been reported by several authors (Dirinck et al. 1981; Forney et al. 2000b; Hancock 1999; Miszczak et al. 1995; Pérez et al. 1997; Pyysalo et al. 1979; Ulrich et al. 1997).

Genotypic variation in flavor characteristics of tomato has also been reported by different authors. Baldwin and others (1991) analyzed flavor components in six Florida-grown tomato cultivars. Fruits were harvested at the mature green stage and left to ripen at 20°C before analysis. Among cultivars, significant differences were found in the levels of glucose, fructose, citric acid, and 9 of 17 aroma volatiles analyzed. Similarly, in the study conducted by Ruiz and others (2005), significant differences were found among very closely related tomato cultivars for three C6-aldehydes (hexanal, *cis*-3-hexenal, and *trans*-2-hexenal) and two C6-alcohols (*cis*-3-hexenol and hexenol). Thus, they proposed the determination of aroma volatiles as a possible tool in tomato breeding programs.

Sensory evaluations should be conducted in parallel with chemical or instrumental analysis of flavor components, since analytic values do not always correlate well with sensory scores (Mattheis and Fellman 1999). Stevens and others (1977) reported differences in SSC, TA, pH, and concentration of individual sugars, acids, and volatile compounds among different tomato cultivars, but no strong correlations were found between analytic data and sensory scores for all cultivars. In contrast, Tandon and others (2003) evaluated 12 tomato selections for their flavor components using sensory and chemical analysis and found a clear relationship between sensory perception of tomatoes and their chemical flavor composition. Tomatoes described as full flavored by the breeder contained higher levels of sugars, soluble solids, aromatic volatile compounds, and lower amounts of organic acids than those considered to be of poorer flavor by the breeder.

Rootstock genotype also influences fruit composition and flavor quality through interactions with soil type, water availability, climate, and scion cultivar. As indicated by Mattheis and Fellman (1999), several reports have shown the influence of rootstock on citrus flavor including orange, grapefruit, and tangerine. They also indicate that bitterness of citrus fruits and juice has been reported to be influenced by rootstock.

According to Schultz (2002), rootstocks affect grape (*V. vinifera*) composition and wine quality through an effect on vine vigor and canopy light exposure. Less vigorous rootstocks (producing less vine growth) yield fruits that produce high-quality wines (Jackson and Lombard 1993). Similarly, in a comparative study, the more vigorous St. George rootstock yielded fruit with higher levels of N, acid, tannin, and K, while the less vigorous 110R rootstock produced fruits with a more balanced composition (Jackson and Lombard 1993).

In spite of the importance that genetic characteristics have in determining fruit flavor, breeding programs have traditionally selected new cultivars and rootstocks based on good yields, adaptability to various environmental and crop pest conditions (Crisosto and Mitchell 2002), and some quality characteristics of fruits such as size, color, SSC, TA, slow ripening, and resistance to transport, neglecting sensorial characteristics such as aroma and taste. It is important that breeders consider primary flavor components (sugars, acids, and aroma compounds) evaluated by both chemical/instrumental and sensory analysis to make a more complete characterization of their breeding populations.

MATURITY

Maturity at harvest strongly influences the flavor of fruits. Fruits harvested at an immature stage develop poor flavor when ripe, and overmature fruits have a limited commercial life before flavor deteriorates. The optimum maturity of a fruit depends on its intended use. For instance, Fellman and others (2003) conducted experiments focused on timing of the optimum harvest for maintaining sweetness, sourness, and aroma-generating capacity during CA storage of "Redchief Delicious" apples. Fruits were sequentially harvested starting at 93 days after full bloom. Firmness, SSC, and TA of fruits from all harvest dates remained at acceptable levels throughout the post-storage ripening period. However, as harvest maturity advanced, the time required to regenerate aroma volatiles to an "optimum" level after removal from CA storage decreased.

There are considerable physiological and chemical changes during the ripening period, but to identify the optimum harvest date is still a challenge to growers, since regional, climatic, and management influences may change fruit physiology and composition and thus alter the optimum harvest date.

CONCLUSIONS

The chemistry of fruit flavor is very complex. Primary flavor compounds including sugars, organic acids, and a wide array of aroma volatiles are present in specific amounts and proportions in fruits. Other constituents such as nonvolatile phenolics and cell wall components influence flavor by affecting the taste perception and the rate at which aroma compounds are released from tissues. Also, fruit temperature and texture can affect the relative vapor pressure of volatiles and aroma release.

Sensory perception of flavor is also very complex. Aside from basic tastes (sweet, salt, sour, and bitter), our tongue can perceive other food characteristics (texture, temperature, astringency, metallic irritation, and chemical heat), and a number of

antagonisms, synergies, and maskings among taste components are present during taste perception (Baldwin 2002; Baldwin et al. 2000; Delwiche 2004). Receptors of the olfactory epithelium can distinguish at least 10,000 different smells, and interactions among volatiles are also present during smell perception. The brain processes information from our sense of taste and smell, and gives an integrated flavor experience where the relative importance of each input is difficult to determine since taste and aroma perceptions interact among themselves and can modify each other (Baldwin 2002; Delwiche 2004).

This complex relationship between flavor chemistry and flavor sensory perception explains why chemical variations in flavor components do not necessarily correlate well with sensory scores. Studies about environmental factors on flavor quality have limited value when sensory evaluations are not conducted in parallel with chemical/instrumental measurements of flavor components.

Fruit flavor becomes more complicated to understand when the effect of environmental factors is considered. The multitude of interacting environmental factors acting simultaneously on plant and fruit development makes it difficult, and sometimes impossible, to distinguish individual contributions of each factor on fruit flavor or to elucidate their integral effect on this important quality attribute (Coombe 1987; Schultz 2002). However, it is possible to recognize the main factors affecting fruit flavor for a given location and specific crop, and obtain good-flavored fruits by appropriately managing some of them. Therefore, it is critical that producers become aware of the multitude of environmental factors that can influence fruit flavor.

In order for growers to take advantage of the market opportunities of our global economy, they have to face the challenge of providing high-quality fruits that meet supermarket requirements and consumer's flavor expectations (Hewett 2006). According to Shewfelt and Henderson (2002), "in the near future more technological innovations to deliver more flavored products will be developed and more research emphasizing preharvest factors in postharvest quality as well as sustainable production to lessen undesirable impact to the environment will be conducted."

REFERENCES

Arpaia ML, Rooyen ZV, Bower JP, Hofman PJ, Woolf AB. 2004. Grower practices will influence postharvest fruit quality. 2° Seminario Internacional de Paltos. September 29–October 1, Sociedad Gardiazabal y Magdahl Ltda, Quillota, Chile.

Baldwin EA. 2002. Fruit and vegetable flavor. USDA/ARS, Citrus and Subtropical Products Laboratory, Winter Haven, FL. http://www.ba.ars.usda.gov/hb66/022flavor.pdf.

Baldwin EA, Nisperos-Carriedo MO, Baker R, Scott JW. 1991. Quantitative analysis of flavor parameters in six Florida tomato cultivars (*Lycopersicon esculentum* Mill). *J Agric Food Chem* 39:1135–40.

Baldwin EA, Scott JW, Shewmaker CK. 2000. Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience* 35(6):1013–21.

Behboudian MH, Dixon J, Pothamshelty K. 1998. Plant and fruit responses of lysimeter-grown "Braeburn" apple to deficit irrigation. *J Hortic Sci Biotechnol* 73:781–5.

Behboudian MH, Mills TM. 1997. Deficit irrigation in deciduous orchards. *Hortic Rev* 21:105–31.

Benbrook M. 2005. Elevating antioxidant levels in food through organic farming and food processing. The Organic Center for Education and Promotion and Organic Center. State of Science Review. http://www.organic-center.org/science.antiox.php?action=view&report_id3.

Boccorh RK, Paterson A, Piggott JR. 1999. Sources of variations in aroma-active volatiles, or flavour components, of blackcurrant concentrates. *Z Lebensm Unters Forsch* 208(5–6):362–8.

Bravdo BA. 2001. Effect of cultural practices and environmental factors on fruit and wine quality. *Agriculturae Conspectus Scientificus* 66(1):13–20.

Bruhn C. 2002. Consumer issues in quality and safety. In Kader AA (ed.), *Postharvest Technology of Horticultural Crops*. Oakland, CA: University of California, Division of Agricultural and Natural Resources, Spec. Publ. 3311, pp. 49–54.

Bussakorn SM, Mpelasoka, Behboudian H. 2002. Production of aroma volatiles in response to deficit irrigation and crop load in relation to fruit maturity for "Braeburn" apple. *Postharvest Biol Technol* 24:1–11.

Carbonell-Barrachina AA, Agustí A, Ruiz JJ. 2006. Analysis of flavor volatile compounds by dynamic headspace in traditional and hybrid cultivars of Spanish tomatoes. *Eur Food Res Technol* 222:536–42.

Coombe BG. 1987. Influence of temperature on composition and quality of grapes. *Acta Hortic* 206:23–36.

Crisosto CH, Johnson RS, DeJong T. 1997. Orchard factors affecting postharvest stone fruit quality. *HortScience* 32(5):820–3.

Crisosto CH, Mitchell JP. 2002. Preharvest factors affecting fruit and vegetable quality. In Kader AA (ed.), *Posharvest Technology of Horticultural Crops*. Oakland, CA: University of California, Division of Agriculture and Natural Resources, Spec. Publ. 3311, pp. 49–54.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol Plant Mol Biol* 42:55–76.

Delwiche J. 2004. The impact of perceptual interactions on perceived flavor. *Food Qual Prefer* 15:137–46.

Dharmadhikari M. 1994. Composition of grapes. *Vineyard and Vintage View*, Missouri State University, 9(7/8):3–8.

Dirinck PJ, De Pooter HL, Willaert GA, Schamp NM. 1981. Flavor quality of cultivated strawberries: The role of the sulfur compounds. *J Agric Food Chem* 29:316–21.

FAO. 2000. Food safety and quality as affected by organic farming. Twenty-Second FAO Regional Conference for Europe, Agenda Item 10.1. July 24–28, Porto, Portugal.

Fellman JK, Miller TW, Mattinson DS. 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. *HortScience* 35(6):1026–33.

Fellman JK, Rudell DR, Mattinson DS, Matteis JP. 2003. Relationship of harvest maturity to flavor regeneration after CA storage of "Delicious" apples. *Postharvest Biol Technol* 27:39–51.

Forney CF, Jordan MA, Nicolas KUKG, DeEll JR. 2000a. Volatile emissions and chlorophyll fluorescence as indicators of freezing injury in apple fruit. *HortScience* 35:1283–7.

Forney CF, Kalt W, Jordan MA. 2000b. The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *HortScience* 35:1022–6.

Forshey CG. 1986. Chemical fruit thinning of apples. *New York's Food and Life Sciences Bulletin No. 116*. New York State Agricultural Experiment Station, Geneva, Cornell University, Ithaca, NY.

Garriz PI, Colavita GM, Alvarez HL. 1998. Fruit and leaf spur growth and quality as influenced by low irradiance levels in pear. *Sci Hortic* 77:195–205.

Hancock JF. 1999. *Strawberries*. Wallingford, UK: CABI Publishing, p. 77.

Harding PL, Fisher DF. 1945. Seasonal changes in Florida grapefruit. US Department of Agriculture. Technical Bulletin 886.

Harding PL, Sunday MB. 1949. Seasonal changes in Florida tangerines. US Department of Agriculture. Technical Bulletin 988.

Harding PL, Sunday MB, Davis PL. 1959. Seasonal changes in Florida tangelos. US Department of Agriculture. Technical Bulletin 1205.

Hewett EW. 2006. An overview of preharvest factors influencing postharvest quality of horticultural products. *Int J Postharvest Technol Innov* 1(1):4–15.

Hofman PJ, Smith LG, Joyce DC, Johnson GI, Meiburg GF. 1997. Bagging of mango (Mangifera indica cv. "Keitt") fruit influences fruit quality and mineral composition. *Postharvest Biol Technol* 12:83–91.

Hui-Juan J, Aritomo A, Goro O. 2005. Influence of fruit bagging on aroma volatiles and skin coloration of "Hakuho" peach (*Prunus persica* Batsch). *Postharvest Biol Technol* 35:61–8.

Jackson DI, Lombard PB. 1993. Environmental and management practices affecting grape composition and wine quality. A Review. *Am J Enol Vitic* 44(4):409–30.

Kasperbauer MJ, Loughrin JH, Wang SY. 2001. Light reflected from red mulch to ripening strawberries affects aroma, sugar and organic acid concentrations. *Photochem Photobiol* 74(1):103–7.

Kimmerer TW, Kozlowski TT. 1982. Ethylene, ethane, acetaldehyde and ethanol production by plants under stress. *Plant Physiol* 69:840–7.

Klieber A, Muchui MN. 2002. Preference of banana flavor and aroma are not affected by time of the year or "winter" chilling. *Aust J Exp Agric* 42(2):201–5.

Lin D, Huang D, Wang S. 2004. Effects of potassium levels on fruit quality of muskmelon in soilless medium culture. *Sci Hortic* 102(1):53–60.

Lopez ML, Lavilla MT, Riba M, Vendrell M. 1998. Comparison of volatile compounds in two seasons in apples: Golden Delicious and Granny Smith. *J Food Qual* 21(2):155–66.

Marini RP, Sowers D, Marini MC. 1991. Peach fruit quality is affected by shade during final swell of fruit growth. *J Am Soc Hortic Sci* 116:383–9.

Mattheis JP, Fellman JK. 1999. Preharvest factors influencing flavor of fresh fruit and vegetables. *Postharvest Biol Technol* 15:227–32.

Maul E, Sargent SA, Sims CA, Baldwin EA, Balaban MO, Huber DH. 2000. Tomato flavor and aroma quality as affected by storage temperature. *J Food Sci* 65(7):1228–37.

McCollum TG, Chellemi DO, Rosskopf, EN, Church GT, Plotto A. 2005. Postharvest quality of tomatoes produced in organic and conventional production systems. *HortScience* 40(4):959.

Miller TW, Fellman JK, Mattheis JP, Mattinson DS. 1998. Factors that influence volatile ester biosynthesis in "Delicious" apples. *Acta Hortic* 464:195–200.

Miszczak A, Forney CF, Prange RK. 1995. Development of aroma volatiles and color during postharvest ripening of "Kent" strawberries. *J Am Soc Hortic Sci* 120:650–5.

Mitcham B. 1996. Quality assurance for strawberries: A case study. *Perishables Handling Newsletter* 85:6–7.

Modise DM, Wright CJ, Atherton JG. 2006. Changes in strawberry aroma in response to water stress. *Bost J Agric Appl Sci* 2(1). http://www.bca.bw/Research/bojaasBca/full2_1_13.htm.

Mpelasoka BS, Bhboudian MH, Dixon J, Neal SM, Caspari HW. 2000. Improvement of fruit quality and storage potential of "Braeburn" apple through deficit irrigation. *J Hortic Sci Biotechnol* 75(5):615–21.

Müller I. 2005. Relationships between preharvest soybean oil application and postharvest behaviour of apples. PhD dissertation, Washington State University Department of Horticulture and Landscape Architecture.

Nestby R. 1998. Effect of N-fertigation on fruit yield, leaf N and sugar content in fruits of two strawberry cultivars. *J Hortic Sci Biotechnol* 73:563–8.

Nestby R. 2002. Accumulation of nutrients in strawberry fruits and their effect on fruit quality. A brief review. Presented at joint meeting of WG 3 and 4 of Cost action 836. June 13–15, Lisboa.

Obenland DM, Aung LH, Bridges DI, Mackey BE. 2003. Volatile emissions of navel oranges as predictors of freeze damage. *J Agric Food Chem* 51:3367–71.

Oke M, TaeHyun A, Schofield A, Palivath G. 2005. Effects of phosphorous fertilizer supplementation on processing quality and functional food ingredients in tomato. *J Agric Food Chem* 53(5):1531–8.

Pantastico EB. 1975. Preharvest factors affecting quality and physiology after harvest. In Pantastico EB (ed.), *Postharvest Physiology, Handling and Utilization of Tropical and Subtropical Fruits and Vegetables*. Westport, CT: AVI Publishing, pp. 25–40.

Parker DD, Zilberman D, Moulton KS. 1991. How quality relates to price in California fresh peaches. *Calif Agric* 45(2):14–16.

Pattern KD, Proebsting EL. 1986. Effect of different artificial shading times and natural light intensities on the fruit quality of "Bing" sweet cherry. *J Am Soc Hortic Sci* 111:360–3.

Peck GM, Andrews PK, Reganold JP, Fellman JK. 2006. Apple orchard productivity and fruit quality under organic, conventional, and integrated management. *HortScience* 41(1):99–107.

Pelayo-Zaldívar C, Ebeler SE, Kader AA. 2005. Cultivar and harvest date effects on flavor and other quality attributes of California strawberries. *J Food Qual* 28:78–97.

Pérez AG, Sanz C, Olías R, Ríos JJ, Olías JM. 1997. Aroma quality evaluation of strawberry cultivars in Southern Spain. *Acta Hortic* 439:337–40.

Poll L, Rindom A, Toldam-Anderson TB, Hansen P. 1996. Availability of assimilates and formation of aroma compounds in apples as affected by the fruit/leaf ratio. *Physiol Plant* 97:223–7.

Pyysalo T, Honkanen E, Hirvi T. 1979. Volatiles of wild strawberries, *Fragaria vesca* L., compared to those of cultivated strawberries, *Fragaria x ananassa* cv. Senga Sengana. *J Agric Food Chem* 27:19–22.

Reganold JP, Glover JD, Andrews PK, Hinman HR. 2001. Sustainability of three apple production systems. *Nature* 410(6831):926–30.

Reuther W. 1973. Climate and citrus behavior. In Reuther W (ed.), *The Citrus Industry. Vol. III. Production Technology*. Oakland, CA: University of California, Division of Agricultural Sciences, pp. 280–337.

Reynolds AG, Wesley D, Lowrey, De Savigny C. 2005. Influence of irrigation and fertigation on fruit composition, vine performance, and water relations of Concord and Niagara grapevines. *Am J Enol Vitic* 56(2):110–28.

Róth E, Berna A, Beullens K, Lammertyn J, Schenk A, Nicolaï B. 2006. The postharvest quality of integrated and organically produced apple fruit. First International Organic Apple and Pear Symposium. Wolfville, Nova Scotia, Canada.

Ruiz JJ, Alonso A, García-Martínez S, Valero M., Blasco P, Ruiz-Bevia F. 2005. Quantitative analysis of flavour volatiles detects differences among closely related traditional cultivars of tomato. *J Sci Food Agric* 85:54–60.

Sala C, Busto O, Guascha J, Zamora F. 2004. Factors affecting the presence of 3-alkyl-2-methoxypyrazines in grapes and wines. A review. http://www.tesisenxarxa.net/TESIS_URV/AVAILABLE/TDX-0626106-110827//15-PaperC2.pdf

Sanz C, Perez AG, Olias R. 2002. Effects of temperature on flavor components in strawberries. *Acta Hortic* 567:365–8.

Schultz HR. 2002. Vineyard management, fruit ripening and flavour development. Proceedings of the 31st New York Wine Industry Workshop, April 3–5, Geneva, NY, pp. 86–89.

Shaw DV. 1988. Genotypic variation and genotypic correlations for sugars and organic acids of strawberries. *J Am Soc Hortic Sci* 113:770–4.

Shaw DV. 1990. Response to selection and associated changes in genetic variance for soluble solids and titratable acids contents in strawberries. *J Am Soc Hortic Sci* 115:839–43.

Shewfelt RL, Henderson JD. 2002. The future of quality. *Acta Hortic* 604:49–59.

Song J, Fan L, Forney CF, Jordan MA. 2001. Using volatile emission and chlorophyll fluorescence as indicators of heat injury in apples. *J Am Soc Hortic Sci* 126:771–7.

Stevens MA, Kader AA, Albright-Holton M, Algazi M. 1977. Genotypic variation for flavor and composition in fresh market tomatoes. *J Am Soc Hortic Sci* 102:680–9.

Stoll M. 2000. Effects of partial rootzone drying on grape vine physiology and fruit quality. PhD dissertation, Department of Horticulture, Viticulture and Oenology. Faculty of Agricultural and Natural Resources Sciences, University of Adelaide.

Tandon KS, Baldwin EA, Scott JW, Shewfelt RL. 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. *J Food Sci* 68(7):2366–71.

Theuer RC. 2006. Do organic fruits and vegetables taste better than conventional fruits and vegetables? Highlights of a State of Science Review: Taste of Organic Food. The Organic Center. http://www.organicagcentre.ca/Docs/OrganicCenter_Taste06.pdf.

Ulrich D, Hoberg E, Rapp A, Kecke S. 1997. Analysis of strawberry flavor. Discrimination of aroma types by quantification of volatile compounds. *Z Lebensm Unters Forsch A Food Res Technol* 205:218–23.

van Hooijdonk BM, Dorji K, Behboudian MH. 2007. Fruit quality of "Pacific Rose" TM apple grown under partial rootzone drying and deficit irrigation. *J Food Agric Environ* 5(3-4):173–8.

Veit-Köhler U, Krumbein A, Kosegarten H. 1999. Effect of different water supply on plant growth and fruit quality of *Lycopersicon esculentum*. *J Plant Nutr Soil Sci* 162(6):583–8.

Wang SY, Bunce JA. 2004. Elevated carbon dioxide affects fruit flavor in field-grown strawberries (*Fragaria* × *ananassa* Duch). *J Sci Food Agric* 84(12):1464–8.

Watson R, Wright CJ, McBurney T, Taylor AJ, Linforth RST. 2002. Influence of harvest date and light integral on the development of strawberry flavour compounds. *J Exp Bot* 53(377):2121–9.

Westwood N. 1978. *Temperate Zone Pomology*. San Francisco, CA: WH Freeman, pp. 203–8 and 237–43.

Wright DH, Harris ND. 1985. Effect of nitrogen and potassium fertilization on tomato flavor. *J Agric Food Chem* 33:355–8.

Yahia EM. 1994. Apple flavor. *Hortic Rev* 16:255–382.

Yang X, Peppard T. 1994. Solid phase microextraction for flavor analysis. *J Agric Food Chem* 42:1925–30.

Yu-Tao W, Shao-Wen H, Rong-Le L, Ji-Yun J. 2007. Effects of nitrogen application on flavor compounds of cherry tomato fruits. *J Plant Nutr Soil Sci* 170(4):461–8.

PART II

FRUIT FLAVORS: BIOTECHNOLOGY

CHAPTER 6

Cell Culture for Flavor Production

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INTRODUCTION

The definition of natural flavors comprises products that are converted by living cells or parts thereof, including enzymes. There is a supplementary regulation established by the Food and Drug Administration (FDA), in which compounds that are known originally as generally recognized as safe (GRAS) should still be classified as GRAS when produced by microbiological or enzymatic processes.

European legislation considers biotechnological aromas as natural substances, on the sole condition that the precursors involved in their synthesis are natural (Sarhy-Bagnon et al. 1997). In the United States, the Code of Federal Regulation (CFR) defines a natural aroma as "the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating and enzymolysis, which contains the flavoring constituents derived from a spice, juice, vegetable or vegetable juice, edible yeast, herb, bud, bark, root, leaf or similar plant material, meat, seafood, poultry, eggs dairy products or fermentation products thereof, whose significant function is food flavoring rather than nutrition (CFR 101.22.0.3)" (Armstrong et al. 1989).

Fragrance and aroma have found widespread application in food, feed, cosmetic, chemical, and pharmaceutical industries, with a worldwide industrial size estimated at US$16 billion in 2003 (Serra et al. 2005). According to the Freedonia Group (*World Flavors and Fragrances to 2008*, 2005), the global demand for flavors and fragrances has increased 4.4% per year to US$18.6 billion in 2008, reaching 7.3% in the Asia/Pacific region (excluding Japan). Demand for fragrance blends and essential oils will benefit from increased interest in natural and exotic aromas, which are more expensive than their synthetic counterparts. Among the major market segments for flavors and fragrances, food and beverages is the largest, accounting for 47% of total demand in 2003.

The potential of biotechnological processes for the production of natural flavor compounds is considered in this chapter, with an emphasis on plant cell and microbial cultures.

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Microbial Flavor Production

Fermentation is a promising biotechnological technique for the production of natural flavors. Attempts are being made for industries to produce natural aroma compounds by fermentation, which allows the recuperation of natural food additives that are preferred by the consumer. Volatile compound production by microorganisms is an easily manageable process, from both a qualitative and quantitative point of view.

Most biotechnological processes that have been reported have not yet been applied in the industrial production of flavors and fragrances. The major reason for this is the low yield. The microbial flavors are often present in low concentrations in fermentation broths, resulting in high costs for downstream processing. Nevertheless, this fact is counterbalanced by the price of the naturally produced compounds, which is 10–100 times higher than that of synthetic ones. For example, synthetic γ-decalactone, the impact flavor compound of peach, costs US$150 for a kilogram (kg), while the same substance extracted from a natural source costs about US$6000/kg. The microbial production of γ-decalatone (peach aroma), involving the bioconversion by *Yarrowia lipolytica* of castor oil yields about 6 g/L (Janssens et al. 1992). The γ-decalactone has its price decreased from US$20,000/kg in the early 1980s down to US$1200/kg in 1995 because of a move to microbial production (Feron et al. 1996). Another example of fermentation process is the production of vanillin, which is widely used as a flavoring agent in a wide range of foods and fragrances. A biotechnological method involving fungi to produce vanillin was employed using ferulic acid as a direct precursor. This bioconversion is a product of the microbial oxidation of lignin, particularly by white-rot basidiomycetes (Lomascolo et al. 1999).

Many microorganisms are capable of synthesizing flavor compounds when grown on a culture media. They have the ability to perform conversions, which would require multiple chemical 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. In this way, biotechnological processes involved in the production of aromatic compounds can be divided in two groups: microbiological and enzymatic processes.

It is important to distinguish the research whose objectives are to obtain complex products with natural characteristics from that which tries to obtain isolated molecules. The first tries to imitate nature and develops a process with one or more microorganisms and enzymes. The second tries to obtain a higher yield of characteristic components. The choice between them determines the methodology, which will be employed *in vivo* or *in vitro* using biosynthesis or bioconversions.

The aim of current biotechnological 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 (SSF), immobilized cells, or genetically modified organisms.

Genetic engineering organisms have improved the yield of desired products by overexpressing genes of interest in microbial systems. Expression of the genes for alcohol acetyltransferases (AATases) in microorganisms for industrial use is

one of many recent advances in the area of flavor compound production in microorganisms. According to Horton and Bennett (2006), the expression of genes for AATases in *Escherichia coli* and *Clostridium acetobutylicum* was a feasible option for the natural production of fruity esters such as butyl acetate, ethyl acetate, and butyl butyrate. *E. coli* cultures were grown aerobically in the nutrient-rich Luria–Bertani (LB) broth at 37°C in a shaker or anaerobically in LB without shaking in airtight sealed 10-mL vials. *C. acetobutylicum* cultures were grown anaerobically at 37°C in the nutrient-rich clostridial growth media.

Vadali and others (2004) used a genetically engineered *E. coli* to produce isoamyl acetate, adding isoamyl alcohol externally to the cell culture medium. The cultures were grown in an orbital shaker under aerobic and anaerobic conditions. At the end of 24 h, the culture flasks were analyzed for isoamyl acetate production using headspace gas analysis. In this study, the authors demonstrated that strategies such as pathway and cofactor manipulations could be implemented to enhance the isoamyl acetate production. Coenzyme A (CoA) and its thioester derivatives are important precursor molecules for many industrially useful compounds such as esters, polyhydroxybutyrates (PHBs), lycopene, and polyketides. The intracellular levels of CoA and acetyl-coenzyme A (acetyl-CoA) could increase the productivity of useful compounds derived from acetyl-CoA.

The development of novel and cheap production processes, such as SSF, may help overcome some of the current limitations of microbial flavor production, as well as widening the spectrum of biotechnologically accessible compounds (Soccol and Vandenberghe 2003).

Bagasse and desiccated coconut were used as solids substrates for the production of 6-pentyl-α-pyrone (6-PP), an unsaturated δ-lactone with a strong odor of coconut, by *Trichoderma harzanium* (Sarhy-Bagnon et al. 1997).

Cassava and sugarcane bagasse, apple pomace, giant palm bran, and coffee husk have been used as substrates for flavor production in SSF (Soccol and Vandenberghe 2003). Fruity flavors were detected in cultures of *Ceratocystis fimbriata* using coffee husk as substrate (Medeiros et al. 2006; Soares et al. 2000). The authors 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. Among the compounds produced, ethanol and ethyl acetate were the most abundant.

Bramorski and others (1998) studied the production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agroindustrial substrates. When *R. oryzae* was grown on a medium containing cassava bagasse plus soybean meal (5:5 w/w), CO_2 production rate was at its highest (200 mL/L), whereas the highest volatile metabolite production was with amaranth grain as the sole substrate (282.8 mL/L). In the headspace, ethanol was the most abundant compound (more than 80%). Acetaldehyde, 1-propanol, ethyl propionate, and 3-methyl butanol were also present. CO_2 and volatile metabolite productions reached their maximum around 20 and 36 h, respectively.

A strain of the yeast *Kluyveromyces marxianus* was used for the production of a fruity aroma in SSF using cassava bagasse as substrate (Medeiros et al. 2000). Experiments were performed with a statistical experimental design. The parameters

studied were cultivation temperature, pH, initial water content, carbon/nitrogen (C/N) ratio of substrate, and inoculum size. The initial pH and the C/N ratio of the medium were statistically significant at 5% level for the production of volatile compounds. Aroma production increased in acidic pH (3.5) medium with a C/N ratio of 100. The results showed the feasibility of using cassava bagasse as substrate to produce a fruity aroma with *K. marxianus* in SSF.

Plant Cell Cultures

The production of aroma compounds by adapted plant cell cultures started in the 1970s; these compounds are characteristic to their plant origin. Plant aroma can be considered as terminal metabolites containing abundant biochemical information; it differs according to metabolism stages. It is independent of climate, geographic location, or political situations, and the recuperation of compounds is also relatively easy. Many differences occur when comparing plant and microbial cells for the production of aroma compounds: plant cells are more fragile and susceptible to shear than microbial cells; duplication time and product accumulation is much faster for microbial cells; production costs are over 10-fold for cell cultures. However, efforts have been made in order to stimulate synthetic activities by selecting strains presenting high productivity, optimizing culture conditions, and also the addition of precursors as well as cell immobilization that may prolong culture viability (Hrazdina 2006).

There are a number of plant cell cultures producing a higher amount of secondary metabolites than in intact plants. However, there are still problems in the production of metabolites by cell cultures resulting from the instability of cell lines, low yields, slow growth, and scale-up problems (Rao and Ravishankar 2002).

Plant cell cultures have an ability to transform exogenous substrates, such as industrial by-products, into products of interest. The bioconversion rates depend on a variety of factors including the solubility of precursors, the amount of enzyme activity present, presence of side reaction, and presence of enzymes degrading the desired product. *Peganum harmala* cell culture converted geranyl acetate to geraniol and linalyl acetate to linalool and α-terpeniol. Immobilized cell cultures of *Capsicum frutescens* produce vanillin and capsaicin when ferulic acid and vanillylamine as precursors were added to the medium. *Coffea arabica* cell culture produces vanillin-β-D-glucoside from vanillin as precursor (Giri et al. 2001).

Several plants showing their characteristic aroma are also good candidates for cell culture. Vanilla is the most used flavor ingredient being the major component cultured in *Vanilla planifolia*, followed by *p*-hydroxybenzaldehyde, vanillic acid, and *p*-hydroxybenzyl methyl ether. Strawberry aroma is very complex; it is constituted by 278 volatile substances, among them are 33 acids, 39 alcohols, 17 aldehydes, 14 ketones, and 103 esters. Tissue culture for strawberry aroma was only performed for component groups like esters. Other flavors produced by plant cell cultures include apple, cinnamic acid, caryophyllen (from *Lindera strychnifolia*), cocoa, garlic, and onion (Longo and Sanromán 2006). According to the authors, the efficiencies of plant cell cultures can further be improved using molecular techniques involving site-directed mutagenesis and gene manipulation for substrate specificity.

CONCLUSION

Aroma biotechnology is increasing rapidly and is becoming a competitive factor in the growing market of natural products. Microorganisms such as yeast and fungi present several opportunities in biosynthesis and biotransformation processes. New perspectives in microbial aroma production can be found with the elucidation of the metabolic pathways, with immobilized cultures, and with genetically modified organisms. Also, as downstream processing is the major drawback in microbial production, future research in this area will open new avenues for technology transfer. Among technologies, SSF is a good choice as it gives a more concentrated product. Although plant cell cultures appear as a viable method to produce flavors, the technology for large-scale production needs to be improved.

REFERENCES

Armstrong DW, Gillies B, Yamazaki H. 1989. Natural flavors produced by biotechnological processing. In Teranishi R, Buttery RG, Shahidi F. (eds.), *Flavour Chemistry: Trends and Developments*, ACS 388. Washington, DC: American Chemical Society, pp. 105–19.

Bramorski A, Christen P, Ramirez M, Soccol CR, Revah S. 1998. Production of volatile compounds by the fungus *Rhizopus oryzae* during solid state cultivation on tropical agro-industrial substrates. *Biotechnol Lett* 20:359–62.

Feron G, Bonnarme P, Durand A. 1996. Prospects for the microbial production of food flavours. *Trends Food Sci Technol* 7:285–93.

Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML. 2001. Biotransformations using plant cells, organ cultures and enzyme systems: Current trends and future prospects. *Biotechnol Adv* 19:175–99.

Horton CE, Bennett GN. 2006. Ester production in *E. coli* and *C. acetobutylicum*. *Enzyme Microb Technol* 38:937–43.

Hrazdina G. 2006. Aroma production by tissue cultures. *J Agric Food Chem* 54:1116–23.

Janssens L, De Poorter HL, Vandamme EJ, Schamp NM. 1992. Production of flavours by microorganisms. *Process Biochem* 27:195–215.

Lomascolo A, Stentelaire C, Asther M, Lesage-Meessen L. 1999. Basidiomycetes as new biotechnological tools to generate natural aromatic flavours for the food industry. *Trends Biotechnol* 17(7):282–9.

Longo MA, Sanromán MA. 2006. Production of food aroma compounds: Microbial and enzymatic methodologies. *Food Technol Biotechnol* 44(3):335–53.

Medeiros ABP, Pandey A, Freitas RJS, Christen P, Soccol CR. 2000. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. *Biochem Eng J* 6:33–9.

Medeiros ABP, Pandey A, Vandenberghe LPS, Pastore GM, Soccol CR. 2006. Production and recovery of aroma compounds produced by solid-state fermentation using different adsorbents. *Food Technol Biotechnol* 44(1):47–51.

Rao SR, Ravishankar GA. 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol Adv* 20:101–53.

Sarhy-Bagnon V, Lozano P, Pioch D, Roussos S. 1997. Coconut-like aroma production by *Trichoderma harzanium* in solid state fermentation. In Roussos S, Lonsane BK, Raimbault M, Viniegra-Gonzales G. (ed.), *Advances in Solid State Fermentation*. Dordrecht: Kluwer

Academic Publishers, pp. 379–91. International symposium on solid state fermentation FMS-95 (Montpellier, France).

Serra S, Fuganti C, Brenna E. 2005. Biocatalytic preparation of natural flavours and fragrances. *Trends Biotechnol* 23:193–8.

Soares M, Christen P, Pandey A, Soccol CR. 2000. Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid-state fermentation. *Process Biochem* 35:857–61.

Soccol CR, Vandenberghe LPS. 2003. Overview of applied solid-state fermentation in Brazil. *Biochem Eng J* 13:205–18.

Vadali RV, Bennett GN, San KY. 2004. Applicability of CoA/acetyl-CoA manipulation system to enhance isoamyl acetate production in *Escherichia coli*. *Metab Eng* 6:294–9.

World Flavors and Fragrances to 2008. 2005. http://www.freedoniagroup.com/DocumentDetails.aspx?DocumentId=46639&ReferrerId=FR-STAF.

CHAPTER 7

Genetic Engineering of Fruit Flavors

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INTRODUCTION

The flavors and aromas of fruits are determined by complex mixtures of often hundreds of volatile compounds. For example, more than 300 compounds contribute in different levels to the characteristic flavor associated with ripe strawberry fruit (Honkanen and Hirvi 1990) and more than 400 volatiles contribute to the characteristic aroma of tomatoes and their products (Petro-Turza 1987). These compounds include metabolites of different chemical groups that include acids, aldehydes, ketones, alcohols, esters, sulfur compounds, furans, phenols, terpenes, epoxides, and lactones and are derived from different biosynthetic pathways (Schwab et al. 2008). Their concentration often varies among different fruit tissues and represents 10–100 ppm or less of the fruit fresh weight. Flavor compounds are generally formed during ripening when fruit metabolism dramatically changes and catabolism of high-molecular-weight molecules such as proteins, polysaccharides, and lipids degrade and are converted to volatile metabolites.

Early research on fruit flavors was focused on identifying components present in different fruit species (Honkanen and Hirvi 1990). The classification of flavor compounds was often accompanied by the identification of the most important substances that convey the characteristic odor unique to a particular fruit (e.g., isoamylacetate for banana and methoxyfuraneol for strawberry). Later, researchers began investigating their biogenesis and the effect that processing and storage imposed on them. In recent years, additional information on fruit flavors and their biosynthesis was obtained by using molecular and biochemical approaches. Genes that directly influence fruit flavor formation have been identified for the main metabolic pathways mainly in tomato, strawberry, and melon fruit. The availability of information on genes and enzymes associated with pathways of flavor formation are a prerequisite for genetic engineering. The authors therefore compiled in this chapter information on key enzymes and genes that affect flavor that have a great potential in attempts of fruit flavor engineering.

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Albeit limited, the information gathered up to date regarding genes and metabolic pathways that generate fruit flavors is crucial for the future manipulation of flavors. Metabolic engineering experiments carried out through the use of genes isolated from non-fruit organs/tissues, as for example leaves and glandular trichomes of herbs, is also important for increasing our capabilities to engineer fruit flavors. While bioengineering of fruit flavor seems to be of minor importance in the efforts for crop improvement, if successful, it might have a large commercial impact. A common, widespread, and often justified consumer dissatisfaction with the flavors of modern commercially available fruit as compared with memories from the past, or garden and heirloom cultivars with limited availability, calls for new research and breeding efforts to introduce the "original flavors" to agricultural produce. At this stage, it is clear that through modern biotechnological tools, we can influence and redirect the biosynthesis of fruit flavor compounds (Davidovich-Rikanati et al. 2007). It is still not clear at this stage how these manipulations will positively influence the public opinion and product acceptability. It is important to note here that genetic engineering of fruit flavor is not restricted to the introduction of new flavors or enhancing existing ones but also includes the removal of undesirable metabolites that generate "off-flavors." Since most of the molecules that compose the flavor profiles of fruit may exhibit antifungal or antibacterial bioactivity, it is conceivable that manipulation of fruit flavor will not only influence the flavor profile of fruit but will also confer resistance to pests and pathogens. In the first part of this chapter, the different attempts to engineer flavors in the model fruit tomato will be described. The second part of this chapter will deal with attempts to engineer flavors in strawberry fruit, while the third part will describe the use of flavor genes isolated from fruit in bioengineering flavor compounds in vegetative tissues of model plant species such as *Arabidopsis* and tobacco.

ENGINEERING FLAVORS IN THE MODEL FRUIT TOMATO

Most attempts carried up to date to manipulate the flavor of fruit were carried out in tomato, partly because tomato is a plant that is easily transformed and carries a big economical importance since it is widely consumed both fresh and processed (Davidovich Rikanati et al. 2008a). Despite the great advances in tomato flavor analysis, breeders and molecular biologists still lack a clear genetic target for selection and manipulation of tomato flavor quality (Galili et al. 2002; Tieman et al. 2006b). These efforts focused on overexpression or downregulation of key enzymes involved in three major routes leading to flavor compounds formation including (1) fatty acid catabolism through the lipoxygenase pathway, (2) the phenylpropanoid pathway, and (3) the isoprenoid pathway. The flavor-associated genes characterized and used for these studies and the outcome of these experiments are described below.

Fatty Acid Catabolism through the Lipoxygenase Pathway

The C6- and C9-aldehydes and alcohols, produced through the fatty acid-derived lipoxygenase pathway, provide "fresh green" odors in numerous fruits. The tomato alcohol dehydrogenase (*ADH2*) that catalyzes the conversion of aldehydes to alcohols was associated with fruit flavor biogenesis (Bicsak et al. 1982; Longhurst et al.

1990). In the same pathway, a tomato lipoxygenase gene (*TomloxC*) was recently characterized and associated with the formation of tomato flavor compounds (Chen et al. 2004).

Manipulation of the fatty acid catabolism pathway was attempted in a few points of the pathway. Expression of a yeast Δ9-desaturase gene driven by the CaMV 35S promoter in tomato led to changes in the levels of unsaturated as well as saturated fatty acids in tomato leaves and fruit (Wang et al. 1996, 2001). Increases in palmitoleic acid, 9,12-hexadienoic acid, and linoleic acid were observed along with a reduction in palmitic acid and stearic acid. Changes in the profile of fatty acids were associated with a significant increase in flavor compounds derived from fatty acids, most notably *cis*-3-hexenol, 1-hexanol, hexanal, and *cis*-3-hexenal. Certain flavor compounds not derived from fatty acids, viz. 6-methyl-5-hepten-2-one and 2-isobutylthiazole, also showed increases in transgenic fruits. These results show that changes in the levels of fatty acids in a plant could lead to changes in its profile of flavor compounds. However, the effect of these alterations in tomato volatiles on the flavor of the fruit was not evaluated.

The tomato gene *TomloxC* (a lipoxygenase) is involved in the generation of volatile C6-aldehyde and alcohol flavor compounds (Chen et al. 2004). Tomatoes with reduced expression level of *TomloxC* mRNA possessed a marked reduction in the levels of known flavor volatiles, including hexanal, hexenal, and hexenol, to as little as 1.5% of those of wild-type controls following maceration of ripening fruit. Addition of linoleic or linolenic acid to fruit homogenates significantly increased the levels of flavor volatiles, but the increase with the *TomloxC*-depleted transgenic fruit extracts was much lower than with the wild-type controls. These results demonstrated that the *TomloxC* lipoxygenase isoform has a key role in the generation of the volatile C6 flavor compounds.

Overexpression of a tomato alcohol-dehydrogenase gene in a fruit-specific manner altered the ratio of short-chain aldehydes to alcohols (Prestage et al. 1999; Speirs et al. 1998). This manipulation resulted in small changes to the fruit volatile profiles that were found to affect the perception of their aroma by a taste panel.

Engineering Flavors Derived from the Phenylpropanoid Pathway

Phenylpropanoid pathway derivatives are also major contributors to the flavors of fruit. The volatile compounds 2-phenyacetaldehyde and 2-phenylethanol are important for the flavor of ripe tomato, strawberry, and grape (Aubert et al. 2005). Recently, genes involved in their biogenesis have been characterized from tomato (Tieman et al. 2006a, 2007). The proteins encoded by a small family of tomato decarboxylases (*LeAADC1A*, *LeAADC1B*, *LeAADC2*) catalyze the first committed step in the pathway in which phenylalanine is directly decarboxylated to phenylethylamine. Tieman and others (2007) reported on the characterization of LePAR1 and LePAR2 that catalyze the conversion of 2-phenylacetaldehyde to 2-phenylethanol. Both compounds are pleasant at low concentrations, whereas 2-phenylacetaldehyde displays an unpleasant nauseating note at high concentrations (Tadmor et al. 2002). Indeed, the tomato wild relative *Solanum pennelli* contains extremely high levels of 2-phenylacetaldehyde and 2-phenylethanol, and this trait (*malodorous*) was probably lost during tomato domestication (Tadmor et al. 2002). While LePAR1, a member of the short-chain dehydrogenase/reductase family, exhibits higher activity

with 2-phenylacetaldehyde (compared with shorter- or longer-chain derivatives), LePAR2 shows similar activity with substrates of varying type and chain length (e.g., benzaldehyde and cinnamaladehyde). Tieman and others (2007) also found that the LePAR1 reaction is not reversible.

Genetic engineering of the phenylalanine catabolism pathway confirmed the biosynthetic route leading to the production of some phenylalanine volatile derivatives (Tieman et al. 2006a). Overexpression of either *Le*AADC1A or *Le*AADC2 resulted in fruits with up to 10-fold increased emissions of the products of the pathway, including 2-phenylacetaldehyde, 2-phenylethanol, and 1-nitro-2-phenylethane (Tadmor et al. 2002). Further, antisense reduction of *Le*AADC2 significantly reduced emissions of these volatiles. These results show that it is possible to change phenylalanine-based flavor and aroma volatiles in tomatoes by manipulating the expression of a single gene. However, in both cases, the effect of these alterations in the tomato volatiles on the flavor of the fruit was not evaluated.

Engineering Flavors Derived from the Isoprenoid (Terpenoid) Pathway

Isoprenoid pathway derivatives (terpenoids), for example, mono- and sesquiterpenes (C_{10} and C_{15}, respectively), are very often part of fruit flavor profiles (Davidovich-Rikanati et al. 2008a). Their carbohydrate precursors are utilized by two parallel pathways in the plastids and the cytosol to generate a myriad of terpenoid compounds with very different flavors. Numerous members of the terpene synthase gene family that catalyze the formation of mono- and sesquiterpenes from their prenyl diphosphate precursors (geranyl diphosphate [GDP] and farnesyl diphosphate [FDP] for mono- and sesquiterpene, respectively) have been characterized from multiple plant species, including fruit. Citrus fruit species are especially known for the production of volatile terpenoids, mainly mono- and sesquiterpenes, *R*-limonene being the most known as it normally accounts for approximately 90% of their essential oils. Apart from the characterization of limonene synthase genes from *Citrus limon* (El Tamer et al. 2003a; Lücker et al. 2002), quite a few more terpene synthases were isolated from various citrus species. These included a γ-terpinene synthase from *Citrus unshui* (Satsuma mandarin; Suzuki et al. 2004), (*E*)-beta farnesene synthase from *Citrus junos* (Maruyama et al. 2001), and β-pinene and γ-terpinene synthases from *C. limon* (Lücker et al. 2002). Sharon-Asa and others (2003) described the characterization of a sesquiterpene synthase that catalyzes the formation of the important orange flavor compound valencene. Several more terpene synthases were isolated from grapes including valencene and germacrene D synthases by Lücker and others (2004a) and α-terpineol synthase by Martin and Bohlmann (2004). An (*E*,*E*)-α-farnesene synthase was isolated from apple fruit peel by Pechous and Whitaker (2004). Terpene synthases from both the cultivated and wild strawberry were suggested to be responsible for the generation of multiple monoterpenes such as α-pinene and linalool and the sesquiterpene nerolidol (Aharoni et al. 2004). While many mono- and sesquiterpenes are direct products of terpene synthases, many others are formed through transformation of the initial products by, for example, oxidation, glycosylation, and acylation (Schwab et al. 2008). Aharoni and others (2004) demonstrated that a strawberry gene encoding a cytochrome P450 enzyme is capable of hydroxylating the primary monoterpene

α-pinene to myrtenol, which is highly abundant in the flavor profile of the wild strawberry species. Since monoterpenes are important aroma constituents in many plant species, many studies have yielded genes that direct their formation (Tholl 2006). Monoterpenes are also important contributors to many floral and fruit fragrances. These volatiles are synthesized from GDP, which is also an intermediate in the pathway leading to the biosynthesis of carotenoids. Therefore, this pathway is highly active in ripening tomato fruits, leading to the production of lycopene. The first attempt to genetically manipulate the tomato terpenoid pathway to produce monoterpenes was the ectopic expression of the *Clarkia breweri* linalool synthase (*LIS*) gene under the control of the tomato late ripening E8 promoter (Lewinsohn et al. 2001). The expression of *LIS* in tomatoes led to the production and accumulation of detectable levels of (*S*)-linalool and 8-hydroxy linalool in ripening fruits, without noticeably affecting the accumulation of fruit carotenoids. These results indicated for the first time that it is possible to increase the levels of monoterpenes in tomatoes. However, the effect of this addition to the tomato aroma perception by consumers remained to be tested.

In a following work, the tomato terpenoid pathway was modified by the ectopic expression of the geraniol synthase (*GES*) gene isolated from lemon basil (*Ocimum basilicum*) (Iijima et al. 2004) under the control of the fruit-ripening-specific tomato polygalacturonase (PG) promoter (Davidovich-Rikanati et al. 2007) (Fig. 7.1). The volatile profiles of transgenic ripe tomato fruit showed high concentrations (5–1800 ng/g fresh weight [FW]) of more than 10 new monoterpene compounds that did not appear or appeared in minute levels in the volatile profile of the control fruits. These compounds included the important aroma volatiles geraniol (possessing a strong rose scent) as well as the geraniol derivatives, citral (geranial and neral, lemon scented), citronellol (rose scented), geranic acid, and neric acid. A taste trial was conducted to study the effect of these added volatiles on the taste and aroma of the fruit. Indeed, taste panelists reported marked differences in the overall aroma and taste of the transgenic tomato fruits (Davidovich-Rikanati et al. 2007). The aroma of the transgenic fruits was often described to have an added "flowery" or "lemony" note.

Recently, another gene isolated from lemon basil, α-zingiberene synthase (*ZIS*), was used to genetically engineer tomato aroma. This gene is a member of the *Tps* family and encodes a gene that encodes an enzyme that catalyzes the formation of α-zingiberene and other sesquiterpenes from FDP, as well as monoterpenes such as α-thujene, α-pinene, β-phellandrene, and γ-terpinene from GDP. Transgenic tomato fruits overexpressing *ZIS* under the control of the fruit-ripening-specific tomato PG accumulated high levels of α-zingiberene and other sesquiterpenes, such as α-bergamotene, 7-*epi*-sesquithujene, β-bisabolene, and β-curcumene, while no sesquiterpenes were detected in nontransformed control fruits. The *ZIS*-transgenic fruits also produced monoterpenes, such as α-thujene, α-pinene, β-phellandrene, and γ-terpinene, that were not detected or were found only in minute concentrations in control fruits (Davidovich-Rikanati et al. 2008b). Since the ZIS protein apparently lacks a transit peptide and is localized in the cytosol, the production of monoterpenes in the transgenic tomatoes suggests that a pool of GDP is available in the cytosol. The phenotype of the *ZIS*-transgenic tomatoes was the same as that for wild-type tomatoes with regard to plant vigor and shape, but transgenic plants exhibited a small decrease in lycopene content.

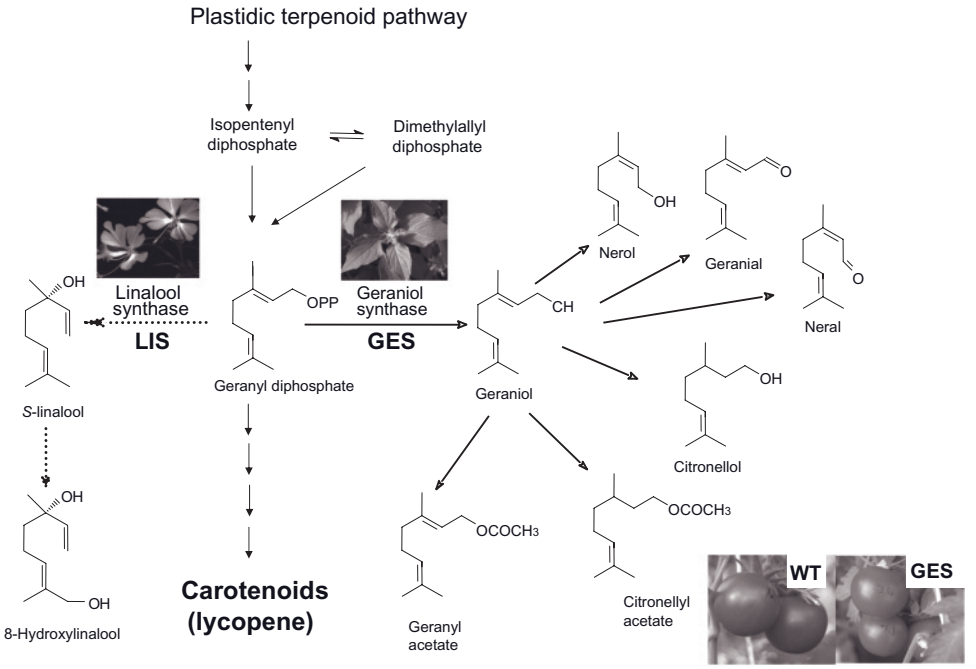

Figure 7.1. Redirection of the plastidial terpenoid pathway in tomato fruit to the production of aroma compounds. The *Clarkia breweri* (*S*)-linalool synthase gene was ectopically expressed in tomato fruit under the direction of the *E8* promoter (right side, broken lines). This resulted in the accumulation of (*S*)-linalool and 8-hydroxy linalool (Lewinsohn et al. 2001) but carried minimal effects on fruit aroma. In subsequent experiments, the *Ocimum basilicum* geraniol synthase gene was ectopically expressed in tomato fruit using the *PG* promoter. This manipulation resulted in the accumulation of geraniol and more than 10 novel volatiles, and a profound change in flavor, as determined by untrained taste panelists (Davidovich-Rikanati et al. 2007).

The tetraterpenoids carotenoids, a different class of isoprenoids, are important plant pigments but also serve as precursors for the formation of apocarotenoids volatiles such as β-ionone that are very potent and important aroma compounds. The cleavage of carotenoids to apocarotenoids at specific sites was shown to be carried out by carotenoid cleavage dioxygenases (CCDs; Schmidt et al. 2006). In tomato, the synthesis of β-ionone, geranyl acetone, and 6-methyl-5-heptene-2-one increases during fruit ripening, and this is mediated by the *LeCCD1* and *LeCCD2* genes (Simkin et al. 2004). Similar genes isolated from grapes and melon were suggested to catalyze the formation of partially similar but also different types of apocarotenoids that contribute to the flavor and aroma of the fruit (Ibdah et al. 2006; Mathieu et al. 2005).

The role of CCDs in the production of carotenoid-derived volatiles of tomato has been demonstrated by the downregulation of *LeCCD1B* gene (Simkin et al. 2004). Transgenic tomatoes exhibited 50% decrease in β-ionone (a β-carotene-derived C13 cyclohexone) and 60% decrease in geranylacetone, a C13 acyclic product likely derived from a lycopene precursor. However, the opposite effect of

the overexpression of CCDs in tomato is still absent. The effect of these alterations in tomato volatiles on the flavor of the fruit was not evaluated.

Concluding, genetic engineering has led to the manipulation of the main biosynthetic pathways that affect tomato aroma, not only confirming the biosynthetic routes but also affording novel flavors in tomato fruit (Davidovich-Rikanati et al. 2008a). With the advent of additional flavor affecting genes, it will be possible to generate tomatoes with varied and enhanced flavors. Still, it is for consumers to decide whether such innovative tomatoes will be accepted.

ENGINEERING THE FLAVOR OF STRAWBERRY FRUIT

Strawberry fruits are well-known for their richness in flavors. The typical flavor of strawberry is most popular in foodstuffs. Nearly 400 of the volatile compounds responsible for strawberry's unique flavor have been identified up to date (Raab et al. 2006). The major compound that generates strawberry flavor is 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol)—a compound that possesses a powerful strawberry aroma, is highly concentrated in strawberry, and has low odor threshold. The rich flavor profile and the abundance of furaneol made strawberry important for deciphering fruit flavor formation. In recent years, several key flavor genes, from different metabolic pathways, have been isolated from this fruit species as described below.

Several 4-hydroxy-3(2H)-furanones are strongly associated with fruit flavor, mainly in strawberry and pineapple (Wein et al. 2001, 2002). These furanones posses a unique biosynthesis route as they are produced directly from carbohydrates without prior degradation of the carbon skeleton. Genes associated with two members of this class of fruit flavors, namely furaneol and methoxyfuraneol, have been identified. The route to furaneol formation starts by the conversion of D-fructose-1,6-diphosphate to 4-hydroxy-5-methyl-2-methylene-3(2H)-furanone by a yet unknown enzyme, and the latter compound serves as a substrate for an enone reductase to form furaneol. Furaneol is further converted to its methoxy form by the action of a promiscuous *O*-methyltransferase such as the one from ripe strawberry fruits (FaOMT; Wein et al. 2002). Antisense expression of the *FaOMT* gene under the control of a constitutive promoter resulted in a near total loss of methoxyfuraneol (Lunkenbein et al. 2006a). The reduced level of methoxyfurnaeol was only perceived by one-third of the panelists, a result consistent with the outcome of aroma extract dilution assay.

In strawberry, methyl and ethyl esters of cinnamate are important fruit flavors (Hirvi and Honkanen 1982). It was suggested that these esters are formed through activated (hydroxy)cinnamoyl Glucose (Glc) ester intermediates. Phenylpropanoid acids such as hydroxycinnamic acids are often found in conjugated forms such as Glc esters, and these have been found in strawberry fruit (Latza et al. 1996). A key gene in this pathway, encoding a UDP-Glc:cinnamate glucosyltransferase (FaGT2) was isolated from strawberry and shown to encode a protein able to generate the 1-*O*-acyl-Glc esters of cinnamic acid *in vitro* (Lunkenbein et al. 2006b). To examine the function of *FaGT2 in vivo*, the same authors generated transgenic strawberry plants silenced for *FaGT2* expression. The results showed that FaGT2 is involved in the production of cinnamoyl-Glc. Nevertheless, since the strawberry cultivar used for the experiments contained only low levels of methyl and ethyl cinnamate, it was

not possible to determine whether the FaGT2 enzyme provided the precursors for these two constituents of strawberry flavor.

The precursors for aliphatic, branched-chain thioesters and aromatic ester formation are derived from fatty acid β-oxidation and amino acid degradation that generates acyl-CoAs and alcohols. Alcohol acyltransferase (AATs) enzymes combine with very high promiscuity various acyl-CoAs and alcohols to form esters. A large number of *AAT* genes were isolated and characterized in recent years from various fruit species including wild and cultivated strawberry, melon, banana, and grapes (Aharoni et al. 2000; Beekwilder et al. 2004; Souleyre et al. 2005; Wang and De Luca 2005; Yahyaoui et al. 2002). Some of these genes have been used with a modicum of success to augment the aromas of flowers (Aranovich et al. 2007; Ben Zvi et al. 2008; Dudareva and Pichersky 2008).

ENGINEERING FRUIT FLAVOR GENES IN ADDITIONAL ORGANS OTHER THAN IN FRUIT

The information gathered along the years with respect to metabolic pathways and genes that are involved in fruit flavor formation was also directed toward the engineering of other plant organs apart from fruit. Since many of the volatiles that are involved in the formation of aromas also have ecological roles in defense and communication, these studies have been targeted at conferring resistance to pests and pathogens to the transgenic plants. Genes involved in flavor production isolated from fruit were used to manipulate their respective biosynthetic pathways in model species such as *Arabidopsis* and tobacco. Although these studies cannot model the outcome of similar engineering strategies in fruit, they could provide valuable information with respect to important engineering issues such as precursor availability, subcellular compartmentization, cross talk, and networking between pathways. Up to date, nearly all of these experiments were carried out with genes related to the isoprenoid (or terpenoid) pathway.

A model plant such as *Arabidopsis* (*Arabidopsis thaliana*) could be extremely helpful for evaluating flavor engineering strategies. Flowers of *Arabidopsis* produce a large number of mono- and sesquiterpenes, whereas leaves produce only trace amounts of limonene and β-myrcene (Aharoni et al. 2003; Chen et al. 2003). Leaves of transgenic *Arabidopsis* plants constitutively overexpressing the strawberry *FaNES1* gene (*Fragaria Ananassa Nerolidol Synthase 1*) encoding a linalool and nerolidol synthase produced and emitted free hydroxylated and glycosylated linalool derivatives (Aharoni et al. 2003). As in other cases, the newly formed monoterpene was converted by endogenous enzymes, most probably hydroxylases and glycosyl transferases to various derivatives. Interestingly, in *Arabidopsis* and potato transformed with the same gene (*FaNES1*), the same 8-hydroxy derivatives were detected (*E*-8-hydroxy linalool, *Z*-8-hydroxy linalool, and *E*-8-hydroxy 6,7-dihydrolinalool), but their glycosylation pattern was different (Aharoni et al. 2006). The different profiles of linalool derivatives identified in these transgenic plants illustrate the dramatic influence of the plant genetic makeup on the outcome of metabolic engineering.

Additional experiments in *Arabidopsis* using the strawberry fruit *FaNES1* gene demonstrated the importance of subcellular localization of enzymes used for

engineering (Aharoni et al. 2005). Expressing the strawberry *FaNES1* gene in *Arabidopsis* using plastidic targeting resulted in the production of small amounts of the sesquiterpene nerolidol (Aharoni et al. 2003). This was unexpected because it is generally assumed that sesquiterpenes are only produced in the cytosol and showed that FDP is also available in the plastids. On the other hand, targeting the same strawberry gene to the mitochondria resulted in the enhanced production of nerolidol that was coupled to the formation of its derivative, the C11 homoterpene 4,8-dimethyl-1,3(*E*),7-nonatriene ((E)-DMNT; Kappers et al. 2005)).

In transgenic tobacco, Lücker and others (2004b) achieved substantial production of three new monoterpene products (γ-terpinene, (+)-limonene, and (−)-β-pinene) by introducing the three corresponding lemon monoterpene synthases to an individual plant. The products were emitted by the leaves as well as the flowers of the transgenics. The same transgenic tobacco plants already expressing three lemon monoterpene synthases, including limonene synthase, were retransformed with a construct designed for the overexpression of a *Mentha spicata* limonene-3-hydroxylase cDNA (Lücker et al. 2004c). Plants combining the expression of the four genes emitted (+)-*trans*-isopiperitenol, the product of limonene C-3 hydroxylation, and some of its derivatives. Because limonene synthase activity in the transgenic tobacco was targeted to the plastids and the cytochrome P450—using the natural targeting—to the endoplasmic reticulum, this result demonstrated that the "normal" transport mechanisms operating in terpene-producing plants and cells are also operable in transgenic plants and hence that multiple-step metabolic engineering of monoterpene biosynthesis involving two (or more) different cellular compartments is feasible. Indeed, high levels of sesquiterpenes in transgenic tobacco plants were obtained by the genetically engineered redirection of cytosolic or plastidic isoprenoid precursors (Wu et al. 2006).

El Tamer and others (2003b) carried out a sensory analysis of transgenic tobacco plants engineered for terpenoid production by a human panel. Transgenic tobacco plants engineered with lemon terpene synthases that produce three additional monoterpenes in their volatile profile (γ-terpinene, (+)-limonene, and (−)-β-pinene described above in Lücker and others 2004b) were used. The human panel detected a significant difference between the transgenic and the control plants, although this difference could not be associated to a series of sensory attributes, probably as a result of insufficient panel training (El Tamer et al. 2003b).

The experiments described above also demonstrated that engineering secondary metabolites such as flavor compounds could have a major impact on plant development, reproduction, and interaction with the environment. Strong and constitutive expression of *FaNES1* in both *Arabidopsis* and potato had a major effect on plant growth (Aharoni et al. 2003, 2006). Depletion of isoprenoid precursors or the possibly toxic effects of the introduced terpenoids may be the reasons for such phenotypes. As mentioned above, most of the molecules that compose a flavor profile might act as defense compounds. Indeed, *Arabidopsis* plants producing (E)-DMNT (Kappers et al. 2005) were attractive to the carnivorous predatory mites *Phytoseiulus persimilis*, natural enemies of spider mites. Linalool-producing *Arabidopsis* plants repelled the aphid *Mysus persicae* in dual-choice assays (Aharoni et al. 2003).

The importance of precursor availability for engineering flavors was demonstrated by Beekwilder and others (2004) who overexpressed the strawberry fruit alcohol acyltransferase (*SAAT*) in transgenic petunia (*Petunia hybrida*) in order to

assess the potential for metabolic engineering of ester production. While the expression of *SAAT* and the activity of the corresponding enzyme were readily detected in transgenic plants, the volatile profile was found to be unaltered. Feeding of alcohols such as isoamyl alcohol to explants of transgenic lines resulted in the emission of the corresponding esters. This confirmed that the availability of alcohol substrates is an important parameter to consider when engineering volatile ester formation in plants.

Thus, learning the lessons from engineering fruit flavor genes in different plant organs and species is important for the future engineering of fruit flavors.

FUTURE PROSPECTS

The significant progress of last years in the isolation and characterization of key genes affecting fruit flavor has made an important addition to our attempts to enhance and modify flavors in fruit using genetic engineering. These advancements were mainly achieved through the development of new genomics tools, including large-scale gene expression analysis and sequencing. The recent developments in metabolic profiling and mass spectrometry have been very important for the identification of new flavor genes. Nevertheless, we still lack a large part of the picture with respect to the metabolic network that generates flavor molecules and to the means of their metabolic control. Metabolic engineering experiments both in fruits as well as in other plant organs demonstrated that it is possible to manipulate known flavor producing pathways. The use of genetic engineering has some major advantages over the use of classical breeding for improving fruit flavors. One of the important advantages is the possibility to modify a plant trait in a surgical manner, in a particular organ, cell layer, and even a single cell at a specific time. This important capability will undoubtedly be used when engineering flavor compounds, in order to avoid major alteration to the growth and fitness of transgenic plants. The introduction of fruit flavor genes to vegetative organs of different species demonstrated that such approaches can provide us with valuable information that will aid in the future design of fruit flavor engineering strategies.

Since the characteristic flavor of a particular fruit is a result of a combination of hundreds of molecules, it will be difficult to predict the outcome of engineering flavors in fruit. Still, promiscuity of enzymes involved in secondary metabolism and the many examples of the formation of many novel compounds upon transgenesis with a single gene indicate that there may not be a need to engineer a large number of genes to achieve a substantial effect in volatile accumulation. In the future, we will be witnessing multidisciplinary work combining genetic engineering and chemical and metabolomic determinations, together with sensory evaluations carried out by professional panelists, to estimate the effect on human perception and preference.

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REFERENCES

Aharoni A, Giri AP, Deuerlein S, Griepink F, de-Kogel W-J, Verstappen FWA, Verhoeven HA, Jongsma MA, Schwab W, Bouwmeester HJ. 2003. Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. *Plant Cell* 15:2866–84.

Aharoni A, Giri AP, Verstappen FW, Bertea CM, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bouwmeester HJ. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–31.

Aharoni A, Jongsma MA, Bouwmeester HJ. 2005. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci* 10(12):594–602.

Aharoni A, Jongsma MA, Kim TY, Ri MB, Giri AP, Verstappen FWA, Schwab W, Bouwmeester HJ. 2006. Metabolic engineering of terpenoid biosynthesis in plants. *Phytochem Rev* 5:49–58.

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun ZK, Alvarez Huerta M, Verhoeven HA, Blaas J, van Houwelingen A, De Vos RCH, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *Plant Cell* 12:647–61.

Aranovich D, Lewinsohn E, Zaccai M. 2007. Postharvest enhancement of aroma in transgenic lisianthus (*Eustoma grandiflorum*) using the *Clarkia breweri* benzyl alcohol acetyl transferase (*BEAT*) gene. *Postharvest Biol Technol* 43:255–60.

Aubert C, Baumann S, Arguel H. 2005. Optimization of the analysis of flavor volatile compounds by liquid-liquid microextraction (LLME). Application to the aroma analysis of melons, peaches, grapes, strawberries, and tomatoes. *J Agric Food Chem* 53(23):8881–95.

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135(4):1865–78.

Ben Zvi MM, Negre-Zakharov F, Masci T, Ovadis M, Shklarman E, Ben-Meir H, Tzfira T, Dudareva N, Vainstein A. 2008. Interlinking showy traits: Co-engineering of scent and colour biosynthesis in flowers. *Plant Biotechnol J* 6(4):403–15.

Bicsak TA, Kann LR, Reiter A, Chase T Jr. 1982. Tomato alcohol dehydrogenase: Purification and substrate specificity. *Arch Biochem Biophys* 216:605–15.

Chen F, Tholl D, D'Auria JC, Farooq A, Pichersky E, Gershenzon J. 2003. Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *Plant Cell* 15(2):481–94.

Chen G, Hackett R, Walker D, Taylor A, Lin Z, Grierson D. 2004. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiol* 136:2641–51.

Davidovich-Rikanati R, Azulay Y, Sitrit Y, Tadmor Y, Lewinsohn E. 2008a. Tomato aroma: Biochemistry and biotechnology. In Havkin Frenkel D, Belanger F (eds.), *Biotechnology in Flavor Production*. Oxford, UK: Blackwell Publishing, pp. 118–29.

Davidovich-Rikanati R, Lewinsohn E, Bar E, Iijima Y, Pichersky E, Sitrit Y. 2008b. Overexpression of the lemon basil α-zingiberene synthase gene (*ZIS*) increases both mono- and sesquiterpene content in tomato fruit. *Plant J* 56:228–38.

Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E. 2007. Enrichment of the aroma and taste of tomatoes by diversion of the plastidial terpenoid pathway. *Nature Biotechnol* 25(8):899–901.

Dudareva N, Pichersky E. 2008. Metabolic engineering of plant volatiles. *Curr Opin Biotechnol* 19:181–9.

El Tamer MK, Lücker J, Bosch D, Verhoeven HA, Verstappen FW, Schwab W, van Tunen AJ, Voragen AG, de Maagd RA, Bouwmeester HJ. 2003a. Domain swapping of *Citrus limon* monoterpene synthases: Impact on enzymatic activity and product specificity. *Arch Biochem Biophys* 411(2):196–203.

El Tamer MK, Smeets M, Holthuysen N, Lücker J, Tang A, Roozen J, Bouwmeester HJ, Voragen AG. 2003b. The influence of monoterpene synthase transformation on the odour of tobacco. *J Biotechnol* 106(1):15–21.

Galili G, Galili S, Lewinsohn E, Tadmor Y. 2002. Genetic, molecular and genomic approaches to improve the value of plant food and feeds. *Crit Rev Plant Sci* 21:167–204.

Hirvi T, Honkanen E. 1982. The volatiles of two new strawberry cultivars, "Annelie" and "Alaska Pioneer," obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, Ruegen and *Fragaria virginiana*. *Z Lebensm Unters Forsch* 175:113–6.

Honkanen E, Hirvi T. 1990. The flavour of berries. In Morton ID, Macleod AJ (eds.), *Food Flavours*. Amsterdam: Elsevier Scientific Publications, pp. 125–93.

Ibdah M, Azulay Y, Portnoy V, Wasserman B, Bar E, Meir A, Burger Y, Hirschberg J, Schaffer AA, Katzir N, Tadmor Y, Lewinsohn E. 2006. Functional characterization of CmCCD1, a carotenoid cleavage dioxygenase from melon. *Phytochemistry* 67:1579–89.

Iijima Y, Gang DR, Fridman E, Lewinsohn E, Pichersky E. 2004. Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiol* 134:370–9.

Kappers IF, Aharoni A, van Herpen TWJM, Luckerhoff LLP, Dicke M, Bouwmeester HJ. 2005. Genetic engineering of terpenoid metabolism attracts bodyguards to *Arabidopsis*. *Science* 309:2070–2.

Latza S, Gansser D, Berger RG. 1996. Identification and accumulation of 1-O-trans-cinnamoyl-β-D-glucopyranose in developing strawberry fruit (*Fragaria* x *ananassa* Duch cv Kent). *J Agric Food Chem* 44:1367–70.

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Hee Nam K, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Picherskey E. 2001. Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 127:1256–65.

Longhurst TJ, Tung HF, Brady CJ. 1990. Developmental regulation of the expression of alcohol dehydrogenase in ripening tomato fruits. *J Food Biochem* 14:421–33.

Lücker J, Bowen P, Bohlmann J. 2004a. *Vitis vinifera* terpenoid cyclases: Functional identification of two sesquiterpene synthase cDNAs encoding (+)-valencene synthase and (–)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. *Phytochemistry* 65(19):2649–59.

Lücker J, Schwab W, Van Hautum B, Blaas J, Van der Plas LHW, Bouwmeester HJ, Verhoeven HA. 2004b. Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. *Plant Physiol* 134:510–9.

Lücker J, Schwab W, Franssen MCR, Van der Plas LHW, Bouwmeester HJ, Verhoeven HA. 2004c. Metabolic engineering of monoterpene biosynthesis: Two-step production of (+)-trans-isopiperitenol by tobacco. *Plant J* 39:135–45.

Lücker J, El Tamer MK, Schwab W, Verstappen FW, van der Plas LH, Bouwmeester HJ, Verhoeven HA. 2002. Monoterpene biosynthesis in lemon (*Citrus limon*). cDNA isolation and functional analysis of four monoterpene synthases. *Eur J Biochem* 269(13):3160–71.

Lunkenbein S, Salentijn EM, Coiner HA, Boone MJ, Krens FA, Schwab W. 2006a. Up- and down-regulation of *Fragaria* × *ananassa* O-methyltransferase: Impacts on furanone and phenylpropanoid metabolism. *J Exp Bot* 57(10):2445–53.

Lunkenbein S, Bellido M, Aharoni A, Salentijn EM, Kaldenhoff R, Coiner HA, Muñoz-Blanco J, Schwab W. 2006b. Cinnamate metabolism in ripening fruit. Characterization of a UDP-glucose:cinnamate glucosyltransferase from strawberry. *Plant Physiol* 140(3): 1047–58.

Martin DM, Bohlmann J. 2004. Identification of *Vitis vinifera* (–)-alpha-terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. *Phytochemistry* 65(9):1223–9.

Maruyama T, Ito M, Honda G. 2001. Molecular cloning, functional expression and characterization of (E)-beta farnesene synthase from *Citrus junos*. *Biol Pharm Bull* 24(10): 1171–5.

Mathieu S, Terrier N, Procureur J, Bigey F, Günata Z. 2005. A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: Functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation. *J Exp Bot* 56(420): 2721–31.

Pechous SW, Whitaker BD. 2004. Cloning and functional expression of an (E, E)-alpha-farnesene synthase cDNA from peel tissue of apple fruit. *Planta* 219(1):84–94.

Petro-Turza M. 1987. Flavor of tomato and tomato products. *Food Rev Int* 2:309–51.

Prestage S, Linforth RST, Taylor AJ, Lee E, Speirs J, Schuch W. 1999. Volatile production in tomato fruit with modified alcohol dehydrogenase activity. *J Sci Food Agric* 79:131–6.

Raab T, López-Ráez JA, Klein D, Caballero JL, Moyano E, Schwab W, Muñoz-Blanco J. 2006. FaQR, required for the biosynthesis of the strawberry flavor compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone, encodes an enone oxidoreductase. *Plant Cell* 18(4):1023–37.

Sharon-Asa L, Shalit M, Frydman A, Bar E, Holland D, Or E, Lavi U, Lewinsohn E, Eyal Y. 2003. Citrus fruit flavor and aroma biosynthesis: Isolation, functional characterization, and developmental regulation of Cstps1, a key gene in the production of the sesquiterpene aroma compound valencene. *Plant J* 36(5):664–74.

Schmidt H, Kurtzer R, Eisenreich W, Schwab W. 2006. The carotenase AtCCD1 from *Arabidopsis thaliana* is a dioxygenase. *J Biol Chem* 281(15):9845–51.

Schwab W, Davidovich-Rikanati R, Lewinsohn E. 2008. Biosynthesis of plant-derived flavor compounds. *Plant J* 54(4):712–32.

Simkin A, Schwartz S, Auldridge M, Taylor M, Klee H. 2004. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β-ionone, pseudoionone, and geranylacetone. *Plant J* 40:882–92.

Souleyre EJ, Greenwood DR, Friel EN, Karunairetnam S, Newcomb RD. 2005. An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. *FEBS J* 272(12):3132–44.

Speirs J, Lee E, Holt K, Yong-Duk K, Scott NS, Loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117:1047–58.

Suzuki Y, Sakai H, Shimada T, Omura M, Kumazawa S, Nakayama T. 2004. Characterization of gamma-terpinene synthase from *Citrus unshiu* (Satsuma mandarin). *Biofactors* 21(1–4):79–82.

Tadmor Y, Fridman E, Gur A, Larkov O, Lastochkin E, Ravid U, Zamir D, Lewinsohn E. 2002. Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. *J Agric Food Chem* 50:2005–9.

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr Opin Plant Biol* 9:297–304.

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ. 2006a.Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci U S A* 103:8287–92.

Tieman DM, Zeigler M, Schmelz EA, Taylor MG, Bliss P, Kirst M, Klee HJ. 2006b. Identification of loci affecting flavor volatile emissions in tomato fruits. *J Exp Bot* 57:887–96.

Tieman DM, Loucas HM, Kim JY, Clark DG, Klee HJ. 2007. Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. *Phytochemistry* 68(21):2660–9.

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE. 1996. Changes of fatty acids and fatty acid derived flavor compounds by expressing the yeast Δ-9 desaturase gene in tomato. *J Agric Food Chem* 44:3399–402.

Wang C, Xing JS, Chin C, Ho C, Martin CE. 2001. Modification of fatty acids changes the flavor volatiles in tomato leaves. *Phytochemistry* 58:227–32.

Wang J, De Luca V. 2005. The biosynthesis and regulation of biosynthesis of Concord grape fruit esters, including "foxy" methylanthranilate. *Plant J* 44(4):606–19.

Wein M, Lavid N, Lunkenbein S, Lewinsohn E, Schwab W, Kaldenhoff R. 2002. Isolation, cloning and expression of a multifunctional O-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2H)-furanone, one of the key aroma compounds in strawberry fruits. *Plant J* 31(6):755–65.

Wein M, Lewinsohn E, Schwab W. 2001. Metabolic fate of isotopes during the biological transformation of carbohydrates to 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry fruits. *J Agric Food Chem* 49(5):2427–32.

Wu S, Schalk M, Clark A, Miles RB, Coates R, Chappell J. 2006. Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. *Nat Biotechnol* 24:1359–61.

Yahyaoui FE, Wongs-Aree C, Latché A, Hackett R, Grierson D, Pech JC. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. *Eur J Biochem* 269(9): 2359–66.

CHAPTER 8

Bioconversion of Flavors

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INTRODUCTION

Products classified as natural, in particular flavors and fragrances, by the European and U.S. food agencies have been preferred by consumers because they consider artificial ingredients to be unhealthy. Even though flavors can be made by chemical transformation of natural substances, the resulting flavor cannot legally be labeled as natural. In addition, chemical methods may lack substrate specificity, resulting in low product purity and yield and resulting in racemic mixtures from which the isolation of the desired isomer will be costly if not impractical. That is why flavor companies are attempting to produce flavors with a biological origin (Janssens et al. 1992; Welsh et al. 1989). For a long time, flavor compounds ranging from highly complex mixtures to single compounds have been extracted from raw materials such as plants and fruits. However, this process is subject to various problems, such as low concentration of the desired flavor, seasonal variation of the materials, and the vagaries of the weather, which can significantly affect the yield and quality of the flavors (Armstrong and Yamazaki 1986).

By biotechnological processes, plant cell and tissue cultures, microorganisms, and enzymes can be used as possible alternatives for the production of natural flavoring compounds (Janssens et al. 1992). Due to the advances in microbial fermentation and enzyme technology, individual flavor compounds or complex flavor mixtures are increasingly becoming targets for production on an industrial scale (Engel and Roling 1996). Biotechnological approaches leading to flavor production can be divided into two classes according to their production means: microbiological or enzymatic methods (Welsh et al. 1989). They can also be divided into two major groups: de novo synthesis in the course of microbial fermentation, by the use of metabolizing cells, and biotransformations/bioconversions of suitable precursors either by microorganisms or by enzymes (Engel and Roling 1996; Welsh et al. 1989). The efficiency of microbial de novo synthesis of flavor compounds can be increased by offering suitable precursors that serve as starting points for biotransformations

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(single-step reactions) or bioconversions (multistep reactions) yielding the desired products. In the present chapter, the bioconversion process will be the focus.

Different reactions such as oxidation, reduction, hydrolytic reactions, dehydration, formation of new C–C bonds, and several degradation reactions can be performed by microorganisms in flavor production if specific substrates are supplied in the medium (Janssens et al. 1992). Natural sources containing the required precursors in high amounts can be used directly as substrates (Engel and Roling 1996). For example, castor oil, which contains in its constitution 80% of a hydroxylated C18 fatty acid (ricinoleic acid) in its esterified form, was used by Gomes and others (2007) as a precursor for the biotransformation of ricinoleic acid into γ-decalactone by *Yarrowia lipolytica*. On the other hand, single compounds from natural sources can be subjected to highly specific microbially catalyzed reaction sequences (Engel and Roling 1996). Terpenes from essential oils are a favorite substrate on which to study bioconversions, as, for example, the sesquiterpene (+)-valencene, which is a fairly cheap component of orange oil that can be converted by some bacteria to a more expensive sesquiterpene, the (+)-nootkatone, an important aroma compound of grapefruit (Sowden and others 2005).

The use of enzymes for single flavor compounds synthesis is also of great importance due to the characteristic of enzymatic reactions such as high substrate specificity, high reaction specificity, mild reaction conditions, and reduction of waste product formation. There are two other features very important in the synthesis of flavor substances: (1) the stability of soma enzymes in organic solvents allows the catalysis of reactions that are not feasible in aqueous medium, for example, esterifications, transesterifications, and lactonizations, thus providing access to a broad spectrum of important volatiles and (2) the increasing knowledge of the influence of absolute configuration on the flavor properties of chiral compounds and analytic progress in the determination of naturally occurring enantiomeric compositions, which have increased the need for biocatalyzed reactions resulting in the "correct" enantiomer (Engel and Roling 1996).

For the purpose of this chapter, microbially or enzymatically produced flavorants are grouped and discussed under the following categories: lactones, esters, terpenes, carbonyls, acids, and alcohols.

LACTONES

Lactones are cyclic esters of primarily γ- and δ-hydroxy acids (Fig. 8.1), being ubiquitously found in food, contributing to taste and flavor nuances such as fruity, coconut like, buttery, creamy, sweet, or nutty. Lactones are generally formed by successive β-oxidations of saturated and unsaturated hydroxyl acids or their

γ-Valerolactone
or 4-methylbutanolide

δ-Hexalactone
or 5-methylpentanolide

Figure 8.1. Common lactone structures.

lipid precursors until this process results in a hydroxylated carbon at the C_4 or C_5 position. γ- and δ-lactones may then be formed through internal esterification (lactonization) of the C_4 (γ) or C_5 (δ) hydroxyl group with a carboxylic acid group on the same molecule (Welsh et al. 1989).

Lactones are mostly produced chemically, but the use of microorganisms can have several advantages in comparison with chemical synthesis, especially for the production of optically active molecules (Janssens et al. 1992). Biocatalysis competes best with chemical catalysis in the following types of reactions: (1) introduction of chirality, (2) functionalization of chemically inert carbons, (3) selective modifications of one functional group in multifunctional molecules, and (4) resolution of racemates (Krings and Berger 1998). A large number of flavors and fragrances are chiral, and often the enantiomers have different sensory properties. Enzymes are not only regio- but also highly stereoselective and are the catalysts *par excellence* for the preparation of enantiopure flavors and fragrances (Franssen et al. 2005).

A variety of microorganisms may carry out de novo lactone biosynthesis (Table 8.1). Whole cells catabolize carbohydrates, fats, and proteins, and further convert the breakdown products to more complex flavor molecules, a property that is traditionally used during the production of fermented foods with their amazing number of aroma chemicals (Krings and Berger 1998). Unfortunately, yields are low, being an exception the *in situ* microbial production in dairy products. The improvement

TABLE 8.1. Main Lactone Producers

Microorganism	Lactone
Candida globiformis	5-Decanolide
Candida pseudotropicalis	5-Decanolide
Ceratocystis moniliformis	4- and 5-Decanolide
Cladosporium butyri	5-Decanolide
Geotrichum fragrans	4-Hexylbutanolide
Ischnoderma bezoinum	4-Butanolide, 4-pentanolide, 4-hexanolide, 2-hexen-4-olide, 4-heptanolide, 2-hetpen-4-olide, 4-octanolide, 2-octen-4-olide
Pityrosporum sp.	4-Hexanolide, 4-heptanolide, 4-octanolide, 4-nonanolide, 4-decanolide, 4-undecanolide, 4-dodecanolide
Polyporus durus	4-Butanolide, 4-pentanolide, 3-penten-4-olide, 4-hexanolide, 2-hexen-4-olide, 5-hexen-4-olide, 5-hexanolide, 2-hetpen-4-olide, 4-heptanolide, 2-, 5-, and 6-octen-4-olide, 4-octanolide, 2-nonen-4-olide, 2-decen-4-olide, 4-decanolide, 4-methoxy-6-methyl-2H-pyran-2-one
Saccharomyces cerevisiae	5-Decanolide
Saccharomyces fermenti	4-Butanolide, 5-ketohexan-4-olide
Saccharomyces fragilis	5-Decanolide
Sarcina lútea	5-Decanolide
Sporobolomyces odorus (salmonicolor)	4-Decanolide, 5-decanolide, *cis*-7-decen-5-olide, *cis*-6-dodecen-4-one
Thricoderma viride	6-(Pent-1-enyl)-α-pyrone, 6-pentyl-α-pyrone
Thricoderma harzianum	6-(Pent-1-enyl)-α-pyrone
Yarrowia lipolytica	4-Hexylbutanolide

of lactone yields by fermentation and on the biotransformation of hydroxy carboxylic acids to lactones direct to studies concerning medium composition, system aeration, and addition of precursor compounds, among other strategies.

Inexpensive, readily available, and renewable natural precursors, such as fatty or amino acids, can be converted to more highly valued flavors. Lipid degradation pathways involve many enzymatic systems, ranging from the hydrolysis of triacylglycerols (which are the major form of oils and fats) to the catabolism and oxidation of single-chain fatty acids. These reactions have been well investigated, and nowadays, the common routes for the production of flavors from triacylglycerols involve, as a first step, the liberation of free fatty acids by lipolytic enzymes. Owing to their high toxicity toward microorganisms, these free fatty acids are converted to coenzyme A (CoA) esters before being catabolized (Feron et al. 1996). The main pathway by which a microorganism catabolizes a fatty acid is the β-oxidation system.

In the case of eukaryotic organisms such as yeasts, the fatty acid is catabolized in a specific subcellular compartment: the peroxisome. The reduction of the aliphatic chain is carried out via the β-oxidation system as described above. After a certain number of cycles of β-oxidation, the CoA ester of the reduced hydroxy fatty acid is released from the β-oxidation complex. This ester can be considered as the first precursor of the lactone; all the metabolic steps leading to the formation of the lactone ring have not yet been clearly identified (Feron et al. 1996).

Decalactone and other lactones are produced commercially in high yields by the biotransformation of ricinoleic acid, the principle fatty acid in castor oil. Ricinoleic acid is thought to be first metabolized to 4-hydroxy decanoic acid, which is then chemically lactonized to γ-decalactone and other lactones. The extent to which lactonization occurs enzymatically without acid catalysis is not clear. Commercial development of the biotransformation of castor oil and ricinoleic acid to γ-decalactone has been the aim of a number of patents. However, very little has been published on the biochemical mechanisms associated with ricinoleic acid oxidation and the formation of γ-decalactone. Presumably, ricinoleic acid is metabolized to a hydroxy acid that accumulates because it cannot be further metabolized by enzymes of the β-oxidation pathway (Hagedorn and Kaphammer 1994).

Yeasts, such as *Candida tropicalis* or *Y. lipolytica*, degrade ricinoleic acid to C16, C14, and C12 acids and, interestingly, accumulate γ-decalactone, which exhibits fruity and oily notes important in the formulation of peach, apricot, or strawberry aromas. However, the yields of this biotransformation are commonly poor, and they rarely reach concentrations over 4–5 g/L in the fermentation broth (Longo and Sanromán 2006). Waché and others (2000) and Groguenin and others (2004) investigated the enzymes involved in γ-decalactone production by *Y. lipolytica* and encountered the reasons for low yields, considering two hypotheses: first, the yeast may reconsume some of the γ-decalactone; second, only a portion of the methyl ricinoleate (methyl δ-12-hydroxy-*cis*-9-octadecenoate) is oxidized to the C10 level, and the C10 product serves as the precursor for several γ-decalactones (Waché et al. 2001).

The authors showed that an increase in the production rate can be achieved, mastering the control of Aox, particularly lowering the impact of short-chain-specific Aox, being a promising way to increase lactone production. Although they are not the rate-limiting enzymes, Aox isozymes are involved in lactone

reconsumption, and mutants with lower β-oxidation fluxes in short-chain acyl-CoA have significantly less γ-decalactone reconsumption. *Y. lipolytica* wild type produces primarily 3-hydroxy-γ-decalactone, but by decreasing Aox activity in a strain, the authors could obtain γ-decalactone instead of 3-hydroxy-γ-decalactone, identifying mutant strains that can produce 26 times more γ-decalactone than the wild-type parents (Waché et al. 2000, 2001).

The compound 6-pentyl-α-pyrone (6-PP) provides a coconut aroma, highly desired by flavorists. It was found to be the major volatile constituent in cultures of the fungus *Trichoderma viride*. Other fungi such as *Tyromyces sambuceus* and *Cladosporium suaveolens* efficiently generate the coconut-flavored lactones γ-decalactone and δ-dodecalactone from ricinoleic acid and linoleic acid, respectively (Longo and Sanromán 2006). In an integrated fermentation process, 6-PP is continuously removed by pervaporation over a selective membrane, since it inhibits growth if it accumulates in the medium. Alternatively, aqueous two-phase systems can be used for *in situ* recovery of 6-PP (Vandamme and Soetaert 2002).

The physiological functionality of lactones is usually dependent on the enantiomeric purity of the lactone, implying that an enantioselective synthesis is important when preparing the desired stereoisomers (Pollock et al. 2007). Biosynthesis can be used for the preparation of lactone precursor compounds that are difficult to prepare chemically. The increase of chiral sulfoxides in asymmetric syntheses has prompted the development of a microbiological method for the preparation of *R*- and *S*-methyl ρ-tolyl sulfoxides. *Mortierella isabellina* could convert methyl ρ-tolyl sulfoxide into *R*-(+)-ρ-tolyl sulfoxide at 60% yield and 100% optical purity. This product can be used to synthesize *R*-mevalonolactone (Welsh et al. 1989).

Identification and enantiodifferentiation of γ-lactones produced during the bioconversion of soybean fatty acids by *Penicillium roqueforti* spores in the presence of an exogenous lipase was performed by Chalier and Crouzet (1998) using gas chromatography–mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode. The authors showed that 4-dodecanolide and 4-hexanolide were first produced with an enantiomeric excess (99%) in favor of the (*R*) form, whereas an enantiomeric excess in favor of the (*S*) form (94%) is found for (6*Z*)-dodecen-4-olide, the major lactone produced by the fungus. If incubation was continued, mixtures of both enantiomers were found, more particularly for 4-decanolide (*R*/*S*: 40/60), which was produced only after 120 h of incubation.

Lactonohydrolases, catalyzing the reversible or irreversible hydrolysis (ring cleaving) of lactone compounds, belong to esterase family enzymes. The reactions catalyzed by lactonohydrolases, as well as those by other esterase family enzymes, are sometimes stereospecific and/or regioselective and thus should be applicable to the synthesis of useful compounds, such as optically active compounds. The activities of the lactonohydrolases have been detected in several organisms (*Fusarium oxysporum*, *Agrobacterium tumefaciens*, *Acinetobacter calcoaceticus*), and they are involved in the synthesis and degradation of various lactone compounds *in vivo* (Shimizu et al. 2001).

Another interesting example of aroma-active lactone synthesis was described by Vanderhaegen and others (2003) concerning the maturation of whiskey, where a dodecalactone with whiskey flavor was formed through the combined activity of lactic acid bacteria and yeast. The bacteria synthesized the precursor hydroxy fatty acid from an unsaturated fatty acid coming from yeast.

ESTERS

Esters are commonly used flavoring agents, very appreciated for the fruity aromas they provide, naturally present in fruits, which are present in fairly low concentrations, mostly between 1 and 100 ppm (Janssens et al. 1992). They are employed in fruit-flavored products (i.e., beverages, candies, jellies, and jams), baked goods, wines, and dairy products (i.e., cultured butter, sour cream, yogurt, and cheese). Acetate esters, such as ethyl acetate, hexyl acetate, isoamyl acetate, and 2-phenylethyl acetate are recognized as important flavor compounds in wine and other grape-derived alcoholic beverages. They may be produced by yeasts, molds, and bacteria, but the amounts and range of esters produced vary with the microbial species.

Several so-called non-*Saccharomyces* wine yeasts are well-known as the producers of acetate ester. Among them, the yeasts *Hanseniaspora guilliermondii* and *Picchia anomala* were found to be potent 2-phenylethyl acetate and isoamyl acetate producers, respectively (Longo and Sanromán 2006).

Two pathways may be used to form esters: (1) the alcoholysis of acyl-CoA compounds and (2) the direct esterification of an organic acid with an alcohol. During *Saccharomyces cerevisiae* fermentation, esters are formed from alcohols and acyl-CoA, and ethyl acetate formation is stimulated by the increased availability of acetyl-CoA. Similarly, ethyl butyrate formation depends on the availability of butyryl-CoA. Normally, acetyl-CoA is formed by oxidative decarboxylation of pyruvate and ATP activation of acetic acid. It is consumed by the citric acid cycle and through carboxylation to malonyl-CoA. When the acetyl-CoA formation is stimulated by lipoic acid addition and its consumption is inhibited by biotin limitation, the size of the acetyl-CoA pool is increased and ethyl acetate synthesis is stimulated. Similarly, cell-free extract of *S. cerevisiae* has been used to synthesize ethyl acetate from ethanol and acetyl-CoA, thus supporting this mechanism of ethyl acetate formation. The ester-synthesizing enzyme has been identified as an alcohol acetyltransferase that is located at the cell membrane, leading to the acetylation of C_1 to C_6 straight- and branched-chain primary alcohols, being less active with the C_3 alcohol (Welsh et al. 1989).

Since ethanol is the alcohol present at the highest concentration in yeast fermentation, the rate of ethyl ester formation is related to the availability of the fatty acid component of the ester. Conversely, acetic acid is the most abundant acid. Thus, ethyl acetate is the major ester constituent, while the formation of other acetate esters depends on the availability of higher alcohols.

The addition of specific amino acids to a fermentation broth was found to increase the level of the corresponding acetate esters. In fact, biochemically, the amino acids valine, leucine, and isoleucine are metabolized by the yeast into the intermediate branched alcohols, isobutanol, 3-methylbutanol, and 2-methylbutanol, respectively. The corresponding volatile branched acetates, isobutylacetate, 3-methylbutylacetate, and 2-methylbutylacetate are then formed via the action of alcohol acetyltransferases. The yeast is able to convert added branched alcohols into the corresponding fruity acetates, thereby drastically improving the yield (Vandamme and Soetaert 2002).

Geotrichum klebahnii produces de novo a broad spectrum of ethyl esters of branched carboxylic acids, generating a pleasant fruity flavor. When supplied with isoleucine, the main product was ethyl-2-methylbutyrate. *Geotrichum fragrans*

partially metabolizes L-leucine by oxidative deamination; addition of ethanol results in esterification, forming flavor esters such as ethylisovalerate, which can be recovered from the bioreactor exit-air by absorption. *Williopsis saturnus* var. mrakii synthesizes important levels of volatile branched acetates, mainly 3-methylbutyl-acetate, the character-impact compound of banana aroma (Vandamme and Soetaert 2002).

Medium composition and system oxygenation appear to be the most important fermentation parameters affecting ethyl ester formation. Yeast grown under anaerobic fermentation conditions has an absolute requirement of unsaturated fatty acids. These acids, when added to the growth medium, stimulate yeast growth and cause a decrease in the formation of medium fatty acids and their ethyl esters. Linoleic acid was shown to repress the membrane-bounded enzymes responsible for ester synthesis without affecting yeast growth. Increased pressure has also been found to reduce both growth and ester formation, due to pyruvate decarboxylation promoted by the increase in partial pressure of carbon dioxide, leading to a decrease in acetyl-CoA formation. Generally, conditions that promote yeast growth increase the biosynthetic demand for acetyl-CoA and decrease the availability of this central compound for the formation of acyl fatty acids required for ester formation (Welsh et al. 1989).

Direct esterification of alcohols and acids is predominantly carried out by bacteria and filamentous fungi. Whole-cell-mediated biosynthesis of flavor esters was first identified in dairy products. In cheese production, ethyl or methyl esters of short-chain fatty acids generally bring about fruity flavors, while thioesters derived from thiols are associated with cabbage or sulfur aromas. The capacity of several lactic acid bacteria and also *Pseudomonas* to synthesize both ethyl esters (ethyl butyrate and ethyl hexanoate) and thioesters has been reported (Longo and Sanromán 2006; Welsh et al. 1989).

The role of a unique esterase from *Lactococcus lactis* in the formation of these aroma compounds has been investigated and ascertained as, at least, partially responsible for the esterification reactions leading to the production of aroma ester compounds. This was undertaken by using an esterase negative mutant of *L. lactis* (Longo and Sanromán 2006).

The main aroma products of the fungus *Ceratocysis moniliformis* are ethyl acetate, propyl acetate, isobutyl acetate, isoamyl acetate, citronellol, and gerianol. To circumvent inhibitory effects, *in situ* product removal using pervaporation was found to decrease product concentrations in the bioreactor and increase microbial growth rates. The total yield of aroma compounds produced is higher in such an integrated bioprocess than in conventional batch cultivation. In addition, permeates obtained from pervaporation consist of highly enriched mixtures of flavors and fragrances (Bluemke and Schrader 2001).

The use of organic solvents as media for whole-cell- and enzyme-catalyzed syntheses has prompted applied research on the development of several organic solvent-containing systems for the production of a variety of esters. Lipases, esterases, proteases, nucleases, and various glycosidases aid flavor-extraction processes and directly hydrolyze flavor molecules from larger progenitors. A good example of reversed lipolysis is the esterification reaction in nonaqueous systems using lipases. These cofactor-independent enzymes have shown potential for use in stereo- and regiospecific hydrolyses and transesterifications to yield optically pure aliphatic and aromatic esters and lactones (Krings and Berger 1998).

TERPENES

Terpenes are often the most important components responsible for the characteristic odors of essential oils (Janssens et al. 1992). These compounds are all composed of 5-carbon isoprene unit (2-methyl-1,3-butadiene) as their skeletal building block. The isoprene unit is linked together in groups of two (monoterpenes), three (sesquiterpenes), four (diterpenes), six (triterpenes), nine (tetraterpenes), or more (polyterpenes), and these structures can be open or closed chain, cyclic, saturated, or unsaturated (Welsh et al. 1989).

The real value of microorganisms for terpene production centers on the biotransformation/bioconversion of inexpensive natural precursors terpenes to higher-value terpenes. Many microorganisms are able to break down terpenes or to carry out specific conversions, creating products with added value (Janssens et al. 1992).

Terpenes are excellent substrates for stereospecific bioconversions. L-Menthol is the only isomer of menthol exhibiting desired flavor. Microbial resolution is accomplished by first esterifying (DL)-menthol with a carboxylic acid to form an ester such as (DL)-menthyl acetate, formate, propionate, or myristate. These esters may be selectively hydrolyzed by stereospecific esterases to produce L-menthol (Yu et al. 2007).

Monoterpenes, widely distributed in nature (more than 400 structures), constitute suitable precursor substrates, which are ideal starting materials for the biotechnological production of flavor compounds (Krings and Berger 1998). Terpenoids, produced from filamentous fungi, are used by various food industries. Readily available monoterpenes, such as α-pirene and limonene, are used as substrates for the conversion of flavoring compounds (Berger et al. 1992). Indeed, many data on terpenoid biotransformation by both fungi and bacteria are published (Cadwallader et al. 1989; Demyttenaere and Kimpe 2001; Demyttenaere and Potter 1996). However, most of the monoterpene biotransformation studies described so far have been of more academic than practical value, and no biotransformation process has been commercialized yet (Krings and Berger 1998).

Patchoulol is a tricyclic sesquiterpene that constitutes 35–40% of Patchouli oil. The first step in the conversion of patchoulol to nor-patchoulol, that is, 10-hydroxypatchoulol production, is very difficult by chemical means (Welsh et al. 1989). Extensive screening of microorganisms with ability to make this conversion has been done. Suhara and others (1981) found four strains of *Pithomyces* species that can carry out region-selective hydroxylation of patchoulol.

Ionones have application as raspberry flavors, in high-value rose perfumes and as tobacco flavoring. Krasnobajew and Helminger (1982) described the microbial transformation of β-ionone by fungi, including *Aspergillus*, *Botryosphera*, and *Lasiodiplodia*. All three genera were able to hydroxylate the ionone ring at positions 4 and 2 (Grivel et al. 1999). They reported that with *Lasiodiplodia theobromae* ATCC 28570, up to 10 g of β-ionone/L of medium was converted with a yield of 90%, giving β-cyclohomogeraniol as the main product.

CARBONYLS

The carbonyl group (C=O) is presented in ketones and aldehydes, which present unique odor characteristics contributing to flavor chemicals.

Ketones contribute primarily to cheese flavors, particular in mold-ripened cheeses (Welsh et al. 1989), which is not the scope of this chapter. Furanones may be produced enzymatically (Cheetham and Quail 1991) or by microorganisms (Dahlen et al. 2001) from naturally occurring sugars. Furaneol® (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone) was first identified as a natural aroma component in pineapples (Rodin et al. 1965). It has since been detected in several fruits such as strawberry, raspberry, mango, and tomato. Due to their low odor threshold and characteristic flavor, Furaneol and its methoxy derivative (methoxyfuraneol and mesifurane) are among the most important aroma compounds in strawberry fruits (Lavid et al. 2002). It can be produced by the soy-sauce-fermenting yeast *Zygosaccharomyces rouxii* after the addition of α-fructose-l,6-diphosphate to yeast–peptone–dextrose nutrient media (Dahlen et al. 2001).

Many aliphatic and aromatic terpenoid aldehyde compounds exhibit distinct organoleptic qualities that are highly valued in the food industry. Generally, the C_2 to C_7 aliphatic aldehydes are volatile and characterized by unpleasant sharply irritating odors, while the fatty aldehydes (C_8 to C_{13}) become less harshly penetrating and more floral and attractive with increasing molecular weight (Welsh et al. 1989).

Natural benzaldehyde is usually liberated from amygdalin, a cyanogenic glycoside present in fruit kernels, and is used as a key ingredient in cherry and other natural fruit flavors. In quantity, benzaldehyde is the second most important flavor molecule after vanillin (Krings and Berger 1998). *A. calcoaceticus* uses benzyl alcohol as a carbon source to produce benzaldehyde (MacKintosh and Fewson 1987). An alternative process is the use of microorganisms that are able to metabolize L-phenylalanine producing this flavor compound. This process is aided by plentiful, cheap supplies of L-phenylalanine, which is now produced extensively as a precursor of the high-intensity sweetener Aspartame® (Cheetham 1993). Comprehensive knowledge about the metabolic pathways of L-phenylalanine is very important for better control, avoiding side reactions and, in turn, better product yield of this biotransformation. Krings and others (1996) used a ring-labeled deutero-L-phenylalanine to elucidate the metabolic pathways of a submerged culture of a basidiomycete, *Ischnoderma benzoinum*. The authors reported an almost complete conversion of L-phenylalanine to benzaldehyde and 3-phenylpropanol, a flowery flavor, and the two different degradation pathways are presented in Figure 8.2.

Geusz and Anderson (1991) patented the process of natural benzaldehyde production via a mandelate pathway by bacteria that metabolize phenylacetate. Benzaldehyde can also be produced enzymatically, using β-glucosidase and mandelonitrile lyase, which are present in crude almond meal (emulsin) (Fig. 8.3) (Cheetham 1993).

ACIDS

The low flavor impact of acids has lessened their perceived value as flavorants. Short-chain fatty acids are characterized by sharp unpleasant, strongly pungent, and irritating odors in high concentration. As acid molecular weight increases, the character is replaced by rancid, buttery, and cheesy notes. Fatty acids above C_{14} are waxy solids with only slight tallow-like odors (Welsh et al. 1989). The acids mainly present

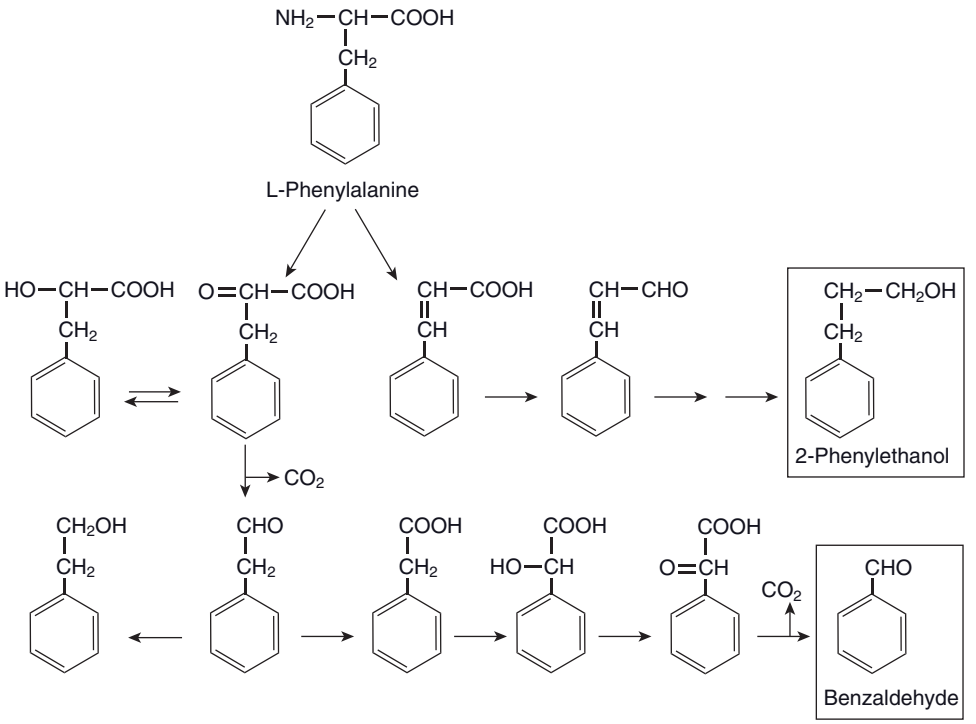

Figure 8.2. Degradation pathway of L-phenylalanine by *I. benzoinum* (modified from Krings et al. 1996).

in food aroma are described as (Longo and Sanromán 2006) acetate, butyrate, caproate, decanoate, isobutyrate, 2-methylbutyric acid, 3-methylbutyric acid, octanoate, phenylacetate, propionate, and valerate.

Although the previous mentioned characteristics, these chemical compounds contribute to complex aromas and accentuate certain aroma characteristics, like C_3 to C_6 acids accentuate fruity notes, while C_4 and C_6 to C_{10} acids provide cheesy flavor. The presence of a hydroxyl group tends to suppress odor, while aromatic acids are faintly balsamic with light, spicy, and floral notes. Di- and tricarboxylic acids are all odorless (Welsh et al. 1989).

Many valuable flavors and fragrances can be produced by microorganisms from fatty acids added as precursors, including compounds that provide green notes, mushroom flavors, specific lactones, and methylketones. Methylketones (2-alkanones) are derived from medium-length fatty acids and confer strong cheese-associated flavors, such as Roquefort, Camembert, and Stilton. Starting from α-linolenic acid (C18:3; found in linseed oil), the fungal plant pathogen *Botryodiplodia theobromae* can form jasmonic acid that can be esterified by commercial lipases to obtain methyl (+)-7-isojasmonic acid, providing a sweet floral, jasmine-like odor. Linoleic acid (C18:2) can also be converted via lipoxygenase action into 1-octene-3-ol, the top note in typical mushroom aroma (Vandamme and Soetaert 2002).

In fact, microbial biogenesis of organic acids may occur by four different routes. Lactic acid and the C_1 to C_5 aliphatic acid series arise primarily as the end products

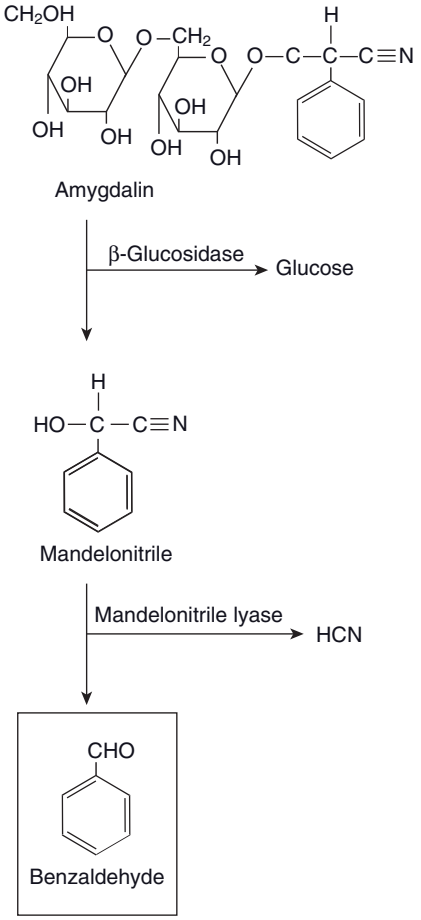

Figure 8.3. The synthesis of benzaldehyde from amygdalin, from citrus wastes using purified enzymes (modified from Cheetham 1993).

of fermentations. Unsaturated fatty acids are precursors of longer C_6 to C_{18} saturated acids through an oxygen-dependent desaturase catalysis. Isoacids are believed to be derived from amino acid biosynthesis pathways, and other free fatty acids are formed by the action of microbial lipases, which degrade glycerides to respective fatty acids and glycerol (Welsh et al. 1989).

The development of microbial processes for acid production has been limited to acetic, propionic, and butyric acids. Acetic acid production is a well-established process performed by various species of *Acetobacter*. Butyric acid is a short-chain fatty acid that bears some important physiological functions. For example, butyric acid esters are the character-impact flavors in tropic fruits and dairy products, being used to supply butter-like notes to flavors and may be used for the production of ethyl, butyl, isobutyl, and amyl butyrate (Kong et al. 2006). Butyric acid is also an important flavor compound in beer. However, at concentrations above its beer flavor threshold, it causes "cheesy" or "sickly" off-flavors. Abnormal concentrations in beer can arise due to infections by anaerobic spore-forming bacteria of the genus

Clostridium (Hawthorne et al. 1991). Natural butyric acid can be produced by anaerobic fermentation using various *Clostridium* and *Butyribacterium* species, being *Clostridium butyricum* and *Clostridium acetobutylicum* the most common sources.

At least, propionic acid bacteria have long been used in the dairy industry. These bacteria play important roles in the development of the characteristic flavor and eye production in Swiss-type cheeses. Three types of bacteria are used in the production of Emmental cheese: *Streptococcus thermophilus*, *Lactobacillus* (*Lactobacillus helveticus* or *Lactobacillus bulgaricus*), and *Propionibacter* (*Propionibacter freudenreichii* or *Propionibacter shermani*). In a late stage of cheese production, *Propionibacter* consumes the lactic acid excreted by the other bacteria and releases carbon dioxide gas, which slowly forms the bubbles that develop the eyes. In general, the larger the eyes in a Swiss cheese, the more pronounced its flavor.

ALCOHOLS

Alcohols play a modest and often indirect role as flavorants. They are essential to flavor quality in wine and distilled beverages and also play indirect role as precursors for the preparation of other flavorants, as the production of aldehydes by oxidation of alcohols (Welsh et al. 1989).

FINAL CONSIDERATIONS

The increase in consumer demand for nutritious and flavorful food supply has led to an increased demand for flavoring materials that may be considered natural. The use of microbial cells or specific enzymes presents great potential to meet this demand. Biosynthetic processes may not yet be industrially worthy, but biotransformations of low valuable precursors are a good alternative to overcome this obstacle. Furthermore, modern methods of gene manipulation and physiological control may be used to increase production levels of desired flavors.

REFERENCES

Armstrong DW, Yamazaki H. 1986. Natural flavours production: A biotechnological approach. *Trends Biotechnol* 4(10):264–8.

Berger RG, Drawert F, Tiefel P. 1992. Naturally occurring flavors from fungi, yeast and bacteria. In Patterson RLS, Charlwood BV, MacLeod G, Williams AA (eds.), *Bioformation of Flavours*. London: Royal Society of Chemistry, pp. 21–32.

Bluemke W, Schrader J. 2001. Integrated bioprocess for enhanced production of natural flavors and fragrances by *Ceratocystis moniliformis*. *Biomol Eng* 17:137–42.

Cadwallader KR, Braddock RJ, Parish ME, Higgins DP. 1989. Bioconversion of (+)-limonene by *Pseudomonas gladioli*. *J Food Sci* 54(5):1241–5.

Chalier P, Crouzet J. 1998. Enantiodifferentiation of four lactones produced by *Penicillium roqueforti*. *Chirality* 10:786–90.

Cheetham PSJ. 1993. The use of biotransformations for the production of flavours and fragrances. *Trends Biotechnol* 35:478–88.

Cheetham PSJ, Quail MA. 1991. Process for preparing L-rhamnose. U.S. Patent 5,077,206.

Dahlen T, Hauck T, Wein M, Schwab W. 2001. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone as a secondary metabolite from D-fructose-l,6-diphosphate metabolism by *Zygosaccharomyces rouxii*. *J Biosci Bioeng* 91(4):352–8.

Demyttenaere JCR, Kimpe N. 2001. Biotransformation of terpenes by fungi study of the pathways involved. *J Mol Catal B Enzym* 11(4-6):265–70.

Demyttenaere JCR, Potter HL. 1996. Biotransformations of geraniol and nerol by spores of *Penicillium italicum*. *Phytochemistry* 41(4):1079–82.

Engel K-H, Roling I. 1996. Generation of flavors by microorganisms and enzymes: An overview. In Takeoka GR, Teranishi R, Williams PJ, Kobayashi A (eds.), *Biotechnology for Improved Foods and Flavors*. Washington DC: American Chemical Society, pp. 120–3.

Feron G, Bonnarme P, Durand A. 1996. Prospects for the microbial production of food flavor. *Trends Food Sci Technol* 7:285–93.

Franssen MCR, Alessandrini L, Terraneo G. 2005. Biocatalytic production of flavors and fragrances. *Pure Appl Chem* 77(1):273–9.

Gomes N, Aguedo M, Teixeira J, Belo I. 2007. Use of castor oil for aroma production by the yeast *Yarrowia lipolytica*: Optimization of operating conditions. *J Biotechnol* 131(2):S169.

Geusz SD, Anderson DM. 1991. Bacteria that metabolize phenylacetate through mandelate. US 4999292 A 910312.

Grivel F, Larroche C, Gros JB. 1999. Determination of the reaction yield during biotransformation of the volatile and chemically unstable compound β-ionone by *Aspergillus niger*. *Biotechnol Prog* 15(4):697–705.

Groguenin A, Waché Y, Garcia EE, Aguedo M, Husson F, LeDall MT, Nicaud JM, Belin JM. 2004. Genetic engineering of the β-oxidation pathway in the yeast *Yarrowia lipolytica* to increase the production of aroma compounds. *J Mol Catal B Enzym* 28:75–9.

Hagedorn S, Kaphammer B. 1994. Microbial biocatalysis in the generation of flavor and fragrance chemicals. *Annu Rev Microbiol* 48:773–800.

Hawthorne DB, Shaw RD, Davine DF, Kavanagh TE, Clarke BJ. 1991. Butyric acid off-flavors in beer: Origins and control. *J Am Soc Brew Chem* 49:0004.

Janssens L, De Pooter HL, Schamp NM, Vandamme EJ. 1992. Production of flavours by microorganisms. *Process Biochem* 27:195–215.

Krasnobajew V, Helminger D. 1982. Fermentation of fragrances: Biotransformation of β-ionone by *Lasiodiplodia theobromae*. *Helv Chim Acta* 65:1590–601.

Kong Q, He GQ, Chen F, Ruan H. 2006. Studies on a kinetic model for butyric acid bioproduction by *Clostridium butyricum*. *Lett Appl Microbiol* 43, 71–7.

Krings U, Berger RG. 1998. Biotechnological production of flavours and fragrances. *Appl Microbiol Biotechnol* 49:1–8.

Krings U, Hinz M, Berger RG. 1996. Degradation of [2H]phenylalanine by the basidiomycete Ischnoderma benzoinum. *J Biotechnol* 51:123–9.

Lavid N, Schwab W, Kafkas E, Koch-Dean M, Bar E, Larkov O, Ravid U, Lewinsohn E. 2002. Aroma biosynthesis in strawberry: *S*-adenosylmethionine:furaneol *O*-methyltransferase activity in ripening fruits. *J Agric Food Chem* 50(14):4025–30.

Longo MA, Sanromán MA. 2006. Production of food aroma compounds: Microbial and enzymatic methodologies. *Food Technol Biotechnol* 44:335–53.

MacKintosh RW, Fewson CA. 1987. Microbial aromatic alcohol and aldehyde dehydrogenases. *Prog Clin Biol Res* 2232:259–73.

Pollock JA, Clark KM, Martynowicz BJ, Pridgeon MG, Rycenga MJ, Stolle KE, Taylor SK. 2007. A mild biosynthesis of lactones via enantioselective hydrolysis of hydroxynitriles. *Tetrahedron Asymmetry* 18(16):1888–92.

Rodin JO, Himmel RM, Silverstein RM, Leeper RW, Gartner WA. 1965. Volatile flavor and aroma components of pineapple. *J Food Sci* 30:280–5.

Shimizu S, Kataoka M, Honda K, Sakamoto K. 2001. Lactone-ring-cleaving enzymes of microorganisms: Their diversity and applications. *J Biotechnol* 92:187–94.

Sowden RJ, Yasmin S, Rees NH, Bell SG, Wong LL. 2005. Biotransformation of the sesquiterpene (+)-valencene by cytochrome $P450_{cam}$ and $P450_{BM-3}$. *Org Biomol Chem* 3:57–64.

Suhara Y, Itoh S, Ogawa S, Yokose K, Sawada T, Sano T, Nimomiya R, Maruyama HB. 1981. Regio-selective 10-hydroxylation of patchoulol, a sesquiterpene, by *Pithomyces* species. *Appl Environ Microbiol* 42:187–91.

Vandamme EJ, Soetaert W. 2002. Bioflavours and fragrances via fermentation and biocatalysis. *J Chem Technol Biotechnol* 77:1323–32.

Vanderhaegen B,·Neven H, Coghe S, Verstrepen KJ, Derdelinckx G, Verachtert H. 2003. Bioflavoring and beer refermentation. *Appl Microbiol Biotechnol* 62:140–50.

Waché Y, Aguedo M, Choquet A, Gatfield IL, Nicaud JM, Belin JM. 2001. Role of β-oxidation enzymes in γ-decalactone production by the yeast *Yarrowia lipolytica*. *Appl Environ Microbiol* 67(12):5700–4.

Waché Y, Laroche C, Bergmark K, Møller-Andersen C, Aguedo M, Dall MT, Wang H, Nicaud JM, Belin JM. 2000. Involvement of acyl coenzyme A oxidase isozymes in biotransformation of methyl ricinoleate into γ-decalactone by *Yarrowia lipolytica*. *Appl Environ Microbiol* 66(3):1233–6.

Welsh FW, Murray WD, Williams RE. 1989. Microbiological and enzymatic production of flavor and fragrance chemicals. *Crit Rev Biotechnol* 9(2):105–69.

Yu L, Xu Y, Wang X, Yu X. 2007. Highly enantioselective hydrolysis of dl-menthyl acetate to l-menthol by whole-cell lipase from *Burkholderia cepacia* ATCC 25416. *J Mol Catal B Enzym* 47:149–54.

CHAPTER 9

Plant Metabolic Pathways and Flavor Biosynthesis

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INTRODUCTION

Although there are many factors that contribute to the flavor of a particular plant product and their interaction is highly complex (Wyllie 2008), it is generally accepted that flavor is a combination of two main sensations: taste and aroma. The main compounds detected by taste in fruits and vegetables are sugars and organic acids that produce sweet and sour sensations, though other minor components may be associated with other taste sensations such as bitterness, astringency, or saltiness. In most fresh products, there is no doubt about the predominance of the aroma sensation over the taste sensation. The chemical nature of most fruit and vegetable flavors has been characterized in the past 40 years by means of new and sensitive isolation methods and the extended use of gas and liquid chromatography coupled to mass spectrometry. However, the knowledge of the biochemical pathways involved in flavor formation is still a step behind.

This chapter aims to present a general vision of the main biochemical pathways involved in the biosynthesis of key flavor compounds produced by fruits and vegetables: aldehydes, alcohols, ketones, esters, terpenes, furanones, glucosinolates, alk(en)yl cysteine sulfoxides, and phenolic compounds. Most of these compounds derive from essential nutrients or health-promoting compounds, suggesting that flavor components could provide important information about the nutritional makeup of plant products (Goff and Klee 2006). The structure of the chapter will follow the classical scheme, initially proposed by Tressl and others (1975), frequently used in reviews on this topic in which flavor formation is studied within the metabolism of the three main groups of flavor precursors in plants: fatty acid (FA) metabolism, amino acid metabolism, and carbohydrate metabolism with an additional section specifically dedicated to the biosynthesis of volatile esters.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

FA METABOLISM

FAs are the major precursors of volatile compounds responsible for the aroma of most plant products. They are catabolized through two main oxidative pathways: β-oxidation and the lipoxygenase (LOX) pathway. It has been suggested that β-oxidation is the main metabolic pathway producing primary aroma in fruits, whereas the LOX pathway may account for the widest variety of aroma compounds from FAs in disrupted fruit tissues. Nevertheless, some studies suggest that increasing availability of FA, along with higher membrane permeability, during fruit ripening might allow the LOX pathway to become active in intact plant tissues and to function as an alternative to β-oxidation (Villatoro et al. 2008).

β-Oxidation

A recent review by Baker and others (2006) describes varied roles for this pathway in relation not only to FA catabolism but also to amino acid metabolism and biosynthesis of hormonal compounds. Unfortunately, the information related to β-oxidation in fruits and vegetables is not based on a detailed study of the enzymes involved but on simple incubation experiments with cold or labeled FA of different fruit tissues (see the review by Sanz et al. 1997). In this metabolic pathway, acyl-coenzyme A (CoA) derivatives of FA are metabolized to shorter-chain acyl-CoAs by loosing two carbons at every round of the cycle involving, in the case of saturated FA, the following enzymes: acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA-dehydrogenase, and acetyl-CoA acetyltransferase. The two hydrogenation steps require FAD and NAD, respectively, while free CoA is needed for the scission step. β-Oxidation of unsaturated FA involved the actuation of auxiliary enzymes, such as enoyl-CoA isomerase (Goepert et al. 2005) and 3-hydroxyacyl-CoA-epimerase that could be implicated in the formation of the different enantiomeric forms of 3-hydroxy acid esters found in tropical fruits. Pear and apple aromas have been two classical examples of volatile formation through the β-oxidation pathway (Paillard 1990).

The biosynthesis of lactones, key aroma components in fruits such as peach and nectarine (γ-decalactone and γ-dodecalactone), pineapple (δ-octalactone), or coconut (γ-octalactone), is also associated with the β-oxidation pathway (Tressl and Albrecht 1986). In fact, most hypotheses on lactone biosynthesis in fruits put in contact the two major pathways producing aroma compounds from FA, β-oxidation, and LOX (Sanz et al. 1997). Despite the importance of these compounds in fruit aroma, there is a lack of enzymatic studies in fruits, and microorganisms serve as a model for studying lactone biosynthesis (see the review by Pérez and Sanz 2008). More recently, it was shown for the first time that enzymes present in apple and artichoke could be used as an effective agent for the lactonization reaction (Olejniczak et al. 2003).

LOX Pathway

The metabolism of polyunsaturated fatty acids (PUFAs), via the first LOX-catalyzed step and the subsequent reactions, is commonly known as the LOX pathway. LOX is the key enzyme in this pathway and catalyzes the regio- and stereospecific

hydroperoxidation of PUFAs with a (1*Z*,4*Z*) pentadiene structure. Linoleic (LA) and linolenic (LNA) acids are the main substrates of LOX in the plant kingdom. The Z double bond attacked by oxygen moves into conjugation with the neighboring Z double bond and assumes an E configuration. Depending on the plant source and the isoenzymes present, oxygen incorporation can occur preferentially at C-9, C-13, or at either C-9 or C-13 in a nonspecific manner (Vick and Zimmerman 1987). In plants, the hydroperoxy-PUFAs initially synthesized by LOX are substrates of different enzyme families that generate an important number of compounds collectively known as oxylipins. In the past few years, the knowledge of the function of LOX and oxylipins in plants, especially in relation to plant defense mechanisms, has increased considerably (Feussner and Wasternak 2002; Matsui 2006). In this chapter, only those metabolic branches related to fruit aroma biosynthesis will be discussed.

An overall view of aroma formation through LOX pathway is shown in Figure 9.1. The enzymatic oxidative degradation is preceded by the action of

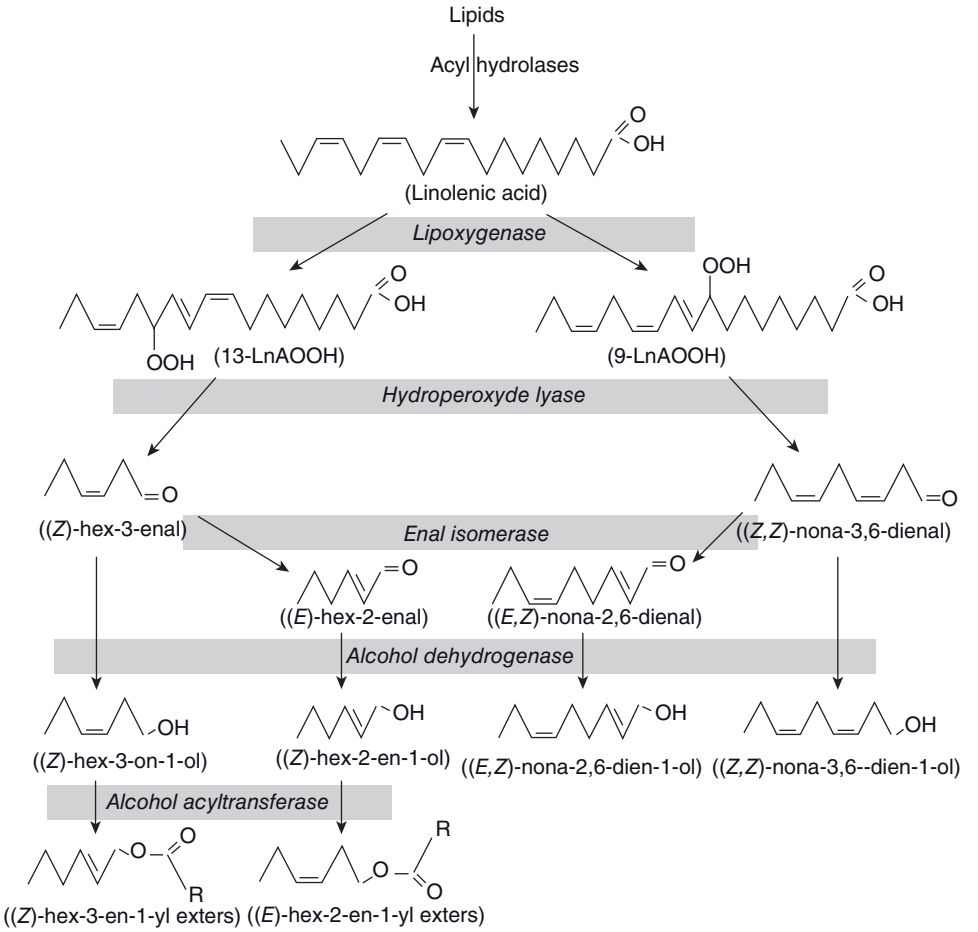

Figure 9.1. Flavor formation through the lipoxygenase pathway.

acylhydrolases, which liberate PUFAs from triacylglycerols, phospholipids, or glycolipids. Though most plant LOXs prefer free FA as substrates, the activity of some LOXs with esterified PUFAs in phospholipids or triglycerides have been demonstrated (Matsui et al. 1998). Typical LOX contains a nonheme iron atom in the active site that alternates between Fe^{2+} and Fe^{3+}, having a molecular weight of around 75–95 kDa (Sanz et al. 1997). A number of plant LOX sequences are now available, making the elucidation of the relationship between both LOX sequences and structures and their regiospecificity and activity possible (Casey and Hughes 2004). According to their overall sequence similarity, plant LOXs can be classified into two families. Those having no transit peptide and having high sequence similarity are type 1-LOX, and those having a putative chloroplast transit peptide sequence are type 2-LOX. All plants contain multiple isoenzymic forms of LOX. Thus, five tomato LOX genes have been shown to be expressed during fruit ripening, four of them mainly producing 9-hydroperoxides, while TomloxC, a chloroplastic isoform, can use both LA and LNA and produces 13-hydroperoxide products critical for biosynthesis of tomato aroma (Chen et al. 2004).

The LOX pathway has long been recognized as the one responsible for the generation of "green" odor notes in plant products such as tomatoes (Chen et al. 2004), apples (Defilippi et al. 2005a), cucumbers (Feussner and Kindl 1992), *Brassica* vegetables (Connor et al. 2008), peas and beans (Casey and Hughes 2004), bell peppers (Shibata et al. 1995), bananas (Jayanty et al. 2002), strawberries (Pérez et al. 1999b), or olives (Olías et al. 1993; Salas et al. 2000). The review by Hatanaka (1993), focusing on volatile biosynthesis in green leaves, is an excellent guide to understand the catalytic and mechanistic aspects related to the LOX pathway in any plant material. Though some readily volatile compounds (C5 carbonyls) might be generated through an additional branch of the LOX pathway from LNA (Gardner et al. 1996; Kondo et al. 1995), the first volatile compounds formed in this pathway come from the cleavage of FA hydroperoxides catalyzed by the enzyme hydroperoxide lyase (HPL). HPL is a membrane-bound enzyme whose presence has been demonstrated in many plant products (Matsui 2006; Vick and Zimmerman 1987). The cleavage catalyzed by HPL gives rise to C6 or C9 carbonyl compounds and the corresponding oxoacids (Hatanaka et al. 1986). Products from 13-hydroperoxylinoleic acid are hexanal and 12-oxo-(9*Z*) dodecenoic acid. When 13-hydroperoxylinolenic acid is the substrate, the same oxoacid is obtained and the aldehyde is (3*Z*)-hexenal. The products of HPL with 9-hydroperoxides of LA and LNA are the 9-oxononaic acid and (3*Z*)-nonenal or (3*Z*,6*Z*)-nonadienal, respectively. HPL enzymes belong to the cytochrome P450 protein family and have been characterized in different plants such tomato (Riley et al. 1996), bell pepper (Shibata et al. 1995), melon (Tijet et al. 2001), potato (Vancanneyt et al. 2001) citrus fruits (Gomi et al. 2003), watermelon (Fukushige and Hildebrand 2005), guava (Tijet et al. 2000), strawberry (Pérez et al. 1999b), and olive (Salas and Sanchez 1999).

In the last few years, several studies have provided new insights of the relative contribution of LOX and HPL in terms of aldehyde production. Thus, it is likely that hexanal and 3-hexenal production is determined by substrate availability to HPL rather than by the abundance of HPL activity, which seems to be constitutively present (Vancanneyt et al. 2001). LOX depletion gives rise to a marked reduction of C6- and C5-aldehydes and alcohols (Leon et al. 2002). Several attempts to modify

tomato aroma, by acting on the LOX pathway have been carried out with different results. Genetic manipulation in order to modify FA composition (Wang et al. 1996), or alcohol dehydrogenase (ADH) (Speirs et al. 1998), effectively changed the aroma profile of transformed tomato fruits, while antisense suppression of LOX (Griffiths et al. 1999) or overexpression of a 9-HPL did not have a significant effect (Matsui et al. 2001). The silencing of LOX and HPL have been performed in potato plants with downregulation of HPL inducing a decrease of C6 compounds and a parallel increase in LOX activity and of the content of most C5 volatiles (Salas et al. 2005). Myung and others (2006), studying the aldehyde biosynthesis in strawberry, hypothesized that fruits may exhibit different metabolic flows through the pathway of LOX and HPL for the production of *Z*,3-hexenal and *E*,2-hexenal. More recently, a genomic study carried out in apple points to a different regulation for LOX and HPL with selected LOX1 and LOX7 genes being induced by ethylene, while none of the identified HPL genes were ethylene regulated (Schaffer et al. 2007).

In relation to product specificity, it is well documented that in most plant products, HPL specificity determines aroma composition despite the specific action of LOX. Thus, cucumber LOX produces 13/9-hydroperoxides in a ratio of 85:15, while cucumber HPL exhibits the higher specificity for the later substrate, explaining the important amount of C9 compounds in the aroma of cucumber fruit (Matsui et al. 2000). The biosynthesis of virgin olive oil aroma through the actuation of olive LOX/HPL is another example of the different importance of LOX and HPL in terms of volatile composition (Luaces et al. 2007; Salas et al. 2000; Sánchez-Ortiz et al. 2007).

In most plants, compounds with a (*Z*,3)-enal structure are rapidly isomerized to the (*E*,2)-enal form. There is no clear consensus on the chemical or enzymatic nature of this isomerization step. Before or after the isomerization of unsaturated carbonyls formed by HPL, ADH catalyzes the reversible reduction of C6-aldehydes to C6-alcohols in a reaction dependent on pyridine nucleotides. In olive pulp, Salas and Sanchez (1998) characterized an NADP-dependent ADH present in the pulp tissue of developing fruits displaying a clear preference for C6- and C9-aldehydes. ADH is a metalloprotein possessing sulfhydryl groups in the catalytic site and with two probably identical subunits of molecular weight 45 kDa that have been characterized in different fruits (Pérez and Sanz 2008). Most ADH genes expressed in fruit isolated so far belong to the medium-chain zinc-binding subfamily of ADHs. In most fruits, the specificity of this enzyme is not a limiting factor for the biosynthesis of volatile compounds from FA metabolism (Defilippi et al. 2005b), though a change in ADH activity effectively alters the balance between C6-aldehydes and alcohols and might affect HPL regulation (Speirs et al. 1998). Very recently, two new ADHs have been isolated and characterized in melon (Manriquez et al. 2006). Both are positively regulated by ethylene and operate preferentially as reductases of aldehydes into alcohols that are indeed substrates for the biosynthesis of volatile esters in melon (El-Sharkawy et al. 2005). Alcohols resulting from ADH activity are natural substrates for the ester-forming enzyme, alcohol acyltransferase (AAT). Thus, saturated and unsaturated C6-alcohols formed through the LOX pathway can be esterified with acyl-CoA moieties to produce hexyl, (2,*E*)-hexenyl, and (3,*Z*)-hexenyl esters (Olías et al. 1993; Salas 2004). The occurrence and characteristics of the enzyme AAT will be described at the end of the chapter.

AMINO ACID METABOLISM

From a quantitative point of view, amino acids represent the second largest source of volatile compounds contributing to the aroma of fruits and vegetables. They should be considered from two different points of view: as direct precursors of aroma compounds, generating aliphatic, branched or aromatic alcohols, carbonyls, acids, and esters; or as indirect precursors, forming nonvolatile compounds that need a second enzymatic transformation upon cell disruption to form volatiles. The latter mechanism is mainly present in vegetables and gives rise to glucosinolates and alkenyl cysteine sulfoxides that will be described at the end of this section.

Radioactive labeling studies have proved the transformation of amino acids such as alanine, leucine, phenylalanine, or aspartic acid into volatile compounds (see the review by Sanz et al. 1997). This aroma biosynthesis pathway comprises three enzymatic activities: aminotransferase, decarboxylase, and ADH. Tressl and Drawert's studies on banana showed that 14C-leucine is converted into labeled 3-methylbutanol, 3-methylbutanoic acid, and 3-methylbutyl esters (Fig. 9.2). The first step of transamination was validated after the identification of the corresponding intermediary 2-oxoacid and the isolation of alanine 2-oxoglutarate aminotransferase in tomato (Gazeu-Reyjal and Crouzet 1976). This enzyme seems to be partially bound to the mitochondrial membrane, exhibits a molecular weight of 100 kDa, and requires pyridoxal-5-phosphate (PALP) as cofactor, although a tight PALP-apoenzyme linkage is demonstrated as no added PALP is needed for *in vitro* maximum activity. The mechanism of action seems to be in good agreement with those elucidated for other plant and animal aminotransferases (Givan 1980). The next step, the decarboxylation of the 2-oxoacid formed after amino acid transamination, occurs via an enzymatic complex similar to pyruvate dehydrogenase or 2-oxoglutarate dehydrogenase from the tricarboxylic acid (TCA) cycle, involving

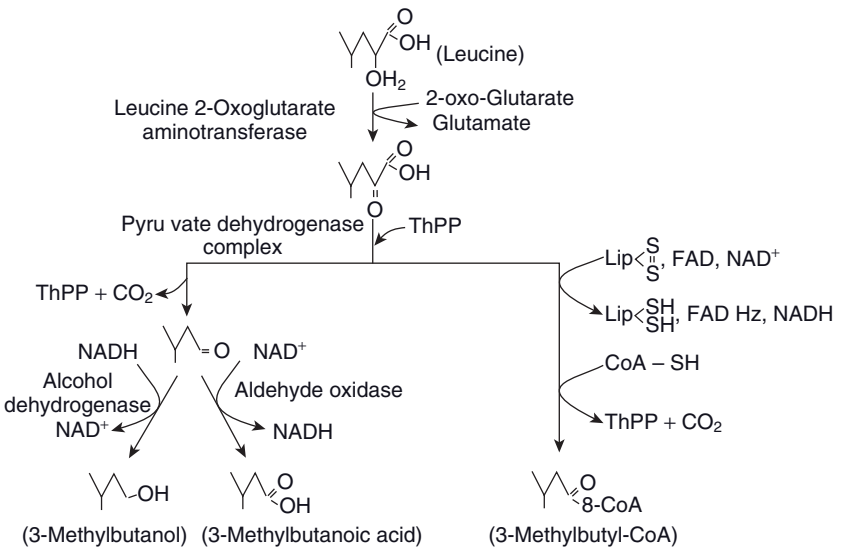

Figure 9.2. Schematic pathway for the formation of volatiles from amino acids (from Sanz et al. 1997).

thiamine pyrophosphate (ThPP), lipoic acid, FAD, NAD, and CoA as cofactors. This complex would produce 3-methylbutanoyl-CoA from leucine, substrate for the biosynthesis of 3-methylbutanoate esters. The involvement of ThPP as the solo cofactor would give rise to 3-methylbutanal, which is reduced to alcohol by ADH using NADH as cofactor (see the review by Sanz et al. 1997). Similar enzymatic transformations have been described for amino acids other than leucine. Isoleucine is a reported precursor of 2-methylbutanol and 2-methylbutyric acid in melon (Wang et al. 1996), apple (Rowan et al. 1996), and strawberry (Pérez et al. 2002). Valine is a reported precursor of 2-methyl propyl esters in banana and tomato (Buttery and Ling 1993; Tressl and Drawert 1973), and alanine is presumably the source of ethyl esters in strawberry (Pérez et al. 1992). Phenylalanine can also be metabolized through this pathway to 2-phenylacetyl-CoA, and this compound is converted into esters of a variety of alcohols, or reduced to 2-phenylethanol and transformed into 2-phenylethyl esters as has been demonstrated in different fruits (Tikunov et al. 2005; Tressl and Albrecht 1986).

It is important to point out that the final product of the decarboxylation, the acyl-CoA or the corresponding aldehyde, could depend on the species (see the review by Pérez and Sanz 2008). Thus, 2-oxopentanoic acid was described as a powerful alkylating agent in strawberry, while incubation of apple disks with this compound produced more butanoate (67%) than butyl esters (21%). Nevertheless, contradictory data have been reported on the relative contribution of each catabolic branch to the aroma of different fruits such as strawberries or apples. Both decarboxylating branches seem to have different regulatory mechanisms. Thus, Bauchot and others (1998), working on 1-aminocyclopropane-1-carboxylate (ACC) oxidase antisense melons, found that those pathways leading to the formation of ethyl esters with branched-chain acyl groups from amino acids were more strongly regulated by ethylene than those forming acetates with branched-chain alcohol moieties.

Although this relationship between the formation of aroma volatiles and the free amino acid pool present in ripening fruits and vegetables is well established, volatile formation is not only determined by substrate availability (Wyllie et al. 1996) but also depends on the relative activities of the 2-oxoacid decarboxylase/dehydrogenase type enzymes in the fruit. These enzymatic activities could be affected by cultivar, maturity stage, and even environmental conditions, either on or off the plant. In this sense, strawberry cultivar variations in two key aroma enzymes, ADH and pyruvate dehydrogenase, account for the different susceptibility to off-flavor development, that is, ethanol, acetaldehyde, and ethyl acetate production (Fernandez-Trujillo et al. 1999; Ke et al. 1994; Watkins et al. 1999). Despite the importance of ADH for fruit aroma biosynthesis, in most fruits, the specificity of ADH is not a limiting factor (Defilippi et al. 2005b; Mitchell and Jelenkovic 1995; Wyllie et al. 1996), while the decarboxylating step seems to be critical for the release of ester precursors. The biochemical and molecular characterization of fruit decarboxylases should provide valuable information to fully elucidate volatile formation from amino acid metabolism (Moyano et al. 2004; Tieman et al. 2006).

Phenolics

Aromatic amino acids may also serve as important precursors leading to a big family containing more then 8000 compounds: plant phenolics (Robbins 2003). Bitter,

sweet, pungent, and astringent tastes have been attributed to individual phenolics, and they can also contribute to aroma (Jones 2008). From a strict point of view, phenolic biosynthesis commences from the shikimate pathway using intermediates from carbohydrate metabolism up to phenylalanine biosynthesis (Herrmann and Weaver 1999). After phenylalanine synthesis, the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5) transforms it into cinnamic acid through nonoxidative deamination in the three steps of general phenylpropanoid metabolism (Robbins 2003). Metabolic pathways of plant phenolics are particularly complex with multiple alternative metabolic fates that may vary markedly from tissue to tissue, from one growing condition to another and in response to environmental stimuli (Macheix et al. 1990; Ryan et al. 2002). A simplified scheme of the biosynthesis of plant phenolics is shown in Figure 9.3. Cinnamic and benzoic acids are present in most plant tissues, linked to structural components such as the cell wall. Chlorogenic acids are esters between *trans*-cinnamic acid derivatives and quinic acid, also known as hydroxycinnamates. The most common is 5-*O*-caffeoyl-quinic acid (5-CQA), but related compounds with many different acyl groups have been identified (Clifford 2000). As shown in Figure 9.3, *p*-coumaric acid is the common precursor of two metabolic branches giving rise to coumarins and flavanones. Caffeic acid derivatives and flavonoids are the main contributors to lettuce flavor (DuPont et al. 2000), and a group of coumarin-like compounds known as secoiridoids determine the flavor of olive fruit and its derivatives such as virgin olive oil (Mateos et al. 2004).

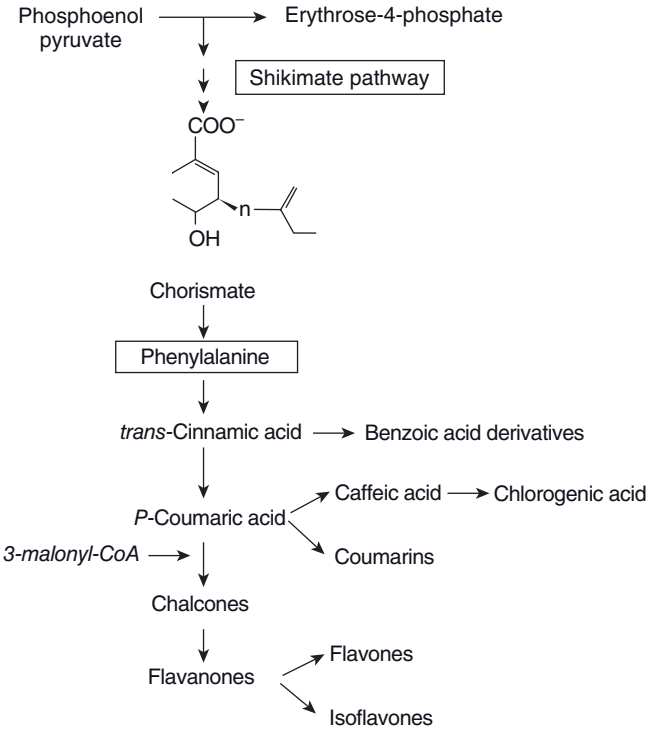

Figure 9.3. Schematic biosynthesis for plant phenolics.

An important consideration about plant phenolics is that most phenolics are stored in plant tissues as glycosidic conjugates so that the actuation of endogenous glycosidases is critical for the release of the flavor-active components.

A phenolic compound, the *p*-coumaric acid, is also involved in the biosynthesis of *p*-hydroxyphenylbutan-2-one (*p*-HPB), also known as raspberry ketone (Borejsza-Wysocki and Hrazdina 1994). *p*-HPB biosynthesis consists of two enzymatic steps identified in raspberry crude extracts. The first one is a condensation reaction of *p*-coumaryl-CoA with malonyl-CoA catalyzed by *p*-hydroxyphenylbut-3-en-2-one synthase, releasing CoA and carbon dioxide. Then, an NADPH reductase gives rise to *p*-HPB.

Glucosinolates

The glucosinolates are the sulfur-containing precursors of volatile and reactive flavor compounds, such as nitriles and isothiocyanates, found within the order Capparales of the Brassicaceae. There are more than 120 glucosinolates known that have been described as defense compounds with well-documented toxicity and feeding-aversion effects, in addition to anticarcinogenic properties. The biosynthetic pathway for glucosinolates starting from aromatic and aliphatic amino acids (alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine) was initially investigated by means of isotopic labeling studies (Sanz et al. 1997) and only recently completed by using information provided by molecular and genomic studies (Grubb and Abel 2006). The first step of glucosinolates biosynthesis (review by Jones 2008) is the oxidation of the amino acids to their aldoximes by substrate-specific cytochrome P450 of the CYP79 family (Fig. 9.4). The next step is the further oxidation of the aldoxime by cytochrome P450 from the CYP83 family

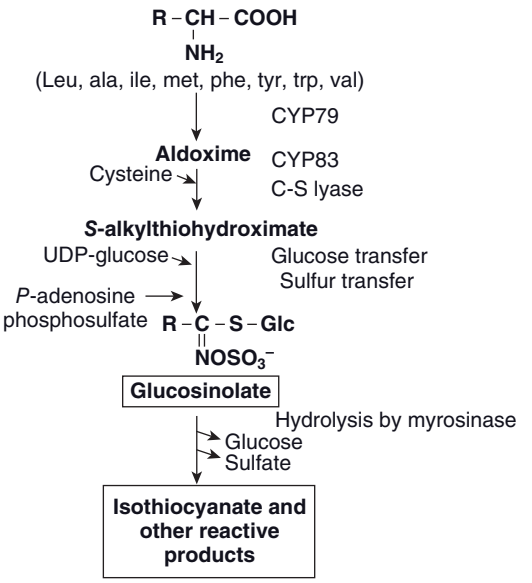

Figure 9.4. Biosynthesis of glucosinolates and their hydrolysis products.

and conjugation to cysteine as S-donor with cleavage, probably by a C-S lyase, to give a thiohydroximic acid. This is followed by transfer of glucose from uridine diphosphate (UDP)-glucose (generating thioglucose) and finally by transfer of sulfate from phosphoadenosine-phosphosulfate to create a sulfonated oxime and to finish the GS core. Only a few enzymes of the glucosinolates biosynthesis pathway have been fully characterized (Halkier and Gershenzon 2006). Plants containing glucosinolates also contain enzymes degrading these compounds called myrosinases. As may be inferred from their systematic name, thioglucohydrolase EC 3.2.3.1., myrosinases catalyze the hydrolysis of the thioglucosidic linkage in glucosinolates. Myrosinases are located within special cells called myrosin cells, separated from glucosinolates. Thus, flavor development in vegetables such as broccoli, cauliflower, or Brussels sprouts depends on tissue damage and rupture of the cell structure (Baik et al. 2003; Engel et al. 2002). Myrosinases have been extensively purified from various plants, and the presence of isoenzymes has been established in a number of cases. Generally speaking, myrosinases are glycoproteins containing sulfhydryl groups, which are essentials for the catalytic activity, with a molecular weight of 125,000–150,000 Da and composed of two or four monomers. Myrosinase isoforms are characterized by their different requirements for ascorbic acid that may promote either activation or inhibition, or may have no effect on myrosinase activity (Sanz et al. 1997). The products of myrosinase hydrolysis are glucose, sulfate, and unstable aglucone intermediates that undergo further changes to yield the real flavor compounds: isothiocyanates, nitriles, and other compounds depending on pH, metal ions, and further protein factors (Bones and Rossiter 2006; Burow et al. 2006).

Alk(en)yl Cysteine Sulfoxides

These sulfoxide-containing cysteine derivatives are the precursors of the characteristic volatile (sulfur-containing) compounds of *Allium*. In fact, the most outstanding characteristic of the chemical composition of *Allium* plants is the unusually large amount of organically bound sulfur (1–5% dry weight) being their flavor dependent on the sweetness provided by sugars and the sensory notes associated to four cysteine sulfoxides: *S*-methyl cysteine sulfoxide (methiin; present in most *Allium*, and some Brassicaceae), *S*-allyl cysteine sulfoxide (alliin; characteristic of garlic), *S-trans*-prop-1-enyl cysteine sulfoxide (isoalliin; characteristic of onion), and *S*-propyl cysteine sulfoxide (propiin; in onion and related species) (Jones et al. 2004). Most of these compounds have been identified in garlic and onion, but they are also present in other plants of the family such as leek, chive, scallion, or shallot (Wang et al. 2008). Cysteine sulfoxides have been found to be present in free and in conjugated forms as *gamma*-glutamyl-*S*-alk(en)yl-*L*-cysteine sulfoxides. These peptides are rapidly hydrolyzed by either *gamma*-glutamyl peptidase or *gamma*-glutamyl transpeptidase, two enzymes that are widely distributed in plants. Lancaster and Shaw (1989) proposed a mechanism for the biosynthesis of various *gamma*-glutamyl-*S*-alk(en)ylcysteine sulfoxides where *gamma*-glutamyl-cysteine and glutathione were the starting compounds and *gamma*-glutamyl peptides were the immediate precursors of cysteine sulfoxides. A second alternative pathway, with analogies to cysteine synthesis, was initially proposed by Granroth (1970), through direct alk(en)ylation of cysteine or thioalk(en)ylation of *O*-acetyl cysteine in a similar way to the biosynthesis of several other secondary metabolites mediated by

O-serine acetyl transferase and cysteine synthase (Ikegami and Murakoshi 1994). Both biochemical pathways are supported by experimental data (Jones et al. 2004; McManus et al. 2005; Shaw et al. 2005), and the discrepancies between both hypotheses may indeed reflect different biosynthetic routes within different tissues as has been recently reviewed by Jones (2008).

The key enzymatic step in relation to the generation of aroma in allium is the cleavage of free alk(en)yl cysteine sulfoxides by the enzyme alliinase (alliin lyase, EC 4.4.1.4). This enzyme, also called alkylcysteine lyase, is a pyridoxal phosphate-dependent glycoprotein that yields pyruvate, ammonia, and reactive, volatile, sulfur compounds. *Allium* alliinases are constituted by monomers of about 51.5 kDa that seem to be associated into dimers, trimers, and tetramers to form a functional holoenzyme. Several alliinases have been purified, and their cDNA clones have been obtained from garlic, onion, and shallot (Do et al. 2004; Lancaster et al. 2000; Shimon et al. 2002). There is a similar enzyme called C-S lyase (EC 4.4.1.8) that differs from alliinase through its ability to cleave L-cystine as well as alk(en)yl cysteine sulfoxides, which is present in the cytosol and vacuole of all tissues of broccoli (Ukai and Sekiya 1999). Alliinases are compartmentalized in the vacuole, while their substrates are in the cytoplasm so that the catalysis products are only produced after tissue homogenization or crushing. Thiosulfinates are the primary sulfur compounds formed by alliinases that rapidly undergo spontaneous nonenzymatic reactions to form the large number of sulfurous compounds characteristics of the headspace of *Allium* and *Brassica* plants (Imai et al. 2002; Wang et al. 2008).

CARBOHYDRATE METABOLISM

There are two main groups of flavor compounds that come directly from carbohydrate metabolism: furanones and terpenes. Furanones, having a pleasant sweet aroma, contribute to the flavor of many fruits and vegetables such as strawberry and tomato. Volatile terpenes are the main components of many fruit essential oils and also contribute to the characteristic flavor of carrots.

Furanone Biosynthesis

The interest in furanones, and particularly in furaneol, the key component of this family of volatiles, during the last three decades has not only been due to their demonstrated contribution to many fruit aromas, but because it has also been detected in many heat-processed foods, for example, beef broth, roasted almonds, roasted coffee, wheat bread crust, or popcorn. Furaneol (2,5-dimethyl-4-hydroxy3[2H]-furanone) and its methylether derivative mesifurane (2,5-dimethyl-4-methoxy3[2H]-furanone) are important aroma compounds that have been identified in many fruits such as pineapple, raspberry, mango, arctic bramble, grapefruit, tomato, and strawberry (Sanz et al. 1997). Furaneol, with a low odor threshold (10 ppb) (Schwab and Roscher 1997), imparts caramel and burnt sugar notes at high concentrations and becomes fruity at lower concentrations, while mesifurane is described as having a more sherry-like aroma. Recent studies have also focused on other furaneol derivatives such as the norfuraneol (4-hydroxy-5-methyl-3[2H]-furanone) identified in raspberry, guava, and tomato (Hauck et al. 2002).

In fruits such as strawberry, at least four furaneol-derived compounds have been detected: free furaneol, mesifurane, furaneol-β-D-glucopyranoside, and the malonyl derivative of this furaneol glucoside (Roscher et al. 1996; Sanz et al. 1995; Zabetakis et al. 1999). Furaneol and mesifurane are considered character-impact compounds in the aroma of many fruits, while furaneol-glucoside and furaneol-malonyl-glucoside are nonvolatile compounds that might influence the overall flavor of fruits (Bood and Zabetakis 2002).

In the last decade, most studies on furaneol biosynthesis have been carried out in strawberry where this compound can reach a high concentration in fully ripe fruits (37 μg/g) (Pérez et al. 1996a). Pérez and others (1999a) found, by means of an *in vitro* growth system, an increase in furaneol content with time in those fruits grown in a medium supplemented with D-fructose-6-phosphate. Experiments using radioactively labeled substrates proved the transformation of the complete carbon chain of D-fructose into furaneol (Schwab 1998), and further studies with D-[2-2H]-glucose demonstrated the involvement of phosphohexose isomerase and confirmed that D-fructose-6-phosphate was a natural precursor of furaneol biosynthesis (Wein et al. 2001). Recently, Raab and others (2006) isolated and characterized an enzyme involved in the last step of furaneol biosynthetic pathway in strawberry. The protein purified from ripe strawberry fruits seems to be an enone oxidoreductase (FaQR) with a 37-kDa molecular mass, an optimum temperature of 37°C, and a broad pH optimum peaking at 7.0. The enzyme catalyzes a two-substrate reaction for which an apparent Km of 3.5 mM for D-fructose-1,6-diphosphate and 30 μM for NADH have been calculated. The ripening-induced increase of this enzymatic activity correlates with the observed furaneol accumulation during ripening. Based on the findings of this study, a new natural precursor (4-hydroxy-5-methyl-2-methylene-3[2H]-furanone) and a new scheme of furaneol biosynthesis have been suggested (see Pérez and Sanz 2008). In a similar way, D-ribulose-5-phosphate seems to be the norfuraneol precursor in tomato through a biosynthesis pathway in which 4,5-dihydroxy-2,3-pentanedione is considered to be a key intermediate (Hauck et al. 2002). More recently, an enone oxidoreductase has been cloned in *Solanum lycopersico* (SlEO), expressed in *Escherichia coli* and characterized by biochemical studies that confirm the involvement of SlEO in the biosynthesis of furaneol in tomato (Klein et al. 2007).

Furaneol is the precursor of mesifurane and furaneol glucoside (Roscher et al. 1997). Lavid and others (2002) identified an *O*-methyltransferase in strawberry capable of transferring a methyl group from *S*-adenosyl-L-methionine (SAM) to furaneol. The activity of this enzyme, with a native molecular mass of 80 kDa, optimum activity at pH 8.5 and 37°C, and an apparent Km of 5 mM for furaneol, also increases with fruit ripening (Lunkenbein et al. 2006). Though furaneol glucoside is formed at the latest stage of strawberry development (Sanz et al. 1995) and most studies proved that furaneol is the precursor of furaneol-glucoside (Roscher et al. 1997), the mechanism regulating the interconversion of furaneol into furaneol-glucoside is not fully understood.

Isoprenoid Pathway

The isoprenoid biosynthetic pathway generates both primary and secondary metabolites. Among the first are phytohormones, photosynthetic pigments such as

chlorophylls and carotenoids, ubiquinones, or sterols, and among those considered to be secondary metabolites are volatile terpenoids. Plants have two biosynthetic pathways leading to the central intermediates for all isoprenoids: the mevalonate (MVA) pathway within the cytosol and the methylerythritol phosphate pathway active in the plastids, both of them forming isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Mahmoud and Croteau 2002; Rodriguez-Concepción and Boronat 2002; Rohdich et al. 2003). Data on mevalonic acid pathway are consistent with the biosynthesis of one molecule of MVA from three of acetate through an enzymatic sequence including acetyl-CoA acetyltransferase, hydroxymethyl-glutaryl-CoA synthethase, and hydroxymethyl-glutaryl-CoA reductase in a two-step reaction requiring NADPH (Kleinig 1989). IPP is formed from MVA by a series of reactions shown in Figure 9.5. The first step is the synthesis

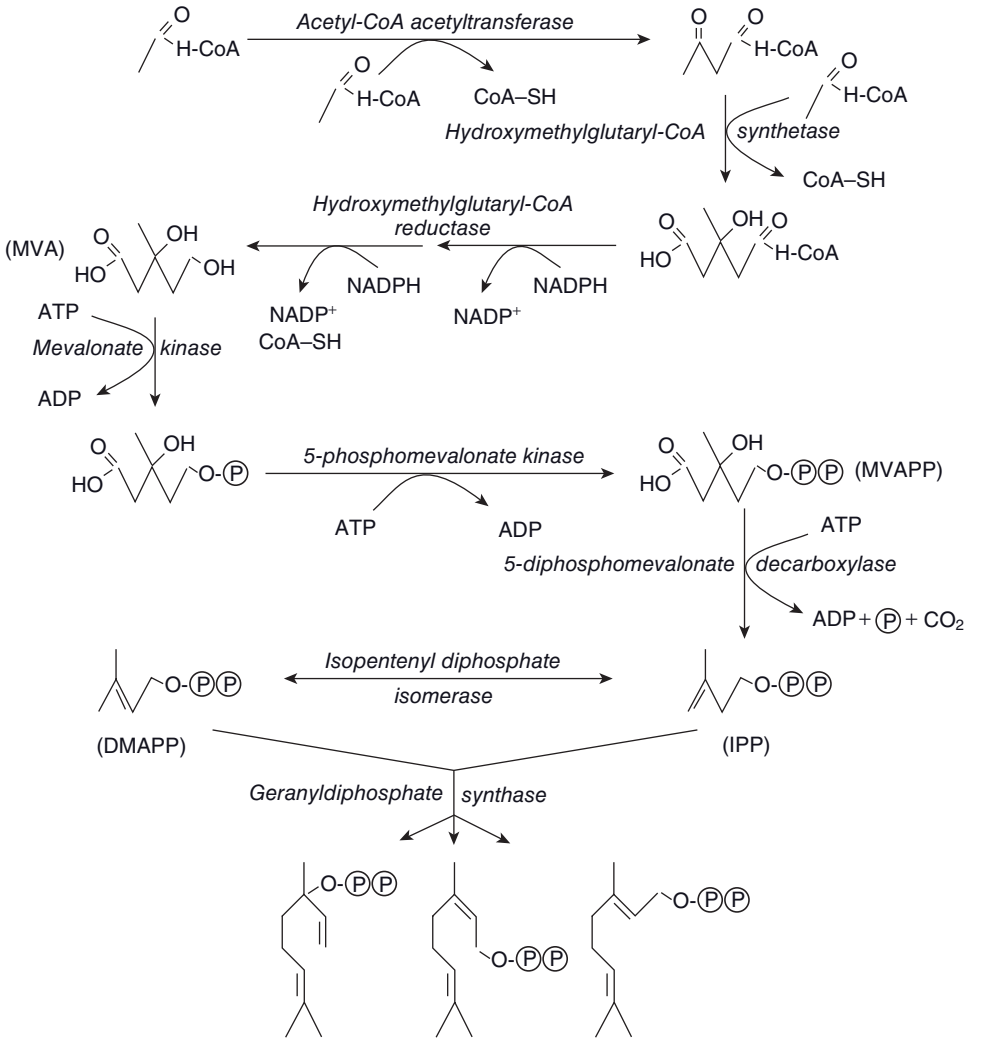

Figure 9.5. Terpene biosynthesis in the cytosol. The mevalonate (MVA) pathway.

of mevalonic acid 5-phosphate by the action of MVA kinase, an enzymatic activity demonstrated to be present in many plants. The final step in IPP biosynthesis is the decarboxylation and dehydration of mevalonic acid diphosphate (MVAPP). The biosynthesis of IPP through the methylerythritol-4-phosphate (MEP) pathway (Fig. 9.6) begins with the formation of 1-deoxy-D-xylulose-5-phosphate (DXP) catalyzed by DXP synthase (Estevez et al. 2001). In a second step, DXP reductoisomerase transforms DXP into MEP (Mahmoud and Croteau 2002). The enzyme catalyzing the last step of this pathway, hydroxymethylbutenyl diphosphate reductase, has been suggested as a rate-limiting step in the MEP pathway in tomato (Botella-Pavia et al. 2004). The MVA pathway is generally considered to supply the precursors for the production of sesquiterpenes and triterpenes, while the MEP pathway seems to supply the precursors for the production of monoterpenes, diterpenes, and tetraterpenes (Aharoni et al. 2005). Little is known, however, about the regulation of the

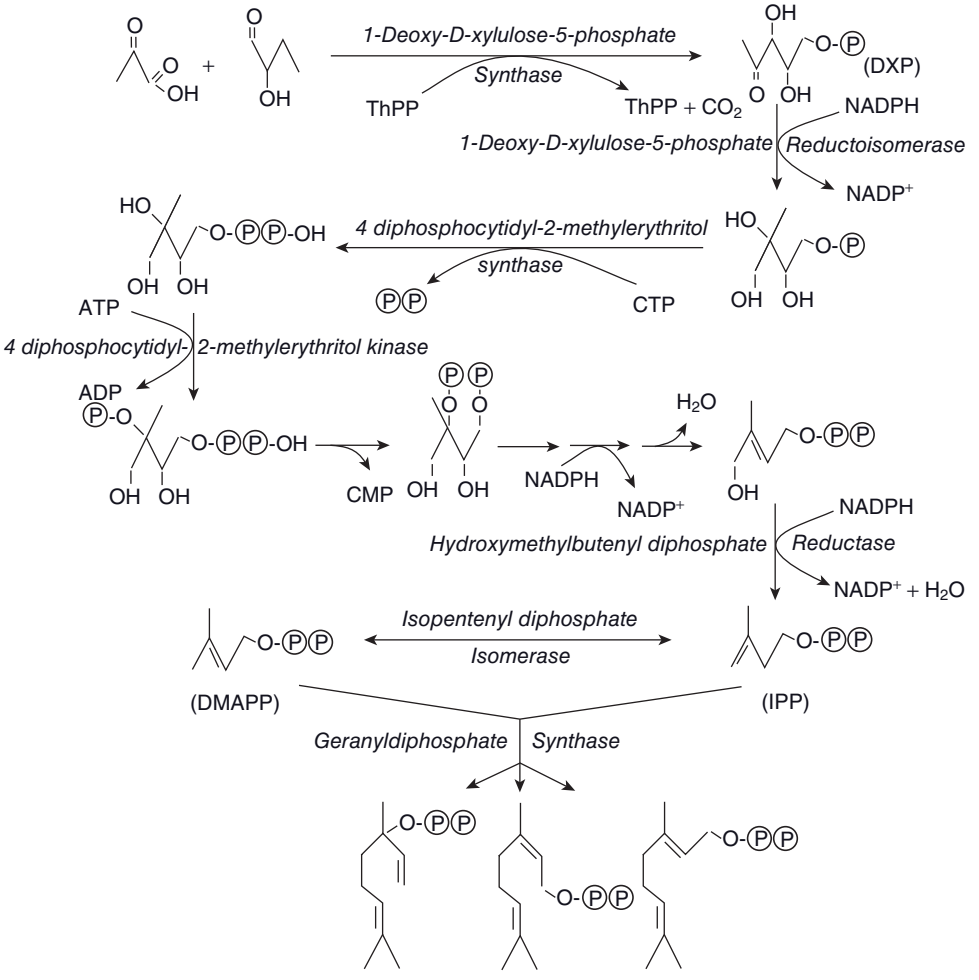

Figure 9.6. Terpene biosynthesis in the plastid. The methylerythritol-4-phosphate (MEP) pathway.

MEP pathway or the possible connection between these two biochemical routes, although limited unidirectional exchange of isoprene units, such as IPP, occurs between the plastid and cytosol. In this sense, an exporter of prenyl diphosphates across the plastid envelope membrane has been characterized in spinach by Bick and Lange (2003), and more recently, IPP, DMAPP, and geranyl diphosphate (GPP) transport has been studied in carrot (Hampel et al. 2005) and raspberry (Hampel et al. 2007).

In both biochemical pathways, IPP is used by prenyltransferases in condensation reactions to produce larger prenyl diphosphates such as GPP. This reaction needs the previous isomerization of IPP to DMAPP carried out by the enzyme IPP isomerase. This enzyme was initially purified and characterized in tomato and red pepper (see Sanz et al. 1997). Prenyltransferases produce GPP when DMAPP and IPP are substrates or farnesyl diphosphate (FPP) when GPP and IPP are substrates. Several terpene synthase genes have been recently identified in citrus fruits (Iijima et al. 2004; Lucker et al. 2002; Sharon-Asa et al. 2003), apple (Pechous and Whitaker 2004), and strawberry (Aharoni et al. 2004).

Monoterpenes are the main fruit aroma components from the isoprenoid family. Their biosynthesis is comparatively well understood. GPP is the direct precursor of their formation through a sequence of reactions including hydrolysis, cyclizations, and oxidoreductions (Lange and Croteau 1999; Schreier 1986). Cyclases, the key enzymes in this sequence, are soluble proteins with molecular weights in the range of 50–100 kDa, and requiring Mn^{2+} or Mg^{2+} as cofactors (Croteau 1992). The crucial role of cyclases in the origin of the different monoterpene structural groups has stimulated several studies on the stereochemical mechanism of cyclization (Schwab et al. 2001). Nonoxygenated monoterpenes are generally oxygenated in a series of steps involving cytochrome P450 systems and molecular oxygen (Sanz et al. 1997).

Significant progress has been made over the past few years on monoterpenoid metabolic engineering (Aharoni et al. 2005). A gene encoding *S*-linalool synthase has been expressed in tomato under the control of a late fruit-ripening promoter resulting in the accumulation of *S*-linalool and 8-hydroxy linalool in ripe tomato (Lewinsohn et al. 2001).

Norisoprenoids, derived from the breakdown of carotenoids generated as primary metabolites of the isoprenoid pathway (Wintelhalter and Rouseff 2002), are also important flavor components of some fruits and vegetables. Thus, although it is widely accepted that citral, a mixture of neral and geranial, is directly formed from GPP, a carotenoid-derived biosynthesis cannot be discounted in some fruits. This might be the case of tomato and watermelon, where citral is not present in fruits from those cultivars devoid of lycopene (Lewinsohn et al. 2005). More recently, Davidovich-Rikanati and others (2007) enriched tomato flavor through heterologous expression of a basil geraniol synthase that increased the accumulation of monoterpenes uncommom in tomato fruit (geraniol, citronellol, neric acid, and limonene). Although the enzymatic or nonenzymatic nature (Wache et al. 2003) of some carotenoid oxidative reactions has yet to be elucidated, some *in vitro* studies point to an effective participation of oxidative enzymatic activities such as peroxidase, LOX, or other dioxygenases in the formation of some classes of volatile terpenes (Bouvier et al. 2003; Giuliano et al. 2003; Wu et al. 1999). Thus, regiospecific carotenoid cleavage enzymes involved in the formation of aroma compounds such as geranyl acetone, pseudoionone, and β-ionone have been isolated in different

plants (Balderman et al. 2005; Fleischman et al. 2002, 2003; Schilling et al. 2008; Sinkim et al. 2004).

ESTER FORMATION

Volatile esters formed by esterification of alcohol and carboxylic acids constitute one of the largest and main group of volatile compounds identified in fruit aroma (Sanz et al. 1997). The biosynthesis of volatile alcohols and acids is generally well explained through the enzymatic routes previously described, but specific knowledge on the final esterification step has only been obtained in the last few years. The mechanism of ester formation was initially studied in microorganisms in which two different enzymes seem to be implicated: AAT and esterase. AAT catalyzes the transfer of an acyl moiety from an acyl-CoA intermediate onto the corresponding alcohol, while esterase functions mainly by hydrolyzing esters, although an ester-forming activity by esterase has also been reported (see the review by Pérez and Sanz 2008). Both enzymatic activities have been described in plants where ester formation seems to be a CoA-dependent reaction since the multiple carboxylesterases studied in plant tissues have exclusively hydrolytic activity against a wide range of esters with varying acyl chain length (Ileperuma et al. 2007). The ester-forming capacity of different fruits was initially studied using whole fruits or tissue disks. These early investigations showed that ester-forming activity was related to fruit ripening and gave initial information on the relationship between substrate specificity and ester composition (see review by Sanz et al. 1997).

The first AAT enzyme characterized in fruits, described as an alcohol acetyltransferase, was localized by Harada and others (1985) in banana pulp cells. A similar enzyme, only active on acetyl-CoA, was identified in apple (Fellman et al. 1991), while the third fruit AAT, active with different acyl-CoAs, was purified and characterized in strawberry (Pérez et al. 1993), where a clear correlation was observed between AAT activity and flavor quality of different strawberry cultivars along ripening (Olías et al. 2002; Pérez et al. 1996b). Olías and others (1995), using crude enzymatic extracts from banana and strawberry fruits, observed important differences in both fruits AAT activities toward short aliphatic alcohols and acyl-CoAs. In a similar study, Ueda and others (1992) also found a clear relationship between specificity of the ester-forming enzyme system of banana, melon, and strawberry and their characteristic aroma pattern. More recently, Holland and others (2005), also working with fruit extracts, found different enzyme levels and substrate specificities in AAT enzymes from two apple cultivars. AAT activity from Granny Smith fruits uses almost exclusively hexanol and *Z*,3-hexenol, while the enzyme from Fuji fruits, being considered one of the most aromatic apple cultivars, accepted a broader range of alcohols.

The level and/or characteristics of an enzyme responsible for the final step of the biosynthesis of a particular volatile are not the only limiting factor. This is particularly true in the case of enzymes such as the AAT that are able to use different substrates so that the final volatile profile of a given fruit might depend on the availability of precursors for those substrates (Dudareva et al. 2004). This is the case of banana, where the substrate specificity of AAT does not explain the composition of the branched-chain esters found in this fruit aroma (Wyllie et al. 1996), and avail-

ability of substrates from amino acid metabolism seems to be the key process in ester biosynthesis (Wyllie and Fellman 2000). In a similar way, Souleyre and others (2005) reported that apple AAT preference for acetate ester formation depends on substrate concentration.

In the past few years, a combination of appropriate biochemical knowledge with gene expression data has provided very valuable information to enable the elucidation of the role of AAT in aroma formation during fruit ripening. AATs belong to a recently discovered family of plant acyltransferases called BADH (St. Pierre and De Luca 2000). BADH acyltransferases directly involved in volatile generation have been investigated in fruits such as strawberry (Aharoni et al. 2000), apple (Defilippi et al. 2005b; Holland et al. 2005; Li et al. 2006; Souleyre et al. 2005), banana (Beekwilder et al. 2004), melon (El-Sharkawy et al. 2005; Shalit et al. 2001; Yahyaoui et al. 2002), and grape (Wang and DeLuca 2005). Though all of them belong to the BADH family, AAT proteins from different fruit species are highly divergent. Curiously, some proteins with very low amino acid identity, for instance, strawberry and melon (SAAT and CmAAT1) having only 22% identity, have quite similar substrate preference (Aharoni et al. 2000; Yahyaoui et al. 2002). On the contrary, AAT genes from wild and cultivated strawberries are closely related, but the activity of both recombinant enzymes is quite different (Beekwilder et al. 2004). The AAT from wild strawberry is much more active on short alcohols in agreement with the substrate preferences reported in fruit enzymatic extracts from both wild and cultivated berries (Olías et al. 2002). In some cases, the substrate preference of recombinant AAT enzymes does not reflect the ester profile of the corresponding fruit. Thus, the banana recombinant enzyme exhibits very low efficiency for synthesizing isoamyl acetate, the key component of banana aroma (Beekwilder et al. 2004). In other fruits such as melon, the different specificity of the multiple AAT proteins codified by the genes identified (CmAAT1, CmAAT3, and CmAAT4) effectively accounts for the great diversity of esters formed in the fruit (El-Sharkawy et al. 2005). Thus, after biochemical characterization of the three recombinant proteins, Cm-AAT1 has the biggest capacity to produce thioesters, the key flavor components of cantaloupe melon (Lucchetta et al. 2007). Despite the low sequence homology found among the AAT genes identified so far, AAT proteins exhibited some common characteristics. All fruit AAT genes identified so far encode proteins ranging from 419 to 479 amino acid residues, which correspond to an average molecular weight of 51–55 kDa. These data are in good agreement with the molecular weight of the native AAT proteins purified in banana (40 kDa), strawberry (48 kDa), grape (50 kDa), or melon (50 kDa). The inhibitory effect of zinc and sulfhydryl reactive compounds on the activity of native and recombinant AATs suggests the implication of cysteine residues in the substrate pocket and/or catalytic region of the enzyme. A threonine residue also seems to be critical for AAT activity as pointed out by El-Sharkawy and others (2005), after the site-directed mutagenesis on a cloned melon AAT gene (CmAAT2) that lacked AAT enzymatic activity. Most of the native and recombinant proteins exhibit a broad pH range of activity between 7 and 9, an optimum temperature around 30°C, and also have similar expression patterns. The expression of genes from wild and cultivated strawberries (Aharoni et al. 2004), banana (Beekwilder et al. 2004), melon (El-Sharkawy et al. 2005), and grape (Wang and DeLuca 2005) is strongly induced during fruit ripening. Ethylene has proved to be a major regulator of the activity in melon, banana, and apple

(Defilippi et al. 2005a; Flores et al. 2002; Li et al. 2006; Schaffer et al. 2007), and an AAT gene identified in mume (*Prunus mume*) has been found to be responsive not only to ripening ethylene but also to biotic stresses such as wounding and may be involved in the synthesis of phytoalexins (Mita et al. 2006).

CONCLUSIONS

Plant flavor formation is a complex process in which quite different pathways are involved. These biochemical routes are interconnected, and most of them are not only exclusively devoted to aroma formation but also give rise to some other important plant metabolites, which have many different biological functions. Major progress has come in the past few years from the use of biochemical and molecular techniques. From a biochemical point of view, it is clear that two main factors control flavor formation: the selectivity of a group of key enzymes and the availability of appropriate substrates. On the other hand, it is also clear that a single enzymatic step will rarely control flavor formation, and in most cases, an entire metabolic pathway should be studied in order to understand the flavor of a given product. Molecular researchers have isolated many genes participating in the formation of flavor compounds, and there is no doubt that development of genomics and proteomics will enhance the knowledge on genes related to these pathways and may contribute to fill the gap on when, where, and how individual genes or groups of genes are switched on or off controlling flavor diversity. Finally, besides the "-omics" techniques (genomics, proteomics, and metabolomics) that provide information on the enzyme systems and precursors involved in aroma formation, many other factors such as soil nutrition, growing environment, stage of maturity, and postharvest conditions might affect the aroma of fresh fruits and vegetables and must be taken into account in any attempt of improving their flavor.

REFERENCES

Aharoni A, Giri AP, Verstappen FWA, Bertea C, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bowmeester HJ. 2004. Gain and loss of flavour compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–31.

Aharoni A, Jongsma MA, Bouwmeester HJ. 2005. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci* 10:594–602.

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun ZK, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen A, De vos RCH, van der Voet H, Cansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP. 2000. Identification of the SAAT gene involved in strawberry flavour biogenesis by use of DNA microarrays. *Plant Cell* 12:647–61.

Baik HY, Juvik J, Jeffery EH, Wallig MA, Kushad M, Klein BP. 2003. Relating glucosinolate content and flavor of broccoli cultivars. *J Food Sci* 68:1043–50.

Baker A, Graham IA, Hodsworth M, Smith SM. 2006. Chewing the fat: β-Oxidation in signalling and development. *Trends Plant Sci* 11:124–32.

Balderman S, Naim M, Fleischman P. 2005. Enzymatic carotenoid degradation and aroma formation in nectarines (*Prunus persica*). *Food Res Int* 38:833–5.

Bauchot AD, Mottram DS, Dodson AT, John P. 1998. Effect of aminoclyclopropane-1-carboxylic acid oxidase antisense gene on the formation of volatile esters in Cantaloupe Charentais melons (cv. Vedrandais). *J Agric Food Chem* 46:4787–92.

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FWA, Bowmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135:1865–78.

Bick JA, Lange BM. 2003. Metabolic crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis: Unidirectional transport of intermediates across the chloroplast envelope membrane. *Arch Biochim Biophys* 415:146–54.

Bones AM, Rossiter J. 2006. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 67:1053–67.

Bood KG, Zabetakis I. 2002. The biosynthesis of strawberry flavour (II): Biosynthetic and molecular biology studies. *J Food Sci* 67:2–8.

Borejsza-Wysocki N, Hrazdina G. 1994. Biosynthesis of p-hydroxyphenyl butan-2-one in raspberry fruit and tissue culture. *Phytochemistry* 35:623–8.

Botella-Pavia P, Besumbes O, Phillips MA, Carretero-Paulet L, Borona A, Rodríguez-Concepción M. 2004. Regulation of carotenoid biosynthesis in plants: Evidence for a key role of hydroxymethylbutenyl diphosphate reductase in controlling the supply of plastidial isoprenoid precursors. *Plant J* 40:188–99.

Bouvier F, Suire C, Mutterer J, Camara B. 2003. Oxidative remodelling of chromoplast carotenoids: Identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in crocus secondary metabolite biogenesis. *Plant Cell* 15:47–62.

Burow M, Markert J, Gershenzon J, Wittstock U. 2006. Comparative biochemical characterization of nitrile-forming proteins from plants and insects that alter myrosinase-catalysed hydrolysis of glucosinolates. *FEBS J* 273:2432–46.

Buttery RG, Ling LC. 1993. Volatile compounds of tomato fruit and plant parts: Relationship and biogenesis. *ACS Symp Ser* 525:23–34.

Casey R, Hughes KH. 2004. Recombinant lipoxygenases and oxylipin metabolism in relation to food quality. *Food Biotechnol* 18:135–70.

Chen G, Hackett R, Taylor A, Lin Z, Griergson D. 2004. Identification of a specific isoform of tomato LOX (tomLoxC) involved in the generation of fatty acid derived flavour compounds. *Plant Physiol* 136:2641–50.

Clifford MN. 2000. Chlorogenic acid and other cinnamates—Nature, occurrence, dietary burden, absorption and metabolism. *J Sci Food Agric* 80:1033–43.

Connor EC, Rott AS, Zeder M, Jüttner F, Dorn S. 2008. ^{3}C-labelling patterns of green leaf volatiles indicating different dynamics of precursors in *Brassica* leaves. *Phytochemistry* 69:1304–12.

Croteau R. 1992. Monoterpene biosynthesis, cyclization of geranyl diphosphate to sabinene. *ACS Symp Ser* 490:8–20.

Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E. 2007. Enrichment of tomato flavour by diversion of the early plastidial terpenoid pathway. *Nat Biotechnol* 25:899–901.

Defilippi B, Dandekar AM, Kader AA. 2005a. Relationship of ethylene biosynthesis to volatile production, related enzymes and precursors availability in apple peel and flesh tissue. *J Agric Food Chem* 53:3133–41.

Defilippi B, Kader AA, Dandekar AM. 2005b. Apple aroma: Alcohol acyltransferase, a rate limiting step for ester biosynthesis is regulated by ethylene. *Plant Sci* 168:1199–210.

Do GS, Suzuki G, Mukai Y. 2004. Genomic organization of a novel root alliinase gene, ALL1, in onion. *Gene* 325:17–24.

Dudareva N, Pichersky E, Gershenzon J. 2004. Biochemistry of plant volatiles. *Plant Physiol* 135:1893–902.

DuPont MS, Mondin Z, Williamson G, Price KR. 2000. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J Agric Food Chem* 48:3957–64.

El-Sharkawy I, Manriquez D, Flores FB, Regad F, Bouzayen M, Latche A, Pech JC. 2005. Functional characterization of a melon AAT gene family involved in the biosynthesis of ester volatiles. Identification of a threonine residue. *Plant Mol Biol* 59:345–62.

Engel E, Baty C, Le Corre D, Souchon I, Marin N. 2002. Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *J Agric Food Chem* 50:6459–67.

Estevez JM, Cantero A, Reindl A, Reichler S, Leon P. 2001. 1-Deoxy-xylulose-5-phosphate synthase: a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *J Biol Chem* 276: 22901–9.

Fellman JK, Mattheis JP, Mattinson DS, Bostick BC. 1991. Assay of acetyl CoA alcohol transferase in Delicious apples. *HortSci* 26:152–61.

Fernandez-Trujillo JP, Nock JF, Watkins B. 1999. Fermentative metabolism and organic acid concentrations in fruits of selected strawberry cultivars with different tolerances to carbon dioxide. *J Am Soc Hortic Sci* 124:696–701.

Feussner I, Kindl H. 1992. A lipoxygenase is the main lipid body protein in cucumber and soybean cotyledons during the stage of triglyceride mobilization. *FEBS Lett* 298:223–5.

Feussner I, Wasternak C. 2002. The lipoxygenase pathway. *Annu Rev Plant Biol* 53:275–97.

Fleischman P, Studer K, Wintelhalter P. 2002. Partial purification and kinetic characterization of a carotenoid cleavage enzyme from quince fruit. *J Agric Food Chem* 50:1677–80.

Fleischman P, Watanabe N, Wintelhalter P. 2003. Enzymatic carotenoid cleavage in star fruit (*Averrhoa carambola*). *Phytochemistry* 62:131–7.

Flores F, El-Yahyaoui F, Billerbeck KG, Romojaro F, Latche A, Bouzayen M, Pech JC, Ambid C. 2002. Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais Cantaloupe melons. *J Exp Bot* 53:201–6.

Fukushige H, Hildebrand DF. 2005. Watermelon (*Citrullus lanatus*) hydroperoxide lyase greatly increases C6 aldehyde formation in transgenic leaves. *J Agric Food Chem* 53:2046–51.

Gardner HW, Grove MJ, Salch YP. 1996. Enzymic pathway to ethyl vinyl ketone and 2-pentanal in soybean preparations. *J Agric Food Chem* 44:882–6.

Gazeu-Reyjal M, Crouzet J. 1976. Identité des formes soluble et solubilisee a partir des mitochondries de L-alanine α-cetoglutarate aminotransferase from tomate. *Phytochemistry* 15:619–22.

Giuliano G, Albabilu S, Von Lintig J. 2003. Carotenoid oxygenases: Cleave or leave it. *Trends Plant Sci* 8:145–9.

Givan CV. 1980. Aminotransferases in higher plants. In Stumpf PK, Conn EE (eds.), *Biochemistry of Plants*, Vol. 5. New York: Academic Press, pp. 329–57.

Goepert S, Vidoudez C, Rezzonico E, Hitunen JK, Poirier Y. 2005. Molecular identification and characterization of the *Arabidopsis* $\Delta^{3,5},\Delta^{2,4}$-dienoyl-coenzyme A isomerase, a peroxisomal enzyme participating in the β-oxidation cycle of unsaturated fatty acids. *Plant Physiol* 138:1947–56.

Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value. *Science* 311:815–9.

Gomi K, Yamasaki Y, Yamamaoto H, Akimitsu K. 2003. Characterization of a hydroperoxide lyase gene and effect of C6-volatiles on expression of genes of the oxylipin metabolism in *Citrus*. *J Plant Physiol* 160:1219–31.

Granroth B. 1970. Biosynthesis and decomposition of cysteine derivatives in onion and other *Allium* species. *Ann Acad Sci Fenn A* 154:1–71.

Griffiths A, Prestage S, Linforth RST, Zhang J, Taylor AJ, Griergson D. 1999. Fruit specific LOX suppression in antisense transgenic tomatoes. *Postharvest Biol Technol* 17:163–73.

Grubb CD, Abel S. 2006. Glucosinolate metabolism and its control. *Trends Plant Sci* 11:89–100.

Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annu Rev Plant Biol* 57:303–33.

Hampel D, Mosandl A, Wüst M. 2005. Biosynthesis of mono and sesquiterpenes in carrots roots and leaves (*Daucus carota* L), metabolic cross talk of cytosolic mevalonate and plastidial methylerythritol phosphate pathways. *Phytochemistry* 66:305–11.

Hampel D, Swatski A, Mosandl A, Wüst M. 2007. Biosynthesis of monoterpenes and norisoprenoids in raspberry fruits (*Rubus idaeus* L.): The role of cytosolic mevalonate and plastidial methylerythritol phosphate pathway. *J Agric Food Chem* 55:9296–304.

Harada M, Ueda Y, Iwata T. 1985. Purification and some properties of alcohol acyltransferase form banana. *Plant Cell Physiol* 26:1067–74.

Hatanaka A. 1993. The biogeneration of green odour by green leaves. *Phytochemistry* 34:1201–18.

Hatanaka A, Kajiwara K, Sekiya J. 1986. Fatty acid hydroperoxide lyase in plant tissues. Volatile aldehyde formation from linoleic and linolenic acids. In Parliament TH, Croteau R (eds.), *Biogeneration of Aromas*. Washington DC: ACS Symposium Series, pp. 167–75.

Hauck T, Hubner Y, Brullman F, Schwab W. 2002. Alternative pathway for the formation of 4,5-dihydroxy-2,3-pentanedione the proposed precursor of 4-hydroxy-5methyl-3(2H) furanone as well as autoinducer-2, and its detection as natural constituent of tomato fruit. *Biochim Biophys Acta* 1623:109–19.

Herrmann KM, Weaver LM. 1999. The shikimate pathway. *Annu Rev Plant Physiol Plant Mol Biol* 50:473–503.

Holland D, Larkov, Yà-akov IB, Bar E, Zax A, Brandeis E, Ravid O, Lewinsohn E. 2005. Developmental and varietal differences in volatile transferase activities in apple (*Malus domestica*). *J Agric Food Chem* 53:7198–203.

Iijima Y, Gang D, Fridman E, Lewinsohn E, Pichersky E. 2004. Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiol* 134:370–9.

Ikegami F, Murakoshi I. 1994. Enzymic synthesis of non-protein β-substituted alanines and some higher homologues in plants. *Phytochemistry* 35:1089–104.

Ileperuma NR, Marshall SDG, Squire CJ, Baker HM, Oakeshott JG, Russell RJ, Plummer KM, Newcomb RD, Baker EN. 2007. High-resolution crystal structure of plant carboxylesterase AeCXE1, from *Actinidia eriantha*, and its complex with a high-affinity inhibitor paraoxon. *Biochemistry* 46:1851–9.

Imai S, Tsuge N, Tomotake M, Nagatome Y, Sawada H, Nagata T, Kumagai H. 2002. An onion enzyme that makes the eyes water. *Nature* 419:685–90.

Jayanty S, Song J, Rubinstein NM, Chong A, Beaudry RM. 2002. Temporal relationship between ester biosynthesis and ripening events in bananas. *J Am Soc Hortic Sci* 127:998–1005.

Jones MG. 2008. Formation of vegetable flavour. In Bruckner B, Wyllie SG (eds.), *Fruit and Vegetable Flavour*. Cambridge, England: Woodhead Publishing, pp. 71–102.

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA. 2004. Biosynthesis of the flavour precursors of onion and garlic. *J Exp Bot* 55:1903–18.

Ke D, Zhou L, Kader AA. 1994. Mode of action of O_2 and CO_2 action on strawberry ester biosynthesis. *J Am Soc Hortic Sci* 119:971–5.

Klein D, Fink B, Arold B, Eisenreich W, Schwab W. 2007. Functional characterization of enone oxidoreductases from strawberry and tomato fruit. *J Agric Food Chem* 55:6705–11.

Kleinig H. 1989. The role of plastids in isoprenoid biosynthesis. *Annu Rev Plant Physiol Plant Mol Biol* 40:39–59.

Kondo Y, Hashidoko Y, Mizutani J. 1995. An enzymatic formation of 13-oxo-trideca-9,11-dienoic acid from 13-hydroperoxylinolenic acid by a homolytic hydroperoxide lyase in elicitor-treated soybean cotyledons. *Biochim Biophys Acta* 1255:9–15.

Lancaster JE, Shaw ML. 1989. γ-Glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. *Phytochemistry* 28:455–60.

Lancaster JE, Shaw ML, Pither Joyce MD, McCallum JA, McManus MT. 2000. A novel alliinase from onion roots. Biochemical characterization and cDNA cloning. *Plant Physiol* 122:1269–80.

Lange BH, Croteau R. 1999. Isopentenyl diphosphate biosynthesis via a mevalonate-independent pathway: Isopentenyl monophosphate kinase catalyzes the terminal enzymatic step. *Proc Natl Acad Sci U S A* 96:13714–9.

Lavid N, Schwab W, Kafkas E, Koch-Dean M, Bar E, Lav O, Ravid U, Lewinsohn E. 2002. Aroma biosynthesis in strawberry. SAM: Furaneol O-methyl transferase activity in ripening fruits. *J Agric Food Chem* 50:4025–30.

Leon J, Royo J, Vancanneyt G, Sanz C, Silkowski H, Griffiths G, Sánchez-Serrano JJ. 2002. Lipoxygenase H1 gene silencing reveals a specific role in supplying fatty acid hydroperoxides for aliphatic aldehyde production. *J Biol Chem* 277:416–23.

Lewinsohn E, Schalechet F, Wilkinsosn J, Matsui K, Tadmor K, Nam KH. 2001. Enhanced levels of the aroma and flavour compounds S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 127:1256–65.

Lewinsohn E, Sitrit Y, Bar E, Azullay Y, Ibdah M, Meir A, Yosef E, Zamir D, Tadmor Y. 2005. Not just color—Carotenoid degradation as a link between pigmentation and aroma in tomato and watermelon fruit. *Trends Food Sci Technol* 16:407–15.

Li D, Xu Y, Xu G, Gu L, Li D, Shu H. 2006. Molecular cloning and expression of a gene encoding alcohol acyltransferase (MdAAT2) from apple (cv. Golden Delicious). *Phytochemistry* 67:658–67.

Luaces P, Sanz C, Pérez A. 2007. Thermal stability of lipoxygenase and hydroperoxide lyase from olive fruit and repercussion on virgin olive oil aroma biosynthesis. *J Agric Food Chem* 55:6309–13.

Lucchetta L, Manriquez D, El Sharkawy I, Flores FB, Sanchez-Bel P, Zouine M, Gines C, Bouzayen M, Rombaldi C, Pech JC, Latche A. 2007. Biochemical and catalytic properties of three recombinant alcohol acyltrasferases of melon. Sulphur containing ester formation, regulatory role of CoASH in activity and sequence elements conferring substrate preference. *J Agric Food Chem* 55:5213–20.

Lucker J, Tamer MK, Schwab W, Verstappen FWA, Van der Plaf LMW, Bowmeester HJ, Verhoeven HA. 2002. Monoterpene biosynthesis in lemon cDNA isolation and functional analysis of four monoterpene synthases. *Eur J Biochem* 269:3160–71.

Lunkenbein S, Salentijn EMJ, Coiner HA, Boone MJ, Krens FA, Schwab W. 2006. Up and down-regulation of *Fragaria* × *ananassa* O-methyltransferase impact on furanone and phenyl propanoid metabolism. *J Exp Bot* 57:2445–53.

Macheix JJ, Fleuriet A, Billot J. 1990. *Fruit Phenolics*. Boca Raton, FL: CRC.

Mahmoud SS, Croteau RB. 2002. Strategies for transgenic manipulation of monoterpene biosynthesis in plants. *Trends Plant Sci* 7:366–73.

Manriquez D, El-Sharkawy I, Flores FB, El-Yahyaoui F. 2006. Two highly divergent ADHs of melon exhibit fruit ripening-specific expression and distinct biochemical characteristics. *Plant Mol Biol* 61:675–85.

Mateos R, Cert A, Perez-Camino MC, García JM. 2004. Evaluation of virgin olive oil bitterness by quantification of secoiridoid derivatives. *J Am Oil Chem Soc* 81:71–5.

Matsui K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Curr Opin Plant Biol* 9:274–80.

Matsui K, Fukutomi S, Wilkinson J, Hiatt B, Kanuf V, Kajwara T. 2001. Effect of overexpression of fatty acid 9-hydroperoxide lyase in tomatoes (*Lycopersicon esculentum* Mill.). *J Agric Food Chem* 49:5418–24.

Matsui K, Nishioka M, Ikeyoshi M, Matsumura Y, Mori T, Kajiwara T. 1998. Cucumber root lipoxygenase can act on acyl groups in phosphatidylcholine. *Biochim Biophys Acta* 1390:8–20.

Matsui K, Ujita C, Fujimoto S, Wilkinson J, Hiatt B, Knauf V, Kajiwara T, Feussner I. 2000. Fatty acid 9- and 13-hydroperoxide lyases from cucumber. *FEBS Lett* 481:183–8.

McManus MT, Leung S, Lambert A, Scott RW, Pither-Joyce M, Chen B, McCallum J. 2005. Molecular and biochemical characterisation of a serine acetyl transferase of onion, *Allium cepa* (L.). *Phytochemistry* 66:1407–16.

Mita S, Nagai Y, Asai T. 2006. Isolation of cDNA clones corresponding to genes differentially expressed in pericarp of mume (*Prunus mume*) in response to ripening ethylene and wounding signals. *Physiol Plant* 128:531–45.

Mitchell WC, Jelenkovic G. 1995. Characterising NAD and NADP-dependent alcohol dehydrogenase enzymes of strawberries. *J Am Soc Hortic Sci* 120:798–801.

Moyano E, Encinas-Villarejo S, Lopez-Raez JA, Redondo-Nevado J, Blanco-Portales R, Bellido ML, Sanz C, Caballero JL, Muñoz-Blanco J. 2004. Comparative study between two strawberry pyruvate decarboxylase genes along development and ripening postharvest and stress conditions. *Plant Sci* 166:835–45.

Myung K, Hamilton-Kemp TR, Archbold DD. 2006. Biosynthesis of trans-2-hexenal in response to wounding in strawberry fruit. *J Agric Food Chem* 54:1442–8.

Olejniczak T, Mironowicz A, Wawrzenczyk C. 2003. Enzymatic lactonization of gamma-epoxy esters by the apple fruit and Jerusalem artichoke bulb. *Bioorg Chem* 31:199–205.

Olías JM, Perez AG, Ríos JJ, Sanz C. 1993. Aroma of virgin olive oil: Biogenesis of the green odour notes. *J Agric Food Chem* 41:2368–73.

Olías JM, Sanz C, Ríos JJ, Perez AG. 1995. Substrate specificity of alcohol acyltransferase from strawberry and banana fruits. In Rouseff RL, Leahy M (eds.), *Fruit Flavors. Biogenesis, Characterization and Authentication*. New York: ACS Symposium Series, pp. 134–41.

Olías R, Perez AG, Sanz C. 2002. Catalytic properties of alcohol acyltransferase in different strawberry species and cultivars. *J Agric Food Chem* 50:4031–6.

Paillard NH. 1990. The flavour of apples, pears and quinces. In Norton ID, McLeod AJ (eds.), *Food Flavor Part C*. Amsterdam: Elsevier, pp. 1–41.

Pechous SW, Whitaker BD. 2004. Cloning and functional expression of an (E,E)alpha-farnesene synthase cDNA from tissue of apple fruit. *Planta* 219:84–94.

Pérez AG, Olías R, Luaces P, Sanz C. 2002. Biosynthesis of strawberry aroma compounds through amino acid metabolism. *J Agric Food Chem* 50:4037–42.

Pérez AG, Olías JM, Sanz C. 1999a. Biosynthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and derivatives in *in vitro* grown strawberries. *J Agric Food Chem* 47:655–8.

Pérez AG, Sanz C, Olías R, Olías JM. 1999b. Lipoxygenase and hydroperoxide lyase activities in ripening strawberry fruits. *J Agric Food Chem* 47:249–53.

Pérez AG, Olías R, Sanz C, Olías JM. 1996a. Furanones in strawberries. Evolution during ripening and postharvest shelf-life. *J Agric Food Chem* 44:3620–4.

Pérez AG, Ríos JJ, Sanz C, Olías JM. 1992. Aroma components and free amino acids in strawberry var. Chandler during ripening. *J Agric Food Chem* 40:2232–5.

Pérez AG, Sanz C. 2008. Formation of fruit flavour. In Bruckner B, Wyllie SG (eds.), *Fruit and Vegetable Flavour*. Boca Raton, FL: CRC Press, pp. 71–102.

Pérez AG, Sanz C, Olías JM. 1993. Partial purification and some properties of alcohol acyltransferase from strawberry fruits. *J Agric Food Chem* 41:1462–6.

Pérez AG, Sanz C, Olías R, Ríos JJ, Olías JM. 1996b. Evolution of strawberry alcohol acyltransferase activity during fruit development and storage. *J Agric Food Chem* 44:3286–90.

Raab T, Lopez-Raez JA, Klein D, Caballero JL, Moyano E, Schwab W, Muñoz-Blanco J. 2006. FaQR required for the biosynthesis of the strawberry flavor compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone encodes an enone oxidoreductase. *Plant Cell* 18:1023–37.

Riley JMC, Willemot C, Thompson JE. 1996. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biol Technol* 7:97–107.

Robbins R. 2003. Phenolic acids in foods: An overview of analytical methodology. *J Agric Food Chem* 51:2866–87.

Rodriguez-Concepción M, Boronat A. 2002. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. *Plant Physiol* 130:1079–89.

Rohdich F, Hecht S, Bacher A, Eisenreich W. 2003. Deoxyxylulose phosphate pathway of isoprenoid biosynthesis. Discovery and function of ispDEFGH genes and their corgnate enzymes. *Pure Appl Chem* 75:393–405.

Roscher R, Herderich M, Steffen JP, Schreier P, Schwab W. 1996. 2,5-Dimethyl-4-hydroxy-(2H)-furanone 6'-O-malonyl-b-D-glucopiranoside in strawberry fruits. *Phytochemistry* 43:155–9.

Roscher R, Schreier P, Schwab W. 1997. Metabolism of 2,5-dimethyl-4-hydroxy-(2H)-furanone in detached ripening strawberry fruits. *J Agric Food Chem* 45:3202–5.

Rowan DD, Lane HD, Allen JM, Fielder S, Hunt MB. 1996. Biosynthesis of 2-methylbutyl, 2-methyl-2-butenyl and 2-methylbutanoate esters in Red Delicious and Granny Smith apples using deuterium labelled substrates. *J Agric Food Chem* 44:3276–85.

Ryan D, Antolovich M, Prenzler P, Robards K, Lavee S. 2002. Biotransformations of phenolic compounds in *Olea europaea* L. *Sci Hortic* 92:147–76.

Salas JJ. 2004. Characterization of alcohol acyltransferase from olive fruit. *J Agric Food Chem* 52:3155–8.

Salas JJ, Sanchez J. 1998. Alcohol dehydrogenase from olive (*Olea europaea*) fruit. *Phytochemistry* 48:35–40.

Salas JJ, Sanchez J. 1999. Hydroperoxide lyase from olive (*Olea europaea*) fruits. *Plant Sci* 48:19–26.

Salas JJ, Sánchez C, García-González D, Aparicio R. 2005. Impact of the suppression of lipoxygenase and hydroperoxide lyase on the quality of the green odour in green leaves. *J Agric Food Chem* 53:1648–55.

Salas JJ, Sanchez J, Ramli US, Manaf AM, Williams M, Harwood JL. 2000. Biochemistry of lipid metabolism in olive and other oil fruits. *Prog Lipid Res* 39:151–80.

Sánchez-Ortiz A, Pérez AG, Sanz C. 2007. Cultivar differences on nonesterified polyunsaturated fatty acids as limiting factor for the biogenesis of virgin olive oil aroma. *J Agric Food Chem* 55:7869–73.

Sanz C, Olías JM, Pérez AG. 1997. Aroma biochemistry of fruits and vegetables. In Tomas-Barberan FA, Robins RJ (eds.), *Phytochemistry of Fruits and Vegetables*. Oxford: Clarendon Press, pp. 125–55.

Sanz C, Richardson DG, Pérez AG. 1995. 2,5-Dimethyl-4-hydroxy-(2H)-furanone and derivatives in strawberries during ripening. In Rouseff RL, Leahy M (eds.), *Fruit Flavors. Biogenesis, Characterization and Authentication*. New York: ACS Symposium Series, pp. 226–75.

Schaffer RJ, Friel EN, Souleyre EJF, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma J-H, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD. 2007. A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiol* 144:1899–912.

Schilling M, Haetzelt F, Schwab W, Schrader J. 2008. Impact of surfactants on solubilization and activity of the carotenoid cleavage dioxygenase, AtCCD1, in an aqueous micellar reaction system. *Biotechnol Lett* 30:701–6.

Schreier P. 1986. Biogeneration of plant aromas. In Birch GG, Lindley MG (eds.), *Development in Food Flavour*. Amsterdam: Elsevier Science Publishing, pp. 89–106.

Schwab W. 1998. Application of stable isotope ratio analysis explaining the bioformation of 2,5-dimethyl-4-hydroxy-(2H)-furanone in plants by a biological Maillard reaction. *J Agric Food Chem* 46:2266–9.

Schwab W, Roscher R. 1997. 4-Hydroxy-3(2H)-furanones. Natural and Maillard products. *Recent Res Dev Phytochem* 1:643–73.

Schwab W, Williams DC, Davis EM, Croteaou R. 2001. Mechanism of monoterpene cyclization: Stereochemical aspects of the transformation of non-cyclizable substrate analogs by recombinant (–)limonene synthase, (+)bornyl diphosphate synthase and (–) pinene synthase. *Arch Biochem Biophys* 392:123–36.

Shalit M, Katzir N, Tadmor Y, Larkov O, Burger Y, Shalekhet F, Lastochkim E, Ravid U, Amar O, Edelstein M. 2001. Acetyl-CoA: Alcohol acetyltransferase activity and aroma formation in ripening melon fruits. *J Agric Food Chem* 49:794–9.

Sharon-Asa L, Shalit M, Frydman A, Bar E, Holland D, Or E, Lavi U, Lewinsohn E, Eyal Y. 2003. Citrus fruit and aroma biosynthesis: Isolation, functional characterization and developmental regulation of Cstps1 a key gene in the sesquiterpene aroma compound valencene. *Plant J* 36:664–74.

Shaw ML, Pither-Joyce MD, McCallum JA. 2005. Purification and cloning of a gamma-glutamyl transpeptidase from onion (*Allium cepa*). *Phytochemistry* 66:515–22.

Shibata Y, Matsui K, Kajiwara T, Hatanaka A. 1995. Purification and properties of fatty acid hydroperoxide lyase from green bell pepper fruits. *Plant Cell Physiol* 36:147–56.

Shimon LJW, Rabinkov A, Miron T, Mirelman D, Wilchek M, Frolow F. 2002. Alliin lyase (alliinase) from garlic (*Allium sativum*): Crystallization and preliminary X-ray characterization. *Acta Crystallogr D Biol Crystallogr* 58:1335–7.

Sinkim AJ, Schwartz SH, Auldrige M, Taylor MG, Klee HJ. 2004. The tomato carotenoid cleavage dioxygenases 1 genes contribute to the formation of the flavour volatiles: β-Ionone, pseudoionone and geranyl acetone. *Plant J* 40:882–92.

Souleyre EJF, Greenwood DR, Friel EN, Karunairetnam S. 2005. An alcohol acyltransferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavour. *FEBS J* 272:3132–44.

Speirs J, Lee E, Holt K, YongDuc K, Scott NS, Loveys B, Schuch W. 1998. Genetic manipulation of ADH levels in ripening tomato affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117:1047–58.

St. Pierre B, De Luca V. 2000. Evolution of acyltransferases genes origins and diversification of the BAHD supefamily of acyltransferases involved in secondary metabolism. In Romeo JJ, Ibrahim R, Varin L, DeLucca V (eds.), *Recent Advances in Phytoevolution of Metabolic Pathway*. Oxford: Elsevier Science, pp. 285–315.

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ. 2006. Tomato aromatic amino acid decarboxylases participate in synthesis of the flavour volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci U S A* 103:8287–92.

Tijet N, Schneider C, Muller BL, Brash AR. 2001. Biogenesis of volatile aldehydes from fatty acid hydroperoxides: Molecular cloning of a hydroperoxide lyase (CYP74C) with specificity for both the 9- and 13-hydroperoxides of linoleic and linolenic acids. *Arch Biochem Biophys* 386:281–9.

Tijet N, Waspi U, Gaskin DJH, Hunziker P, Muller BL, Vulfson EN, Slusarenko A, Brash AR, Whitehead IM. 2000. Purification, molecular cloning and expression of the gene encoding fatty acid 13-hydroperoxide lyase from guava fruit (*Psidium guajava*). *Lipids* 35:709–20.

Tikunov Y, Lommen A, Ric de Vos CH, Verhoeven HA, Bino RJ, Hall RD, Bovy AG. 2005. A novel approach for nontargeted data analysis for metabolimics. Large-scale profiling of tomato fruit volatiles. *Plant Physiol* 39:1125–37.

Tressl R, Albrecht W. 1986. Biogenesis of aroma compounds through acyl pathways. In Parliament TH, Croteau R (eds.), *Biogeneration of Aromas*. Washington, DC: ACS, pp. 114–33.

Tressl R, Drawert F. 1973. Biogenesis of banana volatiles. *J Agric Food Chem* 21:560–5.

Tressl R, Holzer M, Apetz M. 1975. Biogenesis of volatiles in fruit and vegetables. In Maarse H, Groenen PJ (eds.), *Aroma Research*. Wageningen, The Netherlands: Zeist, pp. 41–62.

Ueda Y, Tsuda A, Bai JH, Fujishita N, Chachim K. 1992. Characteristic pattern of aroma ester formation from banana, melon and strawberry with reference to the substrate specificity of ester synthase at alcohol contents in pulp. *J Jpn Soc Food Sci Technol* 39:183–7.

Ukai K, Sekiya J. 1999. Rapid purification and characterization of cystine lyase b from broccoli inflorescence. *Phytochemistry* 51:853–9.

Vancanneyt G, Sanz C, Farmaki T, Paneque M, Ortego F, Castanera P, Sanchez-Serrano JJ. 2001. Hydroperoxide lyase depletion in transgenic plant leads to an increase in aphid performance. *Proc Natl Acad Sci U S A* 98:8139–44.

Vick BA, Zimmerman DC. 1987. Pathways of fatty acid hydroperoxide metabolism in spinach leaf chloroplasts. *Plant Physiol* 85:1073–8.

Villatoro C, Altisent R, Echeverría G, Graell J, López ML, Lara I. 2008. Changes in biosynthesis of aroma volatile compounds during on-tree maturation of "Pink Lady®" apples. *Postharvest Biol Technol* 47:286–95.

Wache Y, Bosser-Deratuld AL, Lhugenot JC, Belin JM. 2003. Effect of cis/trans isomerism of β-carotene on the ratios of volatile compounds produced during oxidative degradation. *J Agric Food Chem* 51:1984–7.

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE. 1996. Changes in fatty acids and fatty acid derived flavour compounds by expressing the yeast delta-9-desaturase in tomato. *J Agric Food Chem* 44:3399–402.

Wang J, DeLuca V. 2005. The biosynthesis and regulation of biosynthesis of Concord grape fruit esters, including foxy methylanthranilate. *Plant J* 44:606–19.

Wang Y, Raghavam S, Ho CT. 2008. Process flavour of allium vegetables. In Bruckner B, Wyllie SG (eds.), *Fruit and Vegetable Flavour*. Cambridge, England: Woodhead Publishing, pp. 200–28.

Watkins B, Manzano-Mendez JE, Nock JF, Zhang JJ, Maloney KE. 1999. Cultivar variation in response of strawberry fruit to high carbon dioxide treatment. *J Sci Food Agric* 79:886–90.

Wein M, Lewinsohn E, Schwab W. 2001. Metabolic fate of isotopes during the biological transformation of carbohydrates to 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry fruits. *J Agric Food Chem* 49:2427–32.

Wintelhalter P, Rouseff R. 2002. *Carotenoid-Derived Aroma Compounds. ACS Symposium Series 802*. Washington, DC: ACS.

Wu Z, Robinson DS, Hughes RK, Casey R, Hardy D, West SI. 1999. Co-oxidation of β-carotene catalyzed by soybean and recombinant pea lipoxygenases. *J Agric Food Chem* 47:4899–906.

Wyllie G, Fellman JK. 2000. Formation of volatile branched chain esters in banana (*Musa sapientum* L). *J Agric Food Chem* 48:3493–6.

Wyllie SG. 2008. Flavour quality of fruit and vegetables: Are we on the brink of major advances? In Bruckner B, Wyllie SG (eds.), *Fruit and Vegetable Flavour*. Boca Raton, FL: CRC Press, pp. 3–10.

Wyllie SG, Leach DN, Nonhebel H, Lusunzi I. 1996. Biochemical pathways for the formation of esters in ripening fruits. In Taylor A, Mottram DS (eds.), *Flavour Science Recent Developments*. Cambridge: Royal Society of Chemistry, pp. 52–7.

Yahyaoui FEL, Wongs-Aree C, Latche A, Hackett R, Griergson D, Pech JC. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyltransferase involved in the biogeneration of aroma volatile esters during melon ripening. *Eur J Biochem* 269: 2359–66.

Zabetakis I, Granshaw JW, Robinson DS. 1999. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone and its derivatives: Analysis, synthesis and biosynthesis. *Food Chem* 65:139–51.

PART III

FRUIT FLAVORS: ANALYTIC METHODOLOGY AND CHEMICAL CHARACTERIZATIONS

CHAPTER 10

History and Principles of Flavor Analysis

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INTRODUCTION

A determining factor in the acceptance or rejection of a foodstuff is its flavor, as this plays a very important role in palatability and is one of the key parameters determining the overall quality of a food product (Carterette and Friedman 1989). Flavor is defined as the combined perception of odor, taste, and mouthfeel (texture) (Ney 1988). It is a multidimensional attribute, which needs to be studied from various points of view, including (1) analytic, synthetic, and organic chemistry of flavor compounds; (2) (bio)chemistry of flavor formation; and (3) biology of flavor perception. Perception by the human sensory system and its measurement are of interest not only to food scientists but also to manufacturers, since it drives the consumers' preference for a product.

Understanding the mechanisms by which flavor compounds are formed can lead to optimized methods of food processing, allowing targeted formation and retention of flavor. Fundamental flavor chemistry information is also essential in genetic engineering of plants and animals for improving flavor in the raw materials of food products (Carterette and Friedman 1989). Advances in analytic methodology have enabled the identification of numerous compounds with known flavor properties. As more compounds are correlated with characteristic flavors, there is a trend to study flavor precursors and to explain how flavor is developed and released. Understanding the chemical reactions involved in the processing and storage of foods helps in achieving optimum consumer acceptability (Teranishi 1989). Besides flavor, other sensory properties with a great impact on the consumer are color and appearance.

The flavor of foods and drinks is perceived via our senses. The basic senses are sight (eyes), taste (tongue), smell (olfactory epithelium—the site of the yellow pituitary of the nose), hearing (outer ear), and feel (fingers and mouth). The sense of feel, responsible for buccal sensation, can be broken down into three sensations: pressure, trigeminal, and kinaesthetic. Pressure is the sensation felt when a force is

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

applied to the surface of a foodstuff; trigeminal refers to the sensation of pain; and kinaesthetic is feedback from the masticatory muscles during mastication (Caterina et al. 1997; Lawless 2004). While odor is preeminent in determining flavor character, the contribution of mouthfeel and taste to flavor impression cannot be ignored.

The basic gustatory sensations are *sweet*, produced by substances such as saccharose; *savory*, by sodium chloride; *sour*, by the pH of various acids; and *bitter*, by substances such as caffeine and quinine (Moruzzi 1977a). A new one has been added—*umami* (tastiness)—which can be represented by the taste of monosodium glutamate (MSG), and the 5′-nucleotides are generally recognized as the primary food components that provide this sensation (Maga 1983; Sugita 2003). MSG is readily measured by ion chromatography or reverse high-performance liquid chromatography (HPLC) (Rounds and Gregory 1998). The 5′-nucleotides are also determined mainly by HPLC (Charalambous et al. 1975; Duran Meras et al. 1993), but other methods have been used, for example, derivative spectrophotometry (Duran Meras et al. 1993). The trigeminal sensations give the descriptors astringency, pungency, and cold. Both the gustatory and trigeminal sensations arise from contact of the food in the mouth, as most of the substances that produce these flavors are polar, water soluble, and nonvolatile. For there to be an aromatic sensation, the corresponding compound has to be volatile enough to be detectable at a distance. The physical interaction between the volatile compound and the corresponding receptor takes place in the nostrils. Those molecules that reach the olfactory receptors either nasally or orally (retronasally) trigger the smell sensations. Aroma is a very complex sensation. The stimuli able to create taste sensations are limited, but more than 7100 volatile compounds have been identified in foods (Boelens 1996; Maarse et al. 1994; Nijssen et al. 1996), each of which may *potentially* contribute to aroma perception, depending upon its concentration and sensory threshold. Some of the more complex food aromas, for instance, strawberries or coffee, may contain over 400 volatile components. Fortunately, the aroma character of most foods can typically be defined by a smaller subset of the total volatile profile.

The human being is exceptionally sensitive to certain volatiles (e.g., 2-isobutyl-3-methoxypyrazine has an odor-detection threshold of 0.002 ppb in water [Leffingwell 1998] and 0.015 ppb in wine [Roujou De Boubee et al. 2000]), but insensitive to many others (e.g., ethanol has an odor threshold of 100,000 ppb in water and a taste threshold of 52,000 ppb in water [Leffingwell 1998]). A person's ability to detect odors is also affected by many other factors, such as genetic variability, olfactory fatigue, and naturally occurring and unpredictable factors such as temperature and humidity. The complexity of food aromas and the sensitivity required, plus the fact that the olfactory system must be able to respond to unknown odorants (it cannot be a learned response), make this a most complex phenomenon.

In our daily lives, a complete flavor experience depends on the combined responses of our senses and the cognitive processing of these inputs. While flavor per se is often thought of as being limited to olfaction, taste, and the somatosenses (irritation, tactile, and thermal), numerous other sensory inputs are processed by the brain to result in flavor perception (Keast et al. 2004). This broad multimodal aspect of flavor perception has only recently been acknowledged and multidisciplinary research directed at its understanding initiated (Taylor and Roberts 2004). Historically, researchers in both academic and industrial settings have viewed flavor as predominantly aroma, with only minor importance given to the contribution of

taste and somatosenses. Current research is proving this to be an unrealistic simplification of human flavor perception.

The very broad nature of flavor perception cannot be addressed in a single chapter, and the book edited by Taylor and Roberts (2004) is recommended for a better appreciation of the overall phenomenon of flavor perception.

CHEMICAL COMPOUNDS RESPONSIBLE FOR FOOD FLAVOR: STRUCTURE, PROPERTIES, AND CLASSIFICATION

The numerous flavors possible in foods are due to interactions of chemical compounds with the smell, taste, and trigeminal receptors. The smell of a food is triggered by an adequate number of molecules of low-molecular-weight (<300) volatile components reaching the olfactory epithelium and being dissolved in the mucosa covering the sensitive olfactory cells, creating bonds with the receptor proteins (Moruzzi 1977b). In a few cases, only one compound is responsible for an odor. These compounds are the so-called *impact compounds* (Jennings and Sevenants 1969). Table 10.1 displays some examples in a selection of foodstuffs, and Figure 10.1 displays the structures of corresponding compounds.

The intensity of the aroma seems to be related more with chemical factors, such as volatility and hydrophobic nature, and the stereochemical structure, such as type and position of the functional groups of the aromatic compounds, than with their concentration. It has been demonstrated that the size, shape, conformational structure, and type and position of the functional groups of the aromatic compounds are important elements in establishing the appropriate bonds to the olfactory receptor proteins present in the olfactory epithelium, thereby giving rise to the perception (Pelosi 1994; Rossiter 1996). The type of aroma is also affected by the cis/trans isomerization. Bedoukian (1971), studying the olfactory characteristics of the seven primary hexanols, found appreciable differences between the cis and trans isomers of each alcohol. The cis forms were more penetrating and green, while the trans isomers smelled more fatty. Moreover, the changes in the position of the double bond could produce different notes of green aroma and different degrees of acceptability. The type of functional group is related more with the intensity of the aroma than with its type. Katanaka and Kaijawara (1992) found that the intensity of aroma of the unsaturated C6-aldehydes is between 10- and 1000-fold stronger than that of

TABLE 10.1. Impact Compounds in a Selection of Foodstuffs

Foodstuff	Impact Compound	Class of Compound
Almond	Benzoic aldehyde	Aldehyde
Vanilla	Vanillin	Phenol, aldehyde
Raspberry	4-Phenyl-2-butanone	Ketone
Strawberry, pineapple	Furaneol	Alcohol
Cucumber	2-*trans*-6-*cis* Nonadienal	Aldehyde
Barlett pear	Ethyl ester of 2,4-decadienoic acid	Ester
Mandarin	Alpha-sinensal	Aldehyde, terpene

Source: Fisher C, Scott T. 1997. Flavores de los Alimentos. Biología y Química.

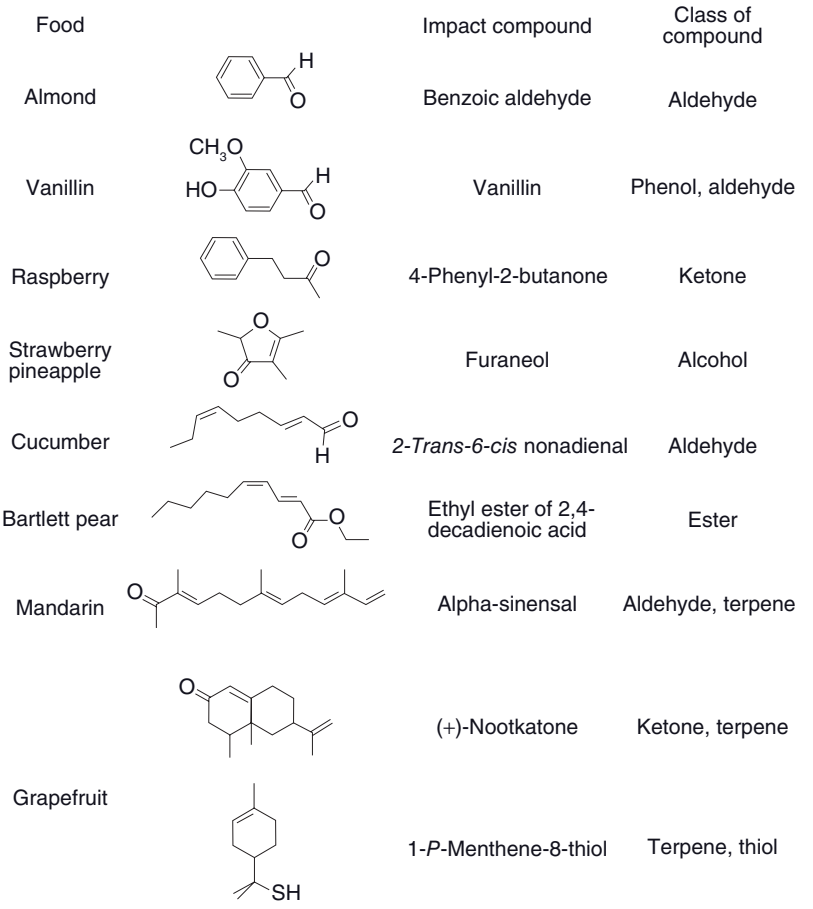

Figure 10.1. Structure of impact compounds in a selection of foodstuffs.

their corresponding hexenols, which have the double bond at the same position and with identical geometry.

These results have been of interest with regard, for example, to virgin olive oils because of the more than 100 volatile compounds identified by gas chromatography (GC) and mass spectrometry (MS) (Camera and Solinas 1990; Morales et al. 1994). Those contributing most to the particular aroma of virgin olive oil are the C6-aldehydes, the alcohols, and the esters (Angerosa 1997; Aparicio et al. 1996; Flath et al. 1973; Morales et al. 1995); however, none of the identified chemical compounds are able to explain all the sensations and notes making up its aroma, although they do contribute.

The volatile components of foods are very diverse. For example, in a study on the chemical characterization of the aroma of dessert and sparkling white wines by gas chromatography–olfactometry (GC/O), the results have shown that all these wines are relatively rich in 3-methylbutanal, phenylacetaldehyde, methional, sotolon, and the ethyl esters of 2-, 3-, and 4-methylpentanoic acids (Campo et al. 2008). In another study analyzing volatile compounds derived from raw and roasted earth

almond, the main flavor compounds in the majority of the volatiles identified in raw earth almond were alcohols, whereas the majority of the volatiles identified in roasted earth almond suggest that the flavor formation is via the Maillard reaction, with pyrazines contributing directly to the roasted flavor (Cantalejo 1997). The characteristic flavor of foods (including trigeminal stimulation) is normally related with a single class of compounds—for instance, the secoiridoids are perhaps the only compounds responsible for the gustatory perceptions of virgin olive oil (Gutiérrez et al. 1989, 2003).

The classes of chemical compounds responsible for flavor may be volatile or nonvolatile. Among the former are aldehydes, alcohols, ketones, acids, esters, ethers, lactones, furans, hydrocarbons, phenols, terpenoids, nitrogen-containing compounds, and sulfur-containing compounds. Among the nonvolatile compounds are amino acids and peptides, organic acids, sugars, salts, flavonoids and alkaloids, phenols, and isocyanates (Fisher and Scott 1997; Reineccius 2006).

The compounds responsible for flavor are very numerous. Some are present naturally in raw foods, but these are often cooked, baked, or toasted to make them more palatable. As knowledge about their flavors increases—in particular, the way to analyze and produce them—foods will appear with stronger and better-balanced flavors to fully meet consumer demand.

FLAVOR ANALYSIS

History

Historically, there was little literature in the public domain on food flavor until the mid-1970s. While flavor research has existed for well over 100 years, the vast majority of early flavor research was done within companies and kept secret. The industry generally did not even disclose findings in patents but chose to hold them as trade secrets, and this is still common today. Ernest Guenther was one of the first individuals to publish a significant work in the public domain, with a six-volume collection on essential oils (Guenther 1972–1998). The book of Fenaroli (1994) is a valuable source, as it provides great information on sensorial thresholds, molecular structures, empirical formulas and molecular weight (MW), specifications, natural frequency, syntheses, uses in foods, and so on, related with the use of chemical aromas.

Benzaldehyde was the first flavor compound to be identified, more than 150 years ago (Beilstein 1925; Flament 1982). This compound was isolated from bitter almonds by Vogel in 1818 and Martres in 1819 (Beilstein 1925) but was not identified until 1832. It was not until the first half of the 20th century that the first attempts were made to identify the main volatile compounds in a number of food products. Identification was based on chemical or physical properties of derivatives such as 3,5-dinitrobenzoates or 2,4-dinitrophenylhydrazones, and on color tests. These studies often took many years and resulted in the identification of not more than 10–30 constituents, since many properties could be measured. The following examples are illustrative. Coppens and Hoejenbos (1939) studied the flavor of strawberries; they started from 445 kg of fruit, which they extracted with diethyl ether, separating the pulp from the solvent by distillation and the acids by extraction with an aqueous solution of sodium carbonate. From the dried ether solution, they

obtained 86 g of oil, in which they identified 12 compounds and tentatively identified five. Reichstein and Staudinger (1950), using classical techniques (derivatives, physical parameters), were able to identify 29 new volatile compounds in coffee. The fact that these included compounds such as alkylpyrazines and 2-furyl methanethiol makes this an outstanding and remarkable achievement.

It is difficult to compare these studies with more recent studies on strawberries and coffee in which sophisticated modern techniques have been used. Good examples of these recent studies are those of Ulrich and others (2007) and Flament (2002).

In evaluating these results, it should be kept in mind that it is not the number of compounds identified that is decisive, but the skill and imagination with which available equipment and techniques are used.

Flavor research has largely meant studying the *volatile* in a food or flavoring. Without aroma, it is very difficult to identify the flavor of a food product. The task of identifying volatile flavor components (aroma compounds), particularly in a food matrix, is one of the most formidable tasks faced by an analytic chemist. The first obstacle is that instruments are less sensitive to many aromas than the human nose. Stuiver (1958) postulated that the nose has a detection limit for volatile compounds far higher than the most sensitive methods of instrumentation analysis. Unfortunately, analytic instrumentation has no sense of taste or smell. Instrument response for the flame ionization detector (FID) (the most commonly used detector in GC) is related to the number of carbon–carbon bonds, whereas the human olfactory system varies greatly in response to different odors. For example, 2-methoxy-3-hexylpyrazine has an odor threshold of 1 part/10^{12} parts water, while pyrazine has an odor threshold of 175,000 parts/10^{12} parts water (Seifert et al. 1970). On pyrazine alone, the human threshold varies by nearly 2×10^8. It could be that the smallest peak in a gas chromatogram may be more important to aroma than the largest peak. It must also be recognized that the instrument is providing no appreciation for aroma character of each component. It is not apparent that, for example, peak 5 is green while peak 50 contributes oxidized notes. There is no question that flavor analysis offers a most challenging analytic problem.

Subjective Versus Objective

The question we might ask before analyzing the flavor of foods is: How to do it? There are two types of technique: (1) chemical and (2) sensorial, and both must be used. Sensorial analysis considers the foodstuff as a whole and gives an overall impression, although some of its techniques—such as quantitative descriptive analysis (QDA)—can provide a description of the main attributes contributing to the flavor of the food. Obviously, as the measuring instrument is a group of tasters, it is subjective, even though it works with standardized and precise techniques. With a good analytic panel, the subjectivity is reduced, and it is possible to obtain reproducible results. Objective chemical analysis depends on the techniques of sampling and/or those of separation prior to the determinations; in addition, it must be remembered what was mentioned in the previous section: some compounds that produce large responses lack odor. Thus, instrumental techniques are complementary to sensorial ones, and of great value, as they can provide the identification of some of the components of most impact in the flavor of foodstuffs.

Psychophysics and Sensorial Evaluation

Psychophysics is the study of the relationship between the psychological perception of a stimulus and the response caused by the perception. Such is the basis of sensorial analysis.

There are different types of test in sensorial analysis, depending on the aim to be achieved:

(1) *Tests of qualitative difference* enable determining the difference between two samples of the same food product that are distinguished by any characteristic. The most used are triangular, paired, duo-trio.
(2) *Tests of preference* enable determining the preference between two products. The most used are paired or hedonic scales.
(3) *Rank tests* enable determining the magnitude of the difference of a particular attribute.
(4) *Scale tests* enable evaluating the magnitude of the difference. The scales used can be structured or nonstructured.
(5) *Descriptive tests* enable identifying and measuring the intensity of the different sensations perceived by the sense organs. They have been developed as various forms: flavor profile, QDA, and profile of free choice. The first two are the most used.

The flavor profile is a purely descriptive test, developed by Caincross and Sjöstron (1950). The test is based on the identification and evaluation of the intensity of each flavor attribute in the foodstuff.

QDA is a technique used for the quantitative characterization of the sensorial attributes of flavor, in order of their appearance. It was developed because of the need to analyze the responses of the tasters of an analytic panel by statistical procedures (Stone and Bloomquist 1974; Stone et al. 1980).

Chemical Analysis of Flavor

Because the aroma components of a foodstuff are distributed in its matrix, the procedures to isolate and concentrate them are complicated. Most of the techniques used in aroma isolation take advantage of either solubility or volatility of the aroma compounds. Inherently, aroma compounds must be volatile to be sensed, so it is logical that volatility is a common basis for separation from a food matrix. Likewise, aroma compounds tend to be more soluble in an organic solvent than in aqueous solution (e.g., a food matrix), thus aroma isolates may be prepared by solvent extraction processes.

Before dealing with the study of the aromatic fraction of foodstuffs, the following points must be considered: (1) the concentration of volatile compounds is usually low, so that only a small amount will be obtained from a large sample; (2) the volatile fraction generally comprises a great number of compounds of differing chemical nature and molecular weight, besides which their concentration can vary over a wide range; (3) there is no direct relationship between the incidence of each compound in the aromatic fraction from the variability of the thresholds of detection of the different compounds; (4) as solvents are used to extract the components, a process

TABLE 10.2. Methods of Separation and Concentration of Volatiles

Methods without Concentration	Methods with Concentration	Others
Direct injection	Distillation	Extraction with solvents
Static headspace	Simultaneous distillation–extraction	
	Dynamic headspace/purge and trap	Membrane dialysis
	Supercritical fluid extraction	
	Solid phase microextraction	

of concentration is necessary; and (5) the formation of artifacts during the analysis must be prevented as they can interfere and produce incorrect results (Flath et al. 1981).

In general, studying the volatile fraction of foods requires four steps: (1) separation, (2) concentration, (3) fractionation of the different compounds, and (4) identification (Mehlitz and Gierchner 1962). All these steps are very important and must be properly chosen (Golovnya 1982).

Separation and Concentration of the Volatile Fraction In accord with the first two sections, there are two great groups of methods: those *not involving concentration* and those *involving concentration* (Morales et al. 1992). Table 10.2 displays the existing methods, and next, a brief description of the most used will be given.

Without Concentration

DIRECT INJECTION This is the simplest technique for the analysis of volatile compounds and requires the least manipulation of the sample. It was developed for the analysis of lipids (Morrison et al. 1981; Warner and Frankel 1985). As described by Dupuy and others (1973), it consists of the introduction of a small amount of sample into a tube filled with glass wool at the injector inlet. The sample is heated to a specific temperature and purged with gas. The volatile compounds are extracted and purged by the carrier gas to the gas chromatograph column. An automatic system of direct injection was developed by Gensic and others (1984), in which a glass tube containing the sample is introduced into an external inlet block connected to a chromatograph. The compounds are isolated in the block and purged to the column. Jackson and Giacherio (1977) developed a direct procedure consisting of placing the oil sample on glass wool filling one end of a U-shaped aluminum tube. This is heated and purged with gas, bringing the volatile compounds to a chromatograph column connected to the other end of the tube.

The technique of direct injection is not very sensitive because the amounts of sample have to be small, and having to use high temperatures between 180 and 200°C, decomposition products can appear in the volatile. A further disadvantage is the need for a thorough cleansing of the chromatograph between samples to prevent the "memory effect" in GC.

STATIC HEADSPACE This is a simple way to analyze the volatile fraction of a foodstuff. It consists of analyzing an aliquot of the vapor phase, which is in equilibrium

with the sample in a sealed vial and subject to a specific temperature for a certain time. The temperature and sampling have to be strictly controlled. The concentrations of the volatile compounds in the two phases do not change with time once equilibrium is reached. Great care must be taken in obtaining the gas aliquot (Droozd and Novák 1979; Nuñez et al. 1984). The most used technique of sample injection is by syringe (Matos and Carbonell 1990); as no foreign substances are introduced, changes in the chemical reactions are minimized, and there is no loss of volatiles. Despite these advantages, the technique has three limitations: (1) it is appropriate only for highly volatile compounds that are present in appreciable amounts in the headspace (Jennings and Fisoof 1977), (2) there may be leaks in filling the syringe (Paillard et al. 1970), and (3) the rubber stopper of the vial can absorb certain volatile compounds (Davis 1970).

The greatest disadvantage of this technique is poor sensitivity, and often, there is a large amount of water in the headspace. Thus, it is unsuitable for the analysis of traces and of compounds with low vapor pressure (Alberola and Izquierdo 1979). It must also be taken into consideration that this technique uses small amounts of sample and that, in most cases, the use of high temperatures—of around 70°C—is necessary. Both factors restrict the use of this technique, although it does have the advantages of being quick and of requiring practically no manipulation of the sample.

With Concentration

DISTILLATION This is one of the techniques most used for the isolation of volatile compounds in foodstuffs. The most common methods are vacuum (Nawar 1988) and steam current (Ceccon 1986). Distillation under reduced pressure is of great interest as it enables working at low temperatures, thereby minimizing possible alterations of the sample. Generally, the vapors from the distillation are condensed on a refrigerant, or are trapped in different cryogenic traps or on absorbent materials.

After distillation, the distillate can be injected directly into the chromatograph, although a concentration step is usually necessary because some of the compounds may be in trace amounts, making their detection impossible otherwise. Such concentration is normally carried out by extraction of the aromatic fraction of the distillate, and drying and concentrating the extract.

SIMULTANEOUS DISTILLATION–EXTRACTION This widely used method was introduced by Likens and Nickerson in 1964 (Rujks 1983). The sample, diluted in water, and the solvent distill separately and condense in the same zone of extraction; the two phases are then separated and recycled. Modifications of the original technique have been described (Godefroot et al. 1981; Weurman 1969).

The method has several advantages: it uses small amounts of solvents, reduces the introduction of contaminants, yields a high concentration of volatile compounds in a short time, and minimizes thermal degradation by working at reduced pressure (Matos and Carbonell 1990). Its disadvantage is that it is not suitable for thermolabile volatile compounds.

Another method of vacuum distillation collects the volatile compounds in cold traps (two cooled with liquid nitrogen and one with acetone/solid carbon dioxide), dissolves them in ethyl ether, and finally concentrates them by distillation and microdistillation (Ullrich and Grosch 1988).

DYNAMIC HEADSPACE This method, introduced by Swinnerton and others (1962a,b), is used to determine the gases dissolved in aqueous samples. It consists of purging the volatile compounds of the sample with an inert gas at a specific temperature and passing the controlled flow through a trap where the compounds are first retained and subsequently desorbed and injected into the chromatograph for their separation. The method has two forms (Nuñez et al. 1984):

1. *Real Dynamic Headspace.* This technique consists of sweeping the surface of the sample with inert gas under shaking.
2. *Purge and Vapor.* This technique consists of bubbling gas through the sample. It can be carried out in open circuit, where the gas passes through the sample and the trap and is then voided, or in closed circuit, where the gas is recycled through the sample and the trap.

   The process can be affected by various factors, such as the diameter and length of the traps, the size and shape of the container used in the isolation, and the particle size of the absorbent (Krost 1982; Senf and Frank 1990), but the three fundamental variables that control it are temperature, time, and the purge flow. The latter two must be prefixed, as values too low can lead to defective purges, and too high to a loss of volatile. The temperature depends on the type of compound to be analyzed. If it is too low (maximum 60°C), the volatile compounds recovered are those actually in the sample at the moment of analysis. However, the amounts are very small, and the analysis is difficult. If high temperatures (up to 160°C) are used, the volatile compounds obtained are not only those actually present in the sample, but also those formed by thermal degradation of certain precursors. The amounts recovered are greater, and the analysis can be carried out more easily.

   Generally, the gaseous samples obtained using the method of Nuñez and others (1984) consist basically of purge gas, steam, and small amounts of volatile compounds. Thus, a concentration step is necessary, using adsorbent and/or cryogenic traps. Such traps can be described as follows:

   - *Absorbent Traps.* These consist of a glass or stainless steel tube filled with a determined amount of adsorbent material (the trap). The volatiles are retained when the inert gas, loaded with the volatile compounds, passes through the trap. Various materials have been used: active carbon and graphitized materials, Tenax series, Porapak series, Chromosorb series, Amberlite series, and other adsorbent materials such as Ostión SP-1, Synachrom, and Spheron, which have been used much less than other trapping materials (Clark and Cronin 1975; Crisp 1980; Dressler 1979; Morales and Aparicio 1993)

     All the traps, depending on the material, require a conditioning to prevent interferences in the analysis (Betti et al. 1985; Macleod and Ames 1986).
   - *Cryogenic Traps.* These consist of a length of column or capillary tube that is cooled to very low temperatures, normally with liquid nitrogen. The volatile compounds pass through the trap and are condensed inside it. The volatile compounds are desorbed by passing a current of inert gas and raising the temperature, and pass to the analytic column. This type of trap has been used to analyze the volatile fractions in samples of virgin olive oil in order to study

the relationship between the composition of the volatile in the headspace and the sensorial analysis (Aparicio and Morales 1994; Servili 1995).

3. *Supercritical Fluid Extraction (SFE).* Recent studies show that the use of supercritical fluids provides a powerful alternative to the traditional extraction techniques. In the last few years, SFE has been applied in the extraction of seed oils but rarely in studying the volatile fraction of other food products. One of the main problems in the analysis of volatiles is leaks, which can cause the loss of part of the compounds responsible for the aroma of the foodstuff. Recently, extra-virgin olive oil and olives have been analyzed using supercritical carbon dioxide for isolating the volatile components (Morales et al. 1998b). For the collection of the volatile compounds of these samples, the SFE system trap was omitted. In its place, the SFE extract, together with the total effluent volume of carbon dioxide, was purged through a removable Tenax trap. The trap was analyzed by thermal desorption and high-resolution gas chromatography/mass spectrometry (HRGC-MS) (Morales et al. 1998a).
4. *Solid Phase Microextraction (SPME).* This is the most recently applied technique in the analysis of volatile compounds. Pawliszyn's group (2001) was the first to develop the SPME method, and they applied it to environmental analysis. Since then, it has become a widely used technique for the analysis of volatiles in foods. Harmon (1997) and Marsili (2002b) provided a comprehensive review and a critical review, respectively, of this technique.

   The technique employs a fiber of adsorbent material, placed inside a modified chromatographic needle, to isolate and concentrate the compounds. The fiber is positioned in the headspace of the sample for a specific time. The volatile compounds are diffused and distributed on the polymer coating in function of their coefficients of distribution. The fiber is removed from the sample and placed in the GC injector, where the compounds are thermally desorbed. In the last few years, this method is being used to analyze the volatile fraction of many food products (Belliardo et al. 2006; Coelho et al. 2006; Fernando and Grün 2001; Servili et al. 2000).

Fractionation and Identification of the Volatile Fraction The analytic method used to analyze an aroma isolate and concentrate depends on the task at hand. For determining the amount of one or more aroma compounds in a food, GC may suffice. If one is looking for odorous compounds (desirable or undesirable) in a food, then GC/O would be used. To identify the aroma compounds in a food would require GC and MS (or GC/O/MS). Other instrumental methods may also be applied (e.g., infrared [IR] or nuclear magnetic resonance [NMR]), but the bulk of aroma research is done by these three methods.

Prefractionation Despite the excellent resolving powers of modern capillary chromatography, there are situations where the analyst may choose to prefractionate the aroma isolate. Some of the more common methods to prefractionate flavor isolates prior to GC analysis are acid/base preparation, HPLC, silicic acid column chromatography, and preparative GC (Parlimen 1981; Schreier 1984; Teitelbaum 1977).

GC The development of GC in the mid-1960s had a decisive effect on aroma research. In 1963, only about 500 aromatic compounds of foodstuffs had been identified—today, some 7000 compounds are known. GC is ideal for aroma study as it has an excellent separating power and great sensitivity in detection (levels of detection in picograms). Resolution and sensitivity are essential for the analysis of the complex aroma isolates encountered in flavor work. The primary disadvantage of capillary columns is their low sample capacity. Sample capacity is needed if the analyst wishes to collect a component for further work, for example, NMR or IR. While capacity is enhanced through the use of thick phase coatings (which can work with up to 500 ng per component), it is still problematic.

GC/O or GC-MS/O GC and GC-MS/O are techniques uniquely applied to aroma studies (Blank 2002). In olfactometric techniques, the nose is used as a GC detector. The GC system may be set up with the column split, so that a portion of the effluent goes to the sniffing pot and the remainder to the GC detector (FID or an MS detector).

GC/O produces what is called an aromagram: a listing of the odor character of each peak in a GC run. More than one sniffer is used in the analysis to minimize data error due to any specific anosmia. While the data obtained are extremely valuable, since they indicate which GC peaks have odors, they do not identify the peak. For that, it is generally desirable to use a GC-MS/O method (as opposed to GC/O). Most commonly, the analyst is interested in identifying components that have odor (which may be a desirable odor or an off-odor). GC/O work enables the assignation of a given odor to a given GC peak, but unfortunately, it is a problem to determine which GC peak is which MS peak. Although making this assignment might be thought simple, MS works under vacuum, and this changes GC column elution time, even when using the same GC column in both GC and MS. A second complication is that the responses to compounds given by an FID are different from those of an MS detector. This means that retention times may shift, and the analytic profiles change between a GC run and the GC-MS run. If an odor profile and an MS profile can be obtained at one time, the identification of odorants is greatly simplified.

MS The inherent very high sensitivity of MS (10–100 pg) and its compatibility with GC make a GC-MS combination extremely valuable. Mass spectrometers can be of low (LR) or high resolution (HR). Those of LR provide the molecular weight of a compound, but not its elemental composition, whereas those of HR do. Most of the earlier studies of flavor used LR.

MS is generally used in the area of flavor either to determine the identity of an unknown or to act as a mass-selective GC detector. As mentioned, MS as an identification tool is unequalled by other instruments. Comprehensive MS libraries and efficient searching algorithms make identification simple; however, herein lies a danger. The neophyte often accepts the proposed identifications without question and obtains incorrect identifications. It is essential that all MS identifications are supported by other data—for example, GC retention time data, Kovasts, index retention, IR, or NMR.

The use of an MS as GC detector can help in some quantifications or in resolving chromatographic problems that would be difficult or impossible by other techniques (Holland and Gadner 2002). In such cases, the MS is operated in either the selected

ion (SIM), multiple ion detection (MID) mode, or full scan mode if sensitivity and scan rate are adequate.

Electronic Noses

In an electronic nose, an odor, *j*, is presented to the active material of a sensor, *i*, which converts a chemical input to an electrical signal. The sensors are key components of this system and have been developed to detect specific gases and vapors since the 1970s. There are many types of sensor, including semiconductor gas sensors (metal oxides), surface acoustic wave devices, biosensors, conducting polymers, and MS-based sensors (Hodgins and Simmonds 1995). However, the requirement for the sensors in an electronic nose is that they have a partial sensitivity; that is, they can respond broadly to rank a class of gases rather than to a specific one.

An electronic nose functions by analyzing a sensory array response to a complete aroma, but there is no separation of the components of the aroma. Although this technique is relatively recent, it has generated a great number of publications (Gardner 1994; Hodgins and Simmonds 1995; Marsili 2002; Rodriguez-Méndez et al. 2005). It is particularly attractive for certain applications of quality control where a decision of acceptable/unacceptable is often necessary.

SUMMARY

This chapter shows that the flavor of a food is a determining factor for their acceptance or rejection. The compounds responsible for the flavor spread over a wide range of chemical compounds, which can be both volatile and nonvolatile. These compounds are detected and transferred into nerve signals in our sense organs, producing a response from its flavor of the food for lunch. The investigation and analysis of the flavor of food must be carried out both chemically and sensorially, and this chapter has collected and described the main techniques that actually exist.

REFERENCES

Alberola J, Izquierdo L. 1979. La fracción aromática del zumo de naranja II. Análisis del espaciode cabeza. *Rev Agroquím Technol Aliment* 19:327–37.

Angerosa F. 1997. Relationship between aroma compounds and malaxation time of olive paste. Proceedings of the 3rd International Symposium on Olive Growing, Chani, Crete.

Aparicio R, Morales MT. 1994. Optimization of a dynamic headspace technique for quantifying virgin olive oil volatiles. Relationship between sensory attributes and volatile peaks. *Food Qual Prefer* 5:109–14.

Aparicio R, Morales MT, Alonso MV. 1996. Relationship between volatile compounds and sensory attributes of olive oils by sensory wheel. *J Am Oil Chem Soc* 73:1253–64.

Bedoukian PZ. 1971. The seven primary hexenols and their olfactory characterists. *J Agric Food Chem* 19:1111–4.

Beilstein S. 1925. Analysis of components of bitter almond. *Handb Org Chem* 7:174–83.

Belliardo F, Bicchi C, Cordero C, Liberto E, Sgorbini B. 2006. Headspace-solid-phase microextraction in the analysis of the volatile fraction of aromatic and medicinal plants. *J Chromatogr Sci* 44(7):416–29.

Betti A, Coppi S, Bichi C. 1985. Pre-concentration of organic pollutants. Potential interference from the use of styrene copolymer adsorbents. *J Chromatogr* 349:181–7.

Blank L. 2002. Gas chromatography-olfactometry in food aroma analysis. In Marsili R (ed.), *Flavor, Fragrance and Odor Analysis*. New York: Marcel Dekker, pp. 297–312.

Boelens NH. 1996. *Volatile Compounds in Food: Qualitative and Quantitative Data*, 7th Ed. Zeist, The Netherlands: TNO Nutrition and Food Research.

Caincross SE, Sjöstron LB. 1950. Flavor profiles: A new approach to flavor problems. *Food Technol* 4:308–11.

Camera L, Solinas M. 1990. Identification of some volatile compounds of olive oil by GLC-MS. Proceedings of International Seminar on Olive Oil and Table Olives: Technology Aspects and Quality, pp. 156–66, Città S., Angelo, Italy.

Campo E, Cacho J, Ferreira V. 2008. The chemical characterization of the aroma of dessert and sparkling white (Pedro Ximenez, Fino, Sauternes, and Cava) by gas chomatography-olfactometry and chemical quantitative analysis. *J Agric Food Chem* 56:2477–84.

Cantalejo MJ. 1997. Analysis of volatile components derived from raw and roasted earth-almond (*Cyperus esculentus* L.). *J Agric Food Chem* 45:1853–60.

Carterette E, Friedman MP. 1989. *Flavor Chemistry. Trends and Development*. Washington, DC: American Chemical Society.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levin JD, Julius D. 1997. The capsaicin receptor: A heat-activate ion channel in the pain pathway. *Nature* 389:816–25.

Ceccon L. 1986. La frazione lipidica nella caratterizazione marceologica dei fromaggi. Acidi gras liberi volatili. Nota IV. *Riv Ital Sostanze Gras* 63:551–4.

Charalambous G, Buckner KJ, Hardwich WA, Weatherby TJ. 1975. Determination of beer flavor compounds by high pressure liquid chromatography: IV Nucleotides. *Tech Q Master Brew Assoc Am* 12(4):203–10.

Clark RG, Cronin DA. 1975. The use of activated charcoal for the concentration and analysis of headspace vapours containing food aroma volatile. *J Sci Food Agric* 26:1615–24.

Coelho E, Rocha SM, Delgadillo I, Coimbra MA. 2006. Headspace-SPME applied to varietal volatile components evolution during *Vitis vinifera* L. cv. "Baga" ripening. *Anal Chim Acta* 563(1–2):204–14.

Coppens A, Hoejenbos L. 1939. Investigation of the volatile constituents of strawberry juice. *Recl Trav Chim Pays Bas* 58:680–90.

Crisp S. 1980. Solid sorbent gas samples. *Ann Occup Hyg* 23:47–76.

Davis PL. 1970. A simple method to prevent loss of volatile during headspace analysis. *J Chromatogr Sci* 8:423–4.

Dressler M. 1979. Extraction of trace amounts of organic compounds from water with porous organic polymers. *J Chromatogr* 165:167–206.

Droozd J, Novăk J. 1979. Headspace gas analysis by gas chromatography. *J Chromatogr* 165:141–65.

Dupuy HP, Fore SP, Goldblatt LA. 1973. Direct gas chromatographic examination of volatiles in salad oil and shortenings. *J Am Oil Chem Soc* 50:340–2.

Duran Meras L, Salinas F, dela Pena AM, López Rosas M. 1993. Simultaneous determination of flavor enhancers inosine 5′-monophosphate and guanosine 5′-monophosphate in food preparations by derivative spectrophotometry. *J Am Oil Chem Soc Int* 76(4):754–63.

Fenaroli G. 1994. *Fenaroli's Handbook of Flavor Ingredients*, 3rd Ed. Boca Raton, FL: CRC Press, p. 504.

Fernando LN, Grün IU. 2001. Headspace-SPME analysis of volatiles of ridge gourd (*Luffa acuntula*) and bitter gourd (*Momordica charantia*) flowers. *Flav Frag J* 16(4):289–93.

Fisher C, Scott TR. 1997. Flavores de los alimentos: Biologia y química. In Acribia SA (ed.), *Flavor Chemistry and Technology*, 2nd Ed. Boca Raton, FL: CRC Press, p. 212.

Flament I. 1982. Propspection des substances aromatisantes perspectives et evolution des connaissances sur la composition desduits naturel. *Labo-Pharma-Probl Techn* 30(321):404–12.

Flament L. 2002. *Coffee Flavor Chemistry*. New York. Wiley Publishing.

Flath RA, Forrey RR, Guadagni DG. 1973. Aroma components of olive oil. *J Agric Food Chem* 21:948–92.

Flath RA, Sugisawa H, Teranishi R. 1981. Problems in flavor research. In Teranishi R, Flath RA (eds.), *Flavor Research: Recent Advances*. New York: Marcel Dekker, pp. 1–10.

Gardner JW. 1994. A brief history of electronic noses. *Sens Actuators B Chem* 18–19:211–20.

Gensic JL, Szuhaj BF, Endress JG. 1984. Automated gas chromatographic system for volatile profile analysis of fats and oils. *J Am Oil Chem Soc* 61:1246–9.

Godefroot M, Sandra P, Verzele H. 1981. New method for quantitative essential oil analysis. *J Chromatogr* 203:325–35.

Golovnya RV. 1982. Some analytical problems in flavor research. *J Chromatogr* 251:249–64.

Guenther E. 1972–1998. The essential oils. http://www.lib.virginia.edu/speccol.

Gutiérrez F, Albi MA, Palma R, Ríos JJ, Olías JM. 1989. Bitter taste of virgin olive oil: Correlation of sensory evaluation and instrumental HPLC analysis. *J Food Sci* 54:68–73.

Gutiérrez F, Ríos JJ, Gómez-Rey ML. 2003. Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionisation mass spectrometry. *J Agric Food Chem* 51:6021–5.

Harmon AD. 1997. Solid-phase microextraction for the analysis of flavors. In Marsili R (ed.), *Techniques for Analysing Food Aroma*. New York: Marcel Dekker, pp. 81–9.

Hodgins D, Simmonds D. 1995. Sensory technology for flavor analysis. *Cereal Foods World* 40(4):186–96.

Holland JF, Gadner BD. 2002. The advantages of GC-TOFMS for flavor and fragrance analysis. In Marsili R (ed.), *Flavor, Fragrance and Odor Analysis*. New York: Marcel Dekker, pp. 107–21.

Jackson HW, Giacherio DJ. 1977. Volatiles and oil quality. *J Am Oil Chem Soc* 54:458–60.

Jennings WG, Fisoof M. 1977. Comparison of sample preparation techniques for gas chromatographic analysis. *J Agric Food Chem* 45:440–5.

Jennings WG, Sevenants MR. 1969. Volatile esters of Bartlett pear. III. *J Food Sci* 29:158–63.

Katanaka A, Kaijawara T. 1992. Odor-structure relationships in n-hexanols and n-hexenales. *Z Naturforsch* 47:183–9.

Keast RS, Dalton PH, Breslin PAS. 2004. Flavor interactions at the sensory level. In Taylor AJ, Roberts D (eds.), *Flavor Perception*. Ames, IA: Blackwell Publishing, pp. 1–21.

Krost KJ. 1982. Collection and analysis of hazardous organic emission. *Anal Chem* 54:810–7.

Lawless HT. 2004. Trigeminal response page. http://zingerone.foodsci.cornell.edu/trigeminal/ 1996.

Leffingwell JC. 1998. Cool without menthol & cooler than menthol. Leffingwell & Associates. http://www.R-pro-ject.org.17 (accessed March 18, 2004).

Maarse H, Visscher CA, Willemsens LC, Nijssen LM, Boelens MH. 1994. *Volatile Compounds in Food: Qualitative and Quantitative Data*. Zeist, The Netherlands: TNO Nutrition and Food Research.

Macleod G, Ames JM. 1986. Comparative assessment of the artefact background on thermal desorption of Tenax Gc and Tenax TA. *J Chromatogr* 355:393–8.

Maga JA. 1983. Flavor potentiators. *CRC Crit Rev Food Sci Nutr* 18(3):231–8.

Marsili R. 2002a. Combining mass spectrometry and multivariate analysis to make a reliable and versatile electronic nose. In Marsili R (ed.), *Flavor, Fragrance and Odor Analysis*. New York: Marcel Dekker, pp. 349–66.

Marsili R. 2002b. SPME comparison studies and what they reveal. In Marsili R (ed.), *Flavor, Fragrance and Odor Analysis*. New York: Marcel Dekker, pp. 205–12.

Matos A, Carbonell E. 1990. Análisis de la fracción aromática de los alimentos. Técnicas de extracción y concentración. *Rev Agroquim Technol Aliment* 30:431–44.

Mehlitz A, Gierchner K. 1962. Ueber bisherige gaschomatographische Untersuchungen der Aromastoffe von Frechten. Proceedings Symposium Volatile Fruit Flavor, International Federation Fruit Juice Producers, p. 25, Bern, Switzerland.

Morales MT, Alonso MV, Ríos JJ, Aparicio R. 1995. Virgin olive oil aroma: Relations between volatile compounds and sensory attributes by chemometrics. *J Agric Food Chem* 43:2925–31.

Morales MT, Aparicio R. 1993. Characterizing some European olive oil varieties by volatiles using statistical tools. *Grasas Aceites* 44:113–5.

Morales MT, Aparicio R, Alonso MV. 1998b. Analysis of virgin olive oil aroma SFE-GC-MS. Proceedings of the 5th International Symposium of Hyphenated Techniques in Chromatography, February 11–13, Brugge, Belgium.

Morales MT, Aparicio R, Gutiérrez F. 1992. Ténicas de aislamiento y concentración de volátiles de aceites vegetales. *Grasas Aceites* 43:164–73.

Morales MT, Aparicio R, Ríos JJ. 1994. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J Chromatogr A* 668:455–62.

Morales MT, Berry AJ, McIntyre PS, Aparicio R. 1998a. Tentative analysis of virgin olive oil aroma by SFE-GC-MS. *J Chromatogr A* 819:267–75.

Morrison WH, Lyon BG, Robertson JA. 1981. Correlation of gas liquid chromatographic volatiles with flavor intensity scores of stored sunflower oils. *J Am Oil Chem Soc* 58:23–7.

Moruzzi G. 1977a. *Il gusto. Fisiologia della vita di relazione*. Torino, Italy: UTEC, pp. 382–9.

Moruzzi G. 1977b. *L'olfatto. Fisiologia della vita di relazione*. Torino, Italy: UTEC, pp. 376–81.

Nawar WW. 1988. A study of the volatile components generated from butter oil by heat. *Rev Fr Corps Gras* 35:117–22.

Ney KH. 1988. Sensogramme Eine Methodische Erweiterung der Aromagramme. *Gordian* 88:19–26.

Nijssen LM, Visscher CA, Maarse H, Willemsens LC, Boelens MH. 1996. *Volatile Compounds in Food: Qualitative and Quantitative Data*, 7th Ed. Zeist, The Netherlands: TNO Nutrition and Food Research, p. 489.

Nuñez A, González LF, Janak J. 1984. Preconcentration of headspace volatiles for trace organic analysis by gas chromatography. *J Chromatogr* 300:127–62.

Paillard N, Pitoloulis S, Mattel A. 1970. Techniques de preparation et analyse de lárome de quelques fruits. *Lebensm Wiss Technol* 3:107–14.

Parlimen TH. 1981. Concentration and fractionation of aromas on reverse-phase absorbents. *J Agric Food Chem* 29(4):836–42.

Pawliszyn J. 2001. Solid phase microextraction. *Adv Exp Med Biol* 488:73–8.

Pelosi P. 1994. Odorant-binding proteins. *Crit Rev Biochem Mol Biol* 3:199–228.

Reichstein T, Staudinger H. 1950. Das Aroma des gerosteten Kaffees. *Experientia* 6:280–5.

Reineccius G. 2006. *Flavor Chemistry and Technology*, 2nd Ed. Boca Raton, FL: CRC Press, p. 467.

Rodriguez-Méndez ML, Apetrei C, de Saja JA. 2005. Modified carbon paste electrodes for discrimination of vegetables oils. *Sens Actuators B Chem* 111–2:403–9.

Rossiter KJ. 1996. Structure-odor relationships. *Chem Rev* 96:3201–40.

Roujou De Boubee D, Leeuwen CV, Dubourdieu D. 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines: Effect of environmental conditions on concentration in grapes during ripening. *J Agric Food Chem* 48:4830–7.

Rounds MA, Gregory JF. 1998. High performance liquid chromatography. In Nielsensen SS (ed.), *Food Analysis*. Gaithersburg, MD: Aspen Publishers, p. 509.

Rujks J. 1983. Possibilities and limitations of steam distillation-extraction as a pre-concentration technique for trace analysis of organics by capillary gas chromatography. *J Chromatogr* 279:395–407.

Schreier P. 1984. The use of HRGC_FTIR in tropical fruit flavors analysis. In Schreier P (ed.), *Analysis of Volatiles: Methods and Applications*. Berlin: Gruyter, pp. 269–79.

Seifert R, Guadagni RG, Black DG, Harris G. 1970. Synthesis of some 2-methoxy-3-alkylpyrazines with strong bell pepper-like aromas. *J Agric Food Chem* 18:246–62.

Senf L, Frank H. 1990. Thermal desorption of organic pollutants enriched on activated carbon. V. Desorption behaviour and temperature profile. *J Chromatogr* 520:131–5.

Servili M. 1995. Sensory characterization of virgin olive oil and relationship with headspace composition. *J Sci Food Agric* 67:61–70.

Servili M, Selvaggini R, Agnese T, Montedoro G. 2000. Volatile compounds of virgin olive oil evaluated by solid phase microextraction: An application in the discrimination of virgin olive oil according to the cultivar and area. In Lanzotti V, Taglialatela-Scafati O (eds.), *Flavor and Fragrance Chemistry*. Dordrecht: Kluwer, pp. 211–20.

Stone H, Bloomquist J. 1974. Sensory evaluation by quantitative descriptive analysis. *Food Technol* 28:24–34.

Stone H, Siedel JH, Bloomquist J. 1980. Quantitative descriptive analysis. *Cereal Foods World* 25:642–4.

Stuiver M. 1958. Biophysics of the sense of smell. Thesis, Groningen University.

Sugita YH. 2003. Flavor enhancers. *Food Sci Technol* 116:409–16.

Swinnerton JW, Linnenbon VJ, Cheef CH. 1962a. Determination of dissolved gases in aqueous solutions by gas chromatography. *Anal Chem* 34:483–5.

Swinnerton JW, Linnenbon VJ, Cheef CH. 1962b. Revised sampling procedure for determination of dissolved gases in solution by gas chromatography solutions by gas chromatography. *Anal Chem* 34:1509–12.

Taylor AJ, Roberts DD. 2004. *Flavor Perception*. Ames, IA: Blackwell Publishing.

Teitelbaum CL. 1977. A new strategy for the analysis of complex flavours. *J Agric Food Chem* 25:466–71.

Teranishi R. 1989. *Trends and Development*. Washington, DC: American Chemical Society, p. 682.

Ullrich F, Grosch W. 1988. Flavor deterioration of soya-bean oil: Identification of intense odour compounds formed during flavor reversion. *Fat Sci Technol* 90:332–6.

Ulrich D, Komes D, Olbricht K, Hoberg E. 2007. Diversity of aroma patterns in wild and cultivated *Fragaria*. *Genet Resour Crop Evol* 54:1185–96.

Warner K, Frankel EN. 1985. Flavor stability of soybean oil based on induction periods for the formation of volatile compounds by gas chromatography. *J Am Oil Chem Soc* 62:100–3.

Weurman C. 1969. Isolation and concentration of volatiles in food odor research. *J Agric Food Chem* 17:370–84.

CHAPTER 11

Advanced Analytic Methodology

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INTRODUCTION

Characterizing and measuring aroma means analyzing odorant volatile compounds that are sensed in the nose at the olfactory epithelium either via the orthonasal (odor) or retronasal (aroma) routes when foods are eaten. Sensory analysis is certainly the most valid means of describing flavor characteristics. Applied to fruit flavor, sensory evaluation is a prominent descriptive tool that is described in Chapter 3. Instrumental analysis of aroma molecules has been the subject of important specialized treatises (for the most relevant literature on the subject, see Ho and Manley 1993; Marsili 1997; Mussinan and Morello 1998; Stephan et al. 2000; van Ruth 2001; Reineccius 2002, 2006; and Deibler and Delwiche 2004). The aroma compounds are mainly hydrophobic, and instrumental analysis of volatiles must consider, as a first step, an extraction method suitable for separating these hydrophobic volatiles from the food matrix. Traditional extraction methods are the subject of some of the following chapters. However, part of the present chapter will focus on recent advances made with sorptive extraction methods: solid phase microextraction (SPME) (Lord and Pawliszyn 2000) and stir bar sorptive extraction (SBSE) (Baltussen et al. 1999), with emphasis on fruit flavor applications.

As no single extraction method yields an "accurate" picture of food aroma (Reineccius 2002, 2006) isolation, analysis of aroma remains challenging (Teranishi 1998). The extraction step may lead to artifacts, and the total volatile content in most cases is very difficult to relate to a sensory profile determined by a panel or experienced by a consumer. Therefore, it appears much more efficient to concentrate efforts on the identification of those compounds that are really relevant to the perceived flavor. As no universal extraction method exists, some authors claim that it is essential to choose a method that yields an extract as representative as possible of the sensory properties of the food (Abbott et al. 1993a,b; Etiévant and Langlois 1998; Etiévant et al. 1994; Mehinagic et al. 2003). In order to identify the key volatiles that contribute significantly to food flavor, gas chromatography coupled

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to olfactometry (GC-O) is used most commonly (Lee 2003; Leland et al. 2001), and the method is described in a following chapter.

As it is still not known how the various volatiles combine to produce an overall sensory impression, it is particularly difficult to predict an aroma perception on the basis of GC-O data only. Demanding recombination experiments have to be undertaken (Buettner and Schieberle 2001a,b; Grosch 2001) even though significant advances have been made recently in the comprehension of odorant mixtures (Atanasova et al. 2004, 2005). Moreover, interactions between taste and aroma (Noble 1996) and interactions of trigeminal sensations with taste and aroma (Green 1996) occur and play an important role in global flavor perception. However, methods that allow direct analysis of flavor molecules released in the mouth during consumption have been developed in the last years (Roberts and Taylor 2000; Taylor and Linforth 1996). Development of instrumental techniques and data obtained recently for volatile flavor compounds will be presented, with a particular attention to fruit flavors.

Finally, specific instrumental techniques have been developed for the global analysis of food flavor. The methods currently used in the quality control of food flavor are still usually based on sensory evaluation by a panel of experts. These panels are able to monitor the quality of a particular food, to detect defects, and to compare samples for classification purposes. Nevertheless, obtaining faster results at lower cost using instruments could be an interesting alternative. The so-called electronic noses based on gas sensor technology, despite important drawbacks for some of them (Schaller et al. 2000), are theoretically able to perform some classification tasks (Schaller et al. 1998), and some applications for the analysis of foodstuffs have been developed (for instance, see Shaw et al. 2000 and Bleibaum et al. 2002 for fruit flavor applications). Two other global analysis methods based on mass spectrometry (MS) seem interesting for classification purposes. The first one analyzes total headspace using a mass spectrometer, without any prior GC separation (Vernat and Berdagué 1995). This method is often referred to as a mass-based electronic nose. Alternatively, headspace sampling may be replaced by SPME of food volatiles (Marsili 1999). Both sampling methods, followed directly by MS (Pérès et al. 2003), have found applications for the rapid characterization of fruit flavor, such as strawberry (Berna et al. 2007), grapefruit juice (Goodner and Rouseff 2001), and mandarin juice (van Ruth et al. 2008). The second method is pyrolysis MS (Aries and Gutteridge 1987), where a small food sample is pyrolyzed up to 500°C. The resulting volatile fraction, characteristic of the flavor and also of t he matrix composition, is analyzed by a mass spectrometer. For all these rapid instrumental methods, a pattern or fingerprint is obtained for each sample, and extensive data treatment, either by conventional multivariate statistics or artificial neural networks, is necessary for classification and quality control purposes (Aries and Gutteridge 1987; Pérès et al. 2003). This technique has also been applied for authentication studies.

SORPTIVE EXTRACTION METHODS

All the extraction procedures used to isolate the volatile fraction from any foodstuff should be adapted to the analysis of trace levels of hydrophobic molecules generally

present in a polyphasic medium, while minimizing losses of highly volatile molecules and preventing reaction of compounds or the formation of artifacts.

SPME, first developed for the extraction of volatile organic compounds in water (see Lord and Pawliszyn 2000 for an authoritative review on the technology), has been applied in the last 10 years to the isolation of aroma compounds from food (Harmon 1997; Kataoka et al. 2000; Pillonel et al. 2002; Reineccius 2002). Numerous applications deal with fruit flavors, for instance, strawberry (Ibanez et al. 1998; Reid et al. 2004; Song et al. 1998; Ulrich et al. 1997a,b), apple (Matich et al. 1996; Schulz et al. 2003), orange juice (Da Porto et al. 2003; Jia et al. 1998; Rega et al. 2003; Steffen and Pawliszyn 1996), kiwifruits (Wan et al. 1999), Brazilian fruits (Augusto et al. 2000), cantaloupe (Beaulieu and Grimm 2001), black currant (Ruiz del Castillo and Dobson 2002), pear, peach, and apricot (Riu-Aumatell et al. 2004). SPME partitions analytes between a liquid or a vapor phase and a thin solid phase adsorbent, of which there are several choices in terms of polarity and film thickness (Kataoka et al. 2000). Adsorbents are coated on inert fibers, generally associated with a syringe, which serves as a direct injection device (Harmon 1997; Kataoka et al. 2000). The method, which is an equilibrium one, can be performed either in the direct extraction mode (immersion of the fiber in the sample, generally an aqueous solution or suspension) or more conventionally in a headspace configuration. It can be automated very easily, but the extraction of the solutes depends on polarity, volatility, partition coefficients, sample volume, temperature, and the nature of the adsorbent-coating material. The mechanisms affecting the analysis of flavor volatiles by SPME have been reviewed (Holt 2001). Therefore, the technique exhibits a certain degree of selectivity, but with the advantages of sensitivity, ease of use, no solvent, and small sample volume (Harmon 1997; Kataoka et al. 2000; Pillonel et al. 2002; Reineccius 2002). Nevertheless, each extraction step, that is, extraction mode (direct or headspace), selection of fiber coatings, extraction setup (concentration, time, agitation, temperature), and desorption, gains through a careful optimization for each application (Bicchi et al. 2000; Doleschall et al. 2003; Ferreira and de Pinho 2003; Kataoka et al. 2000; Liu and Yang 2002). SPME, used for the first time for the analyses of food volatile compounds in the mid-1990s (Chin et al. 1996; Pelusio et al. 1995; Yang and Peppard 1994), has since been used in significant applications on food aroma (Kataoka et al. 2000, Pillonel et al. 2002, Le Quéré and Etiévant 2003, Bredie and Petersen 2006, and references cited therein). SPME has demonstrated several advantages in terms of rapidity and simplicity over conventional extraction methods in some comparative studies carried out in the analysis of strawberry (Ulrich et al. 1995), fruit juices (Miller and Stuart 1999), or olive oils (Cavalli et al. 2003; Kanavouras et al. 2005). Although headspace SPME shows always better retention capacities than static headspace (Miller and Stuart 1999), hence a better sensitivity, liquid–liquid extraction (Ulrich et al. 1995) and dynamic headspace with thermal desorption of Tenax® traps (Kanavouras et al. 2005) provide higher efficiency. Analyzing volatiles directly by immersion of the fiber in highly complex matrices could damage the fiber, and SPME is therefore used almost always in the headspace mode. Comparison of direct SPME and headspace SPME of Camembert cheese volatiles obtained after cryo-trapping of the aqueous phase under vacuum showed only a slight reduction in sensitivity using headspace SPME compared with direct SPME (Jaillais et al. 1999). Compared with other headspace extraction procedures, it is very often concluded that SPME is more appropriate for routine quality control

due to its simplicity, repeatability, and low cost (Cavalli et al. 2003). That is probably why the method has been widely used in recent works on food aroma (Le Quéré and Etiévant 2003, Bredie and Petersen 2006, and references cited therein).

The main limitation of SPME is the relatively low extraction yield due to the relatively small amount of sorbent available on the syringe needle (typically ca. 0.5 μL). Some artifact formations have also been noticed. Artifacts due to the formation of Maillard products during the desorption step was noticed in the flavor analysis of strawberry and apple fruits (Verhoeven et al. 1997). A significant reduction in artifact formation was obtained by rinsing the fiber with water prior to thermal desorption. However, the formation of artifacts is often unavoidable, and intrinsic artifact formation during the analysis of volatile amines (Lestremau et al. 2001) and volatile sulfur compounds (Lestremau et al. 2004) in air has been reported. Recently, new internally cooled fibers have been described for the analysis of five tropical fruits (Carasek and Pawliszyn 2006). By reducing the operating adsorption and desorption temperatures, it is claimed that artifact formation is significantly reduced, and it was found that the cold fiber was the most appropriate one for the purpose of extracting volatile compounds from the five fruit pulps studied.

A novel extraction technique that uses up to 200 μL of the sorbent polydimethylsiloxane (PDMS) was developed to overcome the rather low sensitivity drawback of SPME (Baltussen et al. 1999). The new technique called SBSE consists of a glass-coated magnetic stir bar with a cylindrical coating of PDMS (typically 5-mm film thickness, 10-mm length) maintained in an aqueous medium for a predefined time (Baltussen et al. 1999). After completion of the extraction step, the stir bar is transferred in a thermodesorption system and desorbed at the head of a GC column after cryo-refocusing of the extracted material (Baltussen et al. 1999). A complete set of coated stir bars (called Twister™) and thermodesorption system is commercially available from Gerstel GmbH (Gerstel, Müllheim a/d Ruhr, Germany). As expected, the recoveries were higher for SBSE than for SPME (Baltussen et al. 1999) and the detection limits were found in the low nanogram per liter range for a wide selection of volatile and semivolatile analytes (Baltussen et al. 1999). SBSE has been used for the measurement of volatiles from a wide variety of liquid foods (Pillonel et al. 2002 and references cited therein) including recently coffee brew (Bicchi et al. 2002), wine (Kittel et al. 2004), and lemon beverages (Tredoux et al. 2000). Despite careful optimization of the method, some repeatability problems have been observed with very volatile and/or soluble compounds (Ibañez and Sola 2006). Artifacts due to the thermal desorption step have also been described in the flavor analysis of onions (Granvogl and Schieberle 2006).

As an extension to SBSE, headspace sorptive extraction (HSSE) has been developed (Tienpont et al. 2000) to overcome the limitation of headspace SPME in terms of extraction capacity. Limits of detection in the nanogram per liter range have been obtained for the analyses of volatiles of some food samples (Tienpont et al. 2000). HSSE bars coated with ca. 55 μL PDMS (commercially available from Gerstel GmbH) are suspended in the headspace of the sample (Bicchi et al. 2002), and after sampling completion, the bars are thermally desorbed in a thermal desorption unit connected to a gas chromatograph (Bicchi et al. 2002). As expected, when comparing headspace extractions of coffee (Bicchi et al. 2002) and olive oil (Cavalli et al. 2003) volatiles, HSSE bars showed a higher concentration capacity than SPME fibers due to the higher amount of polymeric coating. However, like SBSE, HSSE needs a

thermal desorption unit to be handled and therefore requires a significant investment, compared with SPME if used in manual mode (automation is possible at cost price), without avoiding possible artifact formation. Nevertheless, as SBSE- and HSSE-coated bars are less subject to deterioration than SPME fibers, they can be applied easily to the analyses of both headspace and liquid (Bicchi et al. 2002).

DYNAMIC METHODS IN FLAVOR ANALYSIS

Supposing that the "best" extraction and identification methods are used, trying to correlate the quantified flavor components in a food to the sensory perception experienced when eating this food is very often unsuccessful. In other words, it is not enough to know the exact composition of food in terms of flavor compounds to understand perfectly the perception of its flavor. Perception of flavor is a dynamic process (Piggott 2000). During food consumption, the concentration of aroma compounds at the olfactory epithelium varies with time as they are released progressively from the food during chewing. Release kinetics depends on the composition of the food matrix and on individual mastication behavior. Sensory methods, such as time–intensity, have been used to study the time-related aspects of flavor perception (Piggott 2000).

Release of Volatiles *In Vivo*

Methods that measure volatiles directly in the mouth or in the nose have been developed to obtain data that could reflect the pattern of aroma molecules released from food and are effectively present at the olfactory epithelium during consumption. These methods have been reviewed in an authoritative edited book (Roberts and Taylor 2000). Among the various approaches aimed at sampling aroma from the nose (nosespace), the collection of expired air samples on Tenax traps provided the first robust results (Linforth and Taylor 1993; Taylor and Linforth 1994). Applied to tomatoes (Linforth et al. 1994) and to strawberry (Ingham et al. 1995), the method showed significant differences compared with headspace sampling, underlining the importance of maceration in the mouth.

By overlapping the sampling time periods, release curves can be constructed, and temporal changes reflecting relative concentrations of volatiles at a particular moment during consumption can be determined (Linforth et al. 1996). Correlation of accumulated data with sensory time–intensity data has been demonstrated (see for instance Delahunty et al. 1996).

Real-time *in vivo* flavor release was demonstrated some time ago using mass spectrometric breath-by-breath analysis with an optimized membrane separator interfaced to a mass spectrometer operated in electron impact mode (Overbosch 1987). Sensory time–intensity data measured in parallel for the perception of 2-pentanone in vegetable oil showed a clear adaptation effect, the stimulus being present in exhaled air long after the perception ended (Overbosch 1987). The method was also used by Soeting and Heridema (1988) and was extensively reviewed by Overbosch and others (1991). However, membrane separator techniques have important drawbacks in terms of selectivity and sensitivity (Taylor et al. 2000).

More recently, atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been developed to monitor aroma release during chewing (reviewed by Taylor et al. 2000). Air from the nose (nosespace) is sampled directly into the APCI-MS source through an interface, making real-time breath-by-breath analysis routinely possible (Taylor and Linforth 1996, Roberts and Taylor 2000, and references cited therein). Therefore, by combining time–intensity sensory studies with nosespace analysis, it is now possible to relate temporal parameters of aroma release to perception (de Kok and Smorenburg 1999; Linforth and Taylor 2006; Linforth et al. 2000; Salles et al. 2003; Taylor and Hort 2004). Perceptual interactions of aroma with sapid compounds may also be studied by this method through controlled delivery of both aroma and sapid molecules to panelists (Cook et al. 2004; Taylor and Hort 2004). The APCI-MS method has been extensively reviewed in detail in specialized treatises (Roberts and Taylor 2000; Taylor 2002; Taylor and Linforth 2003). The method has been applied recently to compare the aroma volatiles emitted *in vivo* from the yellow-fleshed kiwifruit at two different stages of eating ripeness (Friel et al. 2007). APCI sources may also be connected to a tandem mass spectrometer (MS/MS) such as an ion trap with the selectivity and structural capability benefits of MS/MS (Haahr et al. 2003; Le Quere et al. 2006; Sémon et al. 2003).

Another powerful chemical ionization method is proton transfer reaction mass spectrometry (PTR-MS). Originally developed by the Lindinger group (Lindinger et al. 1993) for on-line trace gas analysis, it consists of a three-chamber system. In the first chamber, nearly pure H_3O^+ ion is generated by electrical discharges in H_2O vapor. A small electric field drives H_3O^+ ions through an orifice into a drift tube, where chemical ionization takes place, while neutral volatiles are introduced into the drift tube. Volatile compounds with proton affinities exceeding that of water (166.5 kcal/mol) ionize by proton transfer from H_3O^+ and are accelerated into the third chamber, the mass spectrometer. Specificity of PTR-MS compared with other chemical ionization approaches is that the generation of the reactant ion and the chemical ionization process are spatially and temporally separated. Individual optimization is therefore possible, and quantification is made easier (Yeretzian et al. 2000a). Since the early works on headspace flavor volatiles that used PTR-MS (Yeretzian et al. 2000a,b), applications developed rapidly and a third specific international conference was organized in 2007 in Obergurgl, Austria (Hansel and Märk 2007). Results may be found in the current literature on headspace (Blank et al. 2003; Mayr et al. 2003a); nosespace studies on banana (Mayr et al. 2003b), strawberry (van Ruth et al. 2005), and other foodstuffs (Buettner et al. 2008; Roberts et al. 2003, 2004); and model mouth (van Ruth et al. 2003, 2004) applications.

In Vitro Measurements: Model Mouth Systems

Mechanical devices that aim to mimic the processes that occur in the mouth during eating have been developed (Piggott 2000 and references cited therein). These "model mouths" are often variants of dynamic headspace analysis, but their aim is to obtain time-resolved data similar to those obtained during *in vivo* studies. The various parameters like temperature, air flow, mastication rate, and addition of artificial saliva can be varied to study their effects on volatile flavor release (Rabe et al. 2002; van Ruth and Roozen 2000; van Ruth et al. 1995). The main advantages of model mouths are the large quantities of food samples that can be handled, overcoming some sensitivity problems encountered when monitoring volatiles at

low concentrations (Taylor 2002), and the suppression of interindividual variations, always encountered *in vivo*, that can be detrimental to a robust interpretation of the data. Release of volatile flavor compounds from the retronasal aroma simulator (RAS), originally developed by Roberts and Acree (1995), has been compared with flavor release *in vivo* using APCI-MS detection in both cases (Deibler et al. 2001). While delivering higher concentrations of volatiles than from human breath, the RAS gave a good approximation of time-averaged flavor release in the mouth, with volatile compounds present at similar ratios (Deibler et al. 2001). The model mouth device originally developed by Roozen and coworkers to study the rehydration of bell peppers (van Ruth et al. 1994), French beans, and leeks (van Ruth et al. 1995) has been used to investigate changes in flavor composition in real time during mastication of ripe and unripe bananas (Mayr et al. 2003c) or strawberry (van Ruth et al. 2005). In a recent study, this model mouth system has been compared with the RAS in terms of the effects of oral physiological characteristics on the release of aromas as a function of the physicochemical properties of model emulsions (Geary et al. 2004). Both have been found suitable for the study of oral parameters on aroma release (Geary et al. 2004) with confirmed limits for the temporal dimension of the release (Deibler et al. 2001; Geary et al. 2004). Real-time data, comparable to those obtained *in vivo*, have also been obtained with a computerized apparatus that follows the temporal dimension of flavor release from liquid food (Rabe et al. 2002). More recently, the release of apple volatile compounds has been related to mastication using a specially designed model mouth system (Arvisenet et al. 2006). A new chewing simulator with improved performance to mimic a human mouth has also been recently described (Salles et al. 2007).

Flavor release and flavor perception are dynamic processes and must be studied using dynamic methods (Piggott 2000). Dynamic techniques have been developed to study the parameters of flavor release from foods. Parallel increased applications of dynamic sensory methods provide a better understanding of food flavor. However, further work is needed to improve our knowledge of various interactions arising at different levels in the process of food consumption: for example, interactions between food ingredients (Taylor 2002) and interactions at the perceptual levels such as taste–aroma interactions (Given and Paredes 2002; Noble 1996; Taylor 2002), or trigeminal interferences (Given and Paredes 2002; Green 1996), as these play a fundamental role in overall flavor perception.

GLOBAL AND FAST ANALYSIS OF FLAVOR

The methods currently used to evaluate and control the quality of fruits flavor are still essentially based on sensory evaluation by panels of experts. These trained panels are able to handle such difficult tasks like quality monitoring through descriptive analysis, off-flavor detection, and comparison of samples for classification purposes. It could be interesting for such tasks to substitute humans by instruments that could give quicker answers at reduced costs.

"Electronic Nose"

Evaluation of aroma release from food using gas sensors, the so-called electronic noses or e-noses, is theoretically feasible (Hodgins 1997; Mielle 1996; Schaller et al.

1998). Electronic noses are generally composed of arrays of nonspecific gas sensors, which are based on different physical principles (Hodgins 1997; Mielle 1996; Schaller et al. 1998). The most common sensors are semiconducting metal oxides and conducting organic polymers, and they all give rise to nonspecific responses with particular patterns. Therefore, pattern recognition software, using either standard multivariate data analyses or artificial neural network technology, must be used for data treatment and final presentation of the results (Hodgins 1997; Schaller et al. 1998). The e-nose is particularly attractive for quality control applications where conformity/nonconformity answers are expected (Mielle 1996). Discriminative studies have been conducted on all types of food with some success (Schaller et al. 1998); for example, some results for fruit juices were obtained (Bleibaum et al. 2002; Shaw et al. 2000). However, some problems occurred with the repeatability of the system that could possibly be related to the product itself, the sampling technique, or the moisture content of the air used for sampling, precluding its use in routine tests (Schaller et al. 1998). Metal oxide semiconductors technology, despite some poisoning problems affecting the sensors, seems more reliable than conducting organic polymer sensors that showed a poor sensitivity to volatile components, the main problem of these sensors lying however with their instability (Schaller et al. 2000). The dangers of creating false classifications due to noise in e-nose have also been emphasized (Goodner et al. 2001). Nevertheless, despite some success in some classification tasks conducted to monitor fruit maturity (Benedetti et al. 2008; Gomez et al. 2006; Pathange et al. 2006) and quality during shelf life (Benedetti et al. 2008; Berna et al. 2004; Saevels et al. 2004), electronic noses hardly meet the requirements of the food industry in terms of precision, reproducibility, sensitivity, and stability (Mielle et al. 2000). Moreover, the sensors are known to deteriorate or can be poisoned, therefore changing their response. Even with frequent calibration, the inherent weaknesses of the technique make perenniality of the built databases problematic. Giving a global response, these instruments cannot be used to identify single odorants or to differentiate samples with subtle differences in distinctive sensory attributes. Therefore, in off-flavor studies where identification of the off-flavor compound is a prerequisite and in quality control assessment, they may be used successfully only after recognizing their inherent weaknesses (Mielle et al. 2000; Reineccius 2002).

MS-Based Systems

For classification purposes, two other global and fast analytic methods, based on MS, have been used for food products and seem more powerful and reliable than electronic noses. The first consists of a global analysis of a headspace sample by a mass spectrometer operated in electron ionization mode, without any GC separation. The feasibility of the method was originally demonstrated for rapid classification of four rather different French cheeses (Vernat and Berdagué 1995). This method is often referred to as an "MS-based electronic nose" or "MS e-nose." The mass patterns obtained, considered as fingerprints of the food products analyzed, also need data treatment, either by conventional multivariate analyses or artificial neural networks. SPME may be used as a preconcentration technique instead of headspace sampling (Marsili 1999). A review on the subject appeared in 2003 (Pérès et al. 2003). Applied to rapid assessment of apple quality during shelf life, MS e-nose data

clearly indicated the presence of both shelf life and storage history trend, trend e-nose measurements did not show. Therefore, the e-nose data had worse prediction performance than those based on the MS e-nose data (Saevels et al. 2004). During the study of shelf life and cultivar effect on tomato aroma profile, MS e-nose data indicated a clear change in aroma profile with shelf life, change that could not be demonstrated by a quartz microbalance-based e-nose (Berna et al. 2004). A clear distinction between cultivars based on MS e-nose was obtained; however, based on e-nose measurements only, it was difficult to discriminate between tomato varieties (Berna et al. 2004). Nevertheless, some results demonstrated the potential of the e-nose and the headspace fingerprint MS to complement routine sensory analysis of tomatoes (Berna et al. 2005). Recently, headspace has been directly connected to a PTR-MS with some success to study the influence of dehydration on subsequent reconstitution of mandarin juices (van Ruth et al. 2008). Headspace MS was also used for the classification of red wines with the aim to overcome traditional time-consuming methods (Dirinck et al. 2006).

Developed in the 1980s for food applications, direct pyrolysis-MS is another method that delivers "fingerprints" that can be used for classification/authentication purposes (Aries and Gutteridge 1987). With this method, a tiny food sample is pyrolyzed rapidly up to 530°C and the resulting volatile fraction, characteristic of the flavor and also of the matrix breakdown, is analyzed immediately by a mass spectrometer operated in low-energy electron ionization mode. Here again a complex mass pattern is obtained for each sample, and several data preprocessing steps are often necessary to select a reduced number of mass fragments that allow satisfactory classification. Curie-point pyrolysis MS with associated multivariate data analysis techniques is considered as a powerful classification tool in microbiology for the recognition of microorganisms (Talon et al. 2002 and references cited therein) and food science (Aries and Gutteridge 1987, Pérès et al. 2002, and references cited therein). A clear advantage of the method is that it provides a specific fingerprint of the food matrix that can be potentially related to textural parameters (Pérès et al. 2002). In terms of technical evolution, use of "soft" ionization methods (ionization at atmospheric pressure, chemical ionization) in association with "high-resolution" mass analyzers (i.e., time-of-flight) should allow to get information concerning the molecular origin of the ions and to deduce the identity of certain compounds present in the headspace. Such "fingerprints" should enable not only the characterization of a given product but also the identification of molecules directly responsible for odors or aromas. These advances open new perspectives for the rapid characterization of products by MS in a wide variety of applications (Pérès et al. 2003).

CONCLUSIONS

Developments in extraction methods based on adsorption of aroma molecules on polymers have considerably simplified routine flavor measurements. These sorptive extraction methods (SPME, SBSE, HSSE) are easy to use and fully automatable. Sensory evaluation remains compulsory to study human flavor perception. Relationships between aromas present in a foodstuff and sensory perception of that food are not straightforward to establish. Noticeably, it is still not well understood

how the various flavor-active components combine to produce a particular sensory perception. Developments in dynamic instrumental methods that can follow *in vivo* sequential release of aroma molecules allow the study of time-dependent balance of flavor compounds released from the food in mouth. With more complete and complementary information, combined flavor analysis and sensory evaluation should help understand the relationship between flavor stimuli and perceived flavor and explain the underlying mechanisms of flavor perception.

Authentication of foodstuffs and routine fast analyses for traceability purpose are other challenges. Tools developed recently that combine analytic instruments for global assessment of flavor with multivariate data analyses have demonstrated their usefulness for classification tasks.

REFERENCES

Abbott N, Bayonove C, Etiévant P, Issanchou S. 1993a. Production of wine extracts for chemical and sensory analysis with an odour representative of that of the original wine. In Bayonove C, Flanzy C, Sapis JC (eds.), *Connaissance Aromatique des Cépages et Qualité des Vins*. Montpellier: Revue Française d'Oenologie, pp. 419–26.

Abbott N, Etiévant P, Langlois D, Lesschaeve I, Issanchou S. 1993b. Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing. *J Agric Food Chem* 41:777–80.

Aries RE, Gutteridge CS. 1987. Applications of pyrolysis mass spectrometry to food science. In Gilbert J (ed.), *Applications of Mass Spectrometry in Food Science*. London: Elsevier Applied Science, pp. 377–431.

Arvisenet G, Billy L, Royer G, Prost C. 2006. Role of mastication on the release of apple volatile compounds in a model mouth system. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 465–8.

Atanasova B, Thomas-Danguin T, Chabanet C, Langlois D, Nicklaus S, Etiévant PX. 2005. Perceptual interactions in odour mixtures: Odour quality in binary mixtures of woody and fruity wine odorants. *Chem Senses* 30:209–17.

Atanasova B, Thomas-Danguin T, Langlois D, Nicklaus S, Etiévant P. 2004. Perceptual interactions between fruity and woody notes of wine. *Flav Frag J* 19:476–82.

Augusto F, Valente ALP, Tada ED, Rivellino SR. 2000. Screening of Brazilian fruit aromas using solid-phase microextraction-gas chromatography-mass spectrometry. *J Chromatogr A* 873:117–27.

Baltussen E, Sandra P, David F, Cramers C. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J Microcolumn Sep* 11:737–47.

Beaulieu JC, Grimm CC. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J Agric Food Chem* 49:1345–52.

Benedetti S, Buratti S, Spinardi A, Mannino S, Mignani I. 2008. Electronic nose as a non-destructive tool to characterize peach cultivars and to monitor their ripening stage during shelf-life. *Postharvest Biol Technol* 47:181–8.

Berna AZ, Buysens S, Natale CD, Grun IU, Lammertyn J, Nicolai BM. 2005. Relating sensory analysis with electronic nose and headspace fingerprint MS for tomato aroma profiling. *Postharvest Biol Technol* 36:143–55.

Berna AZ, Geysen S, Li S, Verlinden BE, Larnmertyn J, Nicolai BA. 2007. Headspace fingerprint mass spectrometry to characterize strawberry aroma at super-atmospheric oxygen conditions. *Postharvest Biol Technol* 46:230–6.

Berna AZ, Lammertyn J, Saevels S, Natale CD, Nicolai BM. 2004. Electronic nose systems to study shelf life and cultivar effect on tomato aroma profile. *Sens Actuators B* 97: 324–33.

Bicchi C, Drigo S, Rubiolo P. 2000. Influence of fibre coating in headspace solid-phase microextraction-gas chromatographic analysis of aromatic and medicinal plants. *J Chromatogr A* 892:469–85.

Bicchi C, Iori C, Rubiolo P, Sandra P. 2002. Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted arabica coffee and coffee brew. *J Agric Food Chem* 50:449–59.

Blank I, Devaud S, Matthey-Doret W, Pollien P, Robert F, Yeretzian C. 2003. Formation of odour-active compounds in Maillard model systems based on proline. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, pp. 458–63.

Bleibaum RN, Stone H, Tan T, Labreche S, Saint-Martin E, Isz S. 2002. Comparison of sensory and consumer results with electronic nose and tongue sensors for apple juices. *Food Qual Prefer* 13:409–22.

Bredie WLP, Petersen MA. 2006. *Flavour Science: Recent Advances and Trends. Developments in Food Science 43*. Amsterdam: Elsevier.

Buettner A, Otto S, Beer A, Mestres M, Schieberle P, Hummel T. 2008. Dynamics of retronasal aroma perception during consumption: Cross-linking on-line breath analysis with medico-analytical tools to elucidate a complex process. *Food Chem* 108:1234–46.

Buettner A, Schieberle P. 2001a. Evaluation of aroma differences between hand-squeezed juices from Valencia Late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J Agric Food Chem* 49:2387–94.

Buettner A, Schieberle P. 2001b. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J Agric Food Chem* 49:1358–63.

Carasek E, Pawliszyn J. 2006. Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. *J Agric Food Chem* 54:8688–96.

Cavalli JF, Fernandez X, Lizzani-Cuvelier L, Loiseau AM. 2003. Comparison of static headspace, headspace solid phase microextraction, headspace sorptive extraction, and direct thermal desorption techniques on chemical composition of French olive oils. *J Agric Food Chem* 51:7709–16.

Chin HW, Bernhard RA, Rosenberg M. 1996. Solid phase microextraction for cheese volatile compound analysis. *J Food Sci* 61:1118–22.

Cook DJ, Davidson JM, Linforth RST, Taylor AJ. 2004. Measuring the sensory impact of flavor mixtures using controlled delivery. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 135–49.

Da Porto C, Pizzale L, Bravin M, Conte LS. 2003. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MC. *Flav Frag J* 18:66–72.

de Kok PMT, Smorenburg HE. 1999. Flavor release in the mouth. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry. Thirty Years of Progress*. New York: Kluwer Academic/Plenum, pp. 397–407.

Deibler KD, Delwiche J. 2004. *Handbook of Flavor Characterization*. New York: Marcel Dekker.

Deibler KD, Lavin EH, Linforth RST, Taylor AJ, Acree TE. 2001. Verification of a mouth simulator by in vivo measurements. *J Agric Food Chem* 49:1388–93.

Delahunty CM, Piggott JR, Conner JM, Paterson A. 1996. Comparison of dynamic flavour release from hard cheeses and analysis of headspace volatiles from the mouth with flavour perception during consumption. *J Sci Food Agric* 71:273–81.

Dirinck I, Van Leuven I, Dirinck P. 2006. ChemSensor classification of red wines. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 521–4.

Doleschall F, Recseg K, Kemeny Z, Kovari K. 2003. Comparison of differently coated SPME fibres applied for monitoring volatile substances in vegetable oils. *Eur J Lipid Sci Technol* 105:333–8.

Etiévant PX, Langlois D. 1998. L'amélioration de la représentativité olfactive des extraits aromatiques dans la recherche des composés clefs de l'arôme des aliments. In Peyron L (ed.), *17 èmes Journées Internationales Huiles Essentielles*. Digne les Bains: APPAM, pp. 268–78.

Etiévant PX, Moio L, Guichard E, Langlois D, Lesschaeve I, Schlich P. 1994. Aroma extract dilution analysis (AEDA) and the representativeness of the odour of food extracts. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research: Developments in Food Science*, Vol. 35. Amsterdam: Elsevier, pp. 179–91.

Ferreira ACS, de Pinho PG. 2003. Analytical method for determination of some aroma compounds on white wines by solid phase microextraction and gas chromatography. *J Food Sci* 68:2817–20.

Friel EN, Wang M, Taylor AJ, MacRae EA. 2007. In vitro and in vivo release of aroma compounds from yellow-fleshed kiwifruit. *J Agric Food Chem* 55:6664–73.

Geary MD, van Ruth SM, Delahunty CM, Lavin EH, Acree TE. 2004. Effects of oral physiological characteristics on the release of aroma compounds from oil-in-water emulsions using two mouth simulator systems. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 345–61.

Given P, Paredes D. 2002. *Chemistry of Taste. Mechanisms, Behaviors, and Mimics. ACS Symposium Series 825*. Washington, DC: American Chemical Society.

Gomez AH, Wang J, Hu G, Pereira AG. 2006. Electronic nose technique potential monitoring mandarin maturity. *Sens Actuators B* 113:347–53.

Goodner KL, Dreher JG, Rouseff RL. 2001. The dangers of creating false classifications due to noise in electronic nose and similar multivariate analyses. *Sens Actuators B* 80:261–6.

Goodner KL, Rouseff RL. 2001. Using an ion-trap MS sensor to differentiate and identify individual components in grapefruit juice headspace volatiles. *J Agric Food Chem* 49:250–3.

Granvogl M, Schieberle P. 2006. Facts and "artefacts" in the flavour chemistry of onions. In Bredie WLP, Petersen MA (eds.), *Flavour Science. Recent Developments and Trends*. Amsterdam: Elsevier, pp. 359–62.

Green BG. 1996. Chemesthesis: Pungency as a component of flavor. *Trends Food Sci Technol* 7:415–20.

Grosch W. 2001. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem Senses* 26:533–45.

Haahr AM, Madsen H, Smedsgaard J, Bredie WLP, Stahnke LH, Refsgaard HHF. 2003. Flavor release measurement by atmospheric pressure chemical ionization ion trap mass spectrometry, construction of interface and mathematical modeling of release profiles. *Anal Chem* 75:655–62.

Hansel A, Märk TD. 2007. Contributions. In Habitzel K, Märk TD, Prock S, Stehno B (eds.), *3rd International Conference on Proton Transfer Reaction Mass Spectrometry and Its Applications*. Innsbruck: Innsbruck University Press, p. 258.

Harmon AD. 1997. Solid-phase microextraction for the analysis of flavors. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 81–112.

Ho CT, Manley CH. 1993. *Flavor Measurement*. New York: Marcel Dekker, p. 373.

Hodgins D. 1997. The electronic nose: Sensor array-based instruments that emulate the human nose. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 331–71.

Holt RU. 2001. Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. *J Chromatogr A* 937:107–14.

Ibañez C, Sola J. 2006. Optimisation of stir bar sorptive extraction (SBSE) for flavour analysis. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 577–80.

Ibanez E, Lopez-Sebastian S, Ramos E, Tabera J, Reglero G. 1998. Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem* 63:281–6.

Ingham KE, Linforth RST, Taylor AJ. 1995. The effect of eating on aroma release from strawberries. *Food Chem* 54:283–8.

Jaillais B, Bertrand V, Auger J. 1999. Cryo-trapping/SPME/GC analysis of cheese aroma. *Talanta* 48:747–53.

Jia MY, Zhang QH, Min DB. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *J Agric Food Chem* 46:2744–7.

Kanavouras A, Kiritsakis A, Hernandez RJ. 2005. Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase micro-extraction. *Food Chem* 90:69–79.

Kataoka H, Lord HL, Pawliszyn J. 2000. Applications of solid-phase microextraction in food analysis. *J Chromatogr A* 880:35–62.

Kittel KM, Lavin EH, Deibler KD, Acree TE, Maillard A. 2004. Analysis of off-aromas in wines caused by aging. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 455–62.

Lee S-J. 2003. Finding key odorants in foods: Gas chromatography olfactometry (GC/O). *Food Sci Biotechnol* 12:597–602.

Leland JV, Schieberle P, Buettner A, Acree TE. 2001. *Gas Chromatography-Olfactometry: The State of the Art. ACS Symposium Series*. Washington, DC: American Chemical Society.

Le Quéré JL, Etiévant PX. 2003. *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, p. 780.

Le Quere J-L, Gierczynski I, Langlois D, Sémon E. 2006. Nosespace with an ion trap mass spectrometer—Quantitative aspects. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Advances and Trends*. Amsterdam: Elsevier, pp. 589–92.

Lestremau F, Andersson FAT, Desauziers V. 2004. Investigation of artefact formation during analysis of volatile sulphur compounds using solid phase microextraction (SPME). *Chromatographia* 59:607–13.

Lestremau F, Desauziers V, Fanlo JL. 2001. Formation of artifacts during air analysis of volatile amines by solid-phase microextraction. *Analyst* 126:1969–73.

Lindinger W, Hirber J, Paretzke H. 1993. An ion/molecule-reaction mass spectrometer used for on-line trace gas analysis. *Int J Mass Spectrom Ion Process* 129:79–88.

Linforth R, Taylor A. 2006. The process of flavour release. In Voilley A, Etiévant P (eds.), *Flavour in Food*. Cambridge: Woodhead Publishing, pp. 287–307.

Linforth RST, Hollowood T, Taylor AJ. 2000. Analysis of correlation between temporal aspects of aroma release and perception. In Schieberle P, Engels KH (eds.), *Frontiers of*

Flavour Science. Garching: Deutsche Forschungsanstalt für Lebensmittelchemie, pp. 275–8.

Linforth RST, Ingham KE, Taylor AJ. 1996. Time course profiling of volatile release from foods during the eating process. In Taylor AJ, Mottram DS (eds.), *Flavour Science: Recent Developments*. Cambridge: The Royal Society of Chemistry, pp. 361–8.

Linforth RST, Savary I, Pattenden B, Taylor AJ. 1994. Volatile compounds found in expired air during eating of fresh tomatoes and in the headspace above tomatoes. *J Sci Food Agric* 65:241–7.

Linforth RST, Taylor AJ. 1993. Measurement of volatile release in the mouth. *Food Chem* 48:115–20.

Liu TT, Yang TS. 2002. Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *J Agric Food Chem* 50:653–7.

Lord H, Pawliszyn J. 2000. Evolution of solid-phase microextraction technology. *J Chromatogr A* 885:153–93.

Marsili R. 1997. *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, p. 383.

Marsili RT. 1999. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J Agric Food Chem* 47:648–54.

Matich AJ, Rowan DD, Banks NH. 1996. Solid phase microextraction for quantitative headspace sampling of apple volatiles. *Anal Chem* 68:4114–8.

Mayr D, Hartungen E, Märk T, Margesin R, Schinner F. 2003a. Determination of the spoolage status of meat by aroma detection using proto-transfer-reaction mass-spectrometry. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, pp. 757–60.

Mayr D, Märk T, Lindinger W, Brevard H, Yeretzian C. 2003b. In-vivo analysis of banana aroma by proton transfer reaction-mass spectrometry. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, pp. 256–9.

Mayr D, van Ruth SM, Mark TD. 2003c. Evaluation of the influence of mastication on temporal aroma release of ripe and unripe bananas, using a model mouth system and gas chromatography-olfactometry. *Eur Food Res Technol* 217:291–5.

Mehinagic E, Prost C, Demaimay M. 2003. Representativeness of apple aroma extract obtained by vacuum hydrodistillation: Comparison of two concentration techniques. *J Food Sci* 68:2411–5.

Mielle P. 1996. "Electronic noses": Towards the objective instrumental characterization of food aroma. *Trends Food Sci Technol* 7:432–8.

Mielle P, Marquis F, Latrasse C. 2000. Electronic noses: Specify or disappear. *Sens Actuators B* 69:287–94.

Miller ME, Stuart JD. 1999. Comparison of gas-sampled and SPME-sampled static headspace for the determination of volatile flavor components. *Anal Chem* 71:23–7.

Mussinan CJ, Morello MJ. 1998. *Flavor Analysis. Developments in Isolation and Characterization*. Washington, DC: American Chemical Society.

Noble AC. 1996. Taste-aroma interactions. *Trends Food Sci Technol* 7:439–44.

Overbosch P. 1987. Flavour release and perception. In Martens M, Dalen GA, Russwurm H (eds.), *Flavour Science and Technology*. Chichester: John Wiley, pp. 291–300.

Overbosch P, Afterof WGM, Haring PGM. 1991. Flavor release in the mouth. *Food Rev Int* 7:137–84.

Pathange LP, Mallikarjunan P, Marini RP, O'Keefe S, Vaughan D. 2006. Non-destructive evaluation of apple maturity using an electronic nose system. *J Food Eng* 77:1018–23.

Pelusio F, Nilsson T, Montanarella L, Tilio R, Larsen B, Facchetti S, Madsen JO. 1995. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J Agric Food Chem* 43:2138–43.

Pérès C, Begnaud F, Eveleigh L, Berdagué JL. 2003. Fast characterization of foodstuff by headspace mass spectrometry (HS-MS). *Trends Anal Chem* 22:858–66.

Pérès C, Viallon C, Berdagué JL. 2002. Curie point pyrolysis-mass spectrometry applied to rapid characterisation of cheeses. *J Anal Appl Pyrolysis* 65:161–71.

Piggott JR. 2000. Dynamism in flavour science and sensory methodology. *Food Res Int* 33:191–7.

Pillonel L, Bosset JO, Tabacchi R. 2002. Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review. *Lebensm Wiss Technol* 35:1–14.

Rabe S, Krings U, Banavara DS, Berger RG. 2002. Computerized apparatus for measuring dynamic flavor release from liquid food matrices. *J Agric Food Chem* 50:6440–7.

Rega B, Fournier N, Guichard E. 2003. Solid phase microextraction (SPME) of orange juice flavor: Odor representativeness by direct gas chromatography olfactometry (D-GC-O). *J Agric Food Chem* 51:7092–9.

Reid LM, O'Donnell CP, Downey G. 2004. Potential of SPME-GC and chemometrics to detect adulteration of soft fruit purees. *J Agric Food Chem* 52:421–7.

Reineccius G. 2002. Instrumental methods of analysis. In Taylor AJ (ed.), *Food Flavour Technology*. Sheffield: Sheffield Academic Press, pp. 210–51.

Reineccius G. 2006. Choosing the correct analytical technique in aroma analysis. In Voilley A, Etiévant P (eds.), *Flavour in Food*. Cambridge: Woodhead Publishing, pp. 81–97.

Riu-Aumatell M, Castellari M, Lopez-Tamames E, Galassi S, Buxaderas S. 2004. Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GUMS. *Food Chem* 87:627–37.

Roberts DD, Acree TE. 1995. Simulation of retronasal aroma using a modified headspace technique: Investigating the effects of saliva, temperature, shearing, and oil on flavor release. *J Agric Food Chem* 43:2179–86.

Roberts DD, Pollien P, Antille N, Lindinger C, Yeretzian C. 2003. Comparison of nosespace, headspace, and sensory intensity ratings for the evaluation of flavor absorption by fat. *J Agric Food Chem* 51:3636–42.

Roberts DD, Pollien P, Yeretzian C, Lindinger C. 2004. Nosespace analysis with proton-transfer-reaction mass spectrometry: Intra- and interpersonal variability. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 151–62.

Roberts DD, Taylor AJ. 2000. *Flavor Release. ACS Symposium Series. 763*. Washington, DC: American Chemical Society.

Ruiz del Castillo ML, Dobson G. 2002. Varietal differences in terpene composition of black-currant (*Ribes nigrum* L) berries by solid phase microextraction/gas chromatography. *J Sci Food Agric* 82:1510–5.

Saevels S, Lammertyn J, Berna AZ, Veraverbeke EA, Di Natale C, Nicolai BM. 2004. An electronic nose and a mass spectrometry-based electronic nose for assessing apple quality during shelf life. *Postharvest Biol Technol* 31:9–19.

Salles C, Hollowood TA, Linforth RST, Taylor AJ. 2003. Relating real time flavour release to sensory perception of soft cheeses. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, pp. 170–5.

Salles C, Tarrega A, Mielle P, Maratray J, Gorria P, Liaboeuf J, Liodenot JJ. 2007. Development of a chewing simulator for food breakdown and the analysis of *in vitro* flavor compound release in a mouth environment. *J Food Eng* 82:189–98.

Schaller E, Bosset JO, Escher F. 1998. "Electronic noses" and their application to food. *Lebensm Wiss Technol* 31:305–16.

Schaller E, Bosset JO, Escher F. 2000. Instability of conducting polymer sensors in an electronic nose system. *Analysis* 28:217–27.

Schulz I, Ulrich D, Fischer C. 2003. Rapid differentiation of new apple cultivars by headspace solid-phase microextraction in combination with chemometrical data processing. *Nahrung* 47:136–9.

Sémon E, Gierczynski I, Langlois D, Le Quéré JL. 2003. Analysis of aroma compounds by atmospheric pressure chemical ionisation—Ion trap mass spectrometry. Construction and validation of an interface for in vivo analysis of human breath volatile content. In Ashcroft AE, Brenton G, Monaghan JJ (eds.), *Advances in Mass Spectrometry*, Vol. 16. Amsterdam: Elsevier. CD-ROM Supplement, abstract 324.

Shaw PE, Rouseff RL, Goodner KL, Bazemore R, Nordby HE, Widmer WW. 2000. Comparison of headspace GC and electronic sensor techniques for classification of processed orange juices. *Lebensm Wiss Technol* 33:331–4.

Soeting W, Heridema J. 1988. A mass spectrometric method for measuring flavour concentration/time profiles in human breath. *Chem Senses* 13:607–17.

Song J, Fan LH, Beaudry RM. 1998. Application of solid phase microextraction and gas chromatography time-of-flight mass spectrometry for rapid analysis of flavor volatiles in tomato and strawberry fruits. *J Agric Food Chem* 46:3721–6.

Steffen A, Pawliszyn J. 1996. Analysis of flavor volatiles using headspace solid-phase microextraction. *J Agric Food Chem* 44:2187–93.

Stephan A, Bücking M, Steinhart H. 2000. Novel analytical tools for food flavours. *Food Res Int* 33:199–209.

Talon R, Walter D, Viallon C, Berdagué JL. 2002. Prediction of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* populations in yoghurt by Curie point pyrolysis-mass spectrometry. *J Microbiol Methods* 48:271–9.

Taylor AJ. 2002. Release and transport of flavors in vivo: Physicochemical, physiological, and perceptual considerations. *Compr Rev Food Sci Food Saf* 1:45–57.

Taylor AJ, Hort J. 2004. Measuring proximal stimuli involved in flavour perception. In Taylor AJ, Roberts DD (eds.), *Flavor Perception*. Oxford: Blackwell Publishing, pp. 1–38.

Taylor AJ, Linforth RST. 1994. Methodology for measuring volatile profiles in the mouth and nose during eating. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier, pp. 3–14.

Taylor AJ, Linforth RST. 1996. Flavour release in the mouth. *Trends Food Sci Technol* 7:444–8.

Taylor AJ, Linforth RST. 2003. Direct mass spectrometry of complex volatile and non-volatile flavour mixtures. *Int J Mass Spectrom* 223–4:179–91.

Taylor AJ, Linforth RST, Harvey BA, Blake A. 2000. Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chem* 71:327–38.

Teranishi R. 1998. Challenges in flavor chemistry: An overview. In Mussinan CJ, Morello MJ (eds.), *Flavor Analysis. Developments in Isolation and Characterization*. Washington, DC: American Chemical Society, pp. 1–6.

Tienpont B, David F, Bicchi C, Sandra P. 2000. High capacity headspace sorptive extraction. *J Microcolumn Sep* 12:577–84.

Tredoux AGJ, Lauer HH, Heideman T, Sandra P. 2000. The determination of benzoic acid in lemon flavored beverages by stir bar sorptive extraction-CGC-MS. *J High Resolut Chromatogr* 23:644–6.

Ulrich D, Eunert S, Hoberg E, Rapp A. 1995. Analysis of strawberry aroma with solid phase microextraction. *Dtsch Lebensm Rundsch* 91:349–51.

Ulrich D, Hoberg E, Rapp A, Sandke G. 1997a. Flavour analysis in plant breeding—Solid phase micro extraction of strawberry aroma compounds. Proceedings of the 5th Wartburg Aroma Symposium, Eisenach, March 17–20.

Ulrich D, Krumbein A, Rapp A. 1997b. Analysis of aroma compounds in strawberry, sour cherry and tomato by gas chromatography after solid phase micro extraction. *Dtsch Lebensm Rundsch* 93:311–6.

van Ruth S, Geary MD, Buhr K, Delahunty CM. 2004. Representative sampling of volatile flavor compounds: The model mouth combined with gas chromatogaphy and direct mass spectrometry. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 335–44.

van Ruth SM. 2001. Aroma measurement: Recent developments in isolation and characterisation. In De Cuyper M, Bulte JWM (eds.), *Physics and Chemistry Basis of Biotechnology*. Amsterdam: Kluwer Academic Publishers, pp. 305–28.

van Ruth SM, Buhr K, Delahunty CM. 2005. Strawberry flavour analysis by PTR-MS: Various low and high time resolution applications. In Hansel A, Märk TD (eds.), *2nd International Conference on Proton Transfer Reaction Mass Spectrometry and Its Applications*. Innsbruck: Innsbruck University Press, pp. 81–4.

van Ruth SM, Buhr K, Geary M, Dings L, Odake S. 2003. Effect of oral parameters on dynamic aroma release under mouth conditions. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec&Doc, pp. 176–81.

van Ruth SM, Frasnelli J, Carbonell L. 2008. Volatile flavour retention in food technology and during consumption: Juice and custard examples. *Food Chem* 106:1385–92.

van Ruth SM, Roozen JP. 2000. Influence of mastication and saliva on aroma release in a model mouth system. *Food Chem* 71:339–45.

van Ruth SM, Roozen JP, Cozijnsen JL. 1994. Comparison of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier, pp. 59–64.

van Ruth SM, Roozen JP, Cozijnsen JL. 1995. Volatile compounds of rehydrated french beans, bell peppers and leeks. Part 1. Flavour release in the mouth and in three mouth model systems. *Food Chem* 53:15–22.

Verhoeven H, Beuerle T, Schwab W. 1997. Solid-phase microextraction: Artefact formation and its avoidance. *Chromatographia* 46:63–6.

Vernat G, Berdagué JL. 1995. Dynamic headspace-mass spectrometry (DHS-MS): A new approach to real-time characterization of food products. In Etiévant P, Schreier P (eds.), *Bioflavour 95*. Versailles: INRA Editions, pp. 59–62.

Wan XM, Stevenson RJ, Chen XD, Melton LD. 1999. Application of headspace solid-phase microextraction to volatile flavour profile development during storage and ripening of kiwifruit. *Food Res Int* 32:175–83.

Yang X, Peppard T. 1994. Solid-phase microextraction for flavor analysis. *J Agric Food Chem* 42:1925–30.

Yeretzian C, Jordan A, Brevard H, Lindinger W. 2000a. Time-resolved headspace analysis by proton-transfer-reaction mass-spectrometry. In Roberts DD, Taylor AJ (eds.), *Flavor Release*. Washington, DC: American Chemical Society, pp. 58–72.

Yeretzian C, Jordan A, Brevard H, Lindinger W. 2000b. On-line monitoring of coffee roasting by proton-transfer-reaction mass-spectrometry. In Roberts DD, Taylor AJ (eds.), *Flavor Release*. Washington, DC: American Chemical Society, pp. 112–23.

CHAPTER 12

Extraction and Distillation

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EXTRACTION

Introduction

The consumption of foods and beverages is closely related to the stimulation of the human chemical senses, odor, and taste. The flavor of food, along with its appearance and texture, is considered to be decisive for the consumer in the selection and ingestion of a particular food (Zellner et al. 2008).

The perception of flavor depends on a multifaceted series of sensory responses, but the factor having the greatest influence is odor. The concept of odor is multivariate, and its analysis has involved the separation sciences. Analysis generally involves the determination of an analyte or group of analytes that correlates the human perception of odor/off-odor, product freshness, and quality. A technique that is applicable to the analysis of aroma in food/essential oils must be able to quantitatively isolate relevant aroma compounds (Sides et al. 2000).

Extraction is a process of great importance for anyone interested in natural products, because it enables the separation or concentration of a variety of substances from natural feedstock. Among these substances, the group related to flavors receives a special attention as a function of its economic importance. Nowadays, the amount of solvent used in industry within conventional extractive processes is estimated to be about 1 million tons/year. The development of extraction techniques can reduce this amount of solvent and also improve the product quality. Thus, there is a need not only to update conventional techniques but also to take into account the commercial adoption of new techniques, which are studied and used by research groups.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Extraction Techniques

Extraction techniques vary according to where the flavor compound is located inside the natural product. Simões and Spitzer (2003) cited various methods of extraction, treatment, and conservation applied in the recovery of flavor compounds. The most common are presented.

Stripping Using Water Vapor Volatile oils have vapor pressure higher than water; therefore, they can be stripped by water vapor. In a small scale, the Clevenger apparatus, a traditional apparatus used in hydrodistillation, can be used. The obtained volatile oil, after separation from water, must be dried using anhydrous sodium sulfates. Along this classical procedure, the formation of new compounds is possible via degradation, due to the high temperatures used during stripping or water separation stages. Preferentially, in flavor extraction, this technique is used to extract oils from fresh plants.

In a large scale, using batch or continuous operation, this process can be used in the deodorization of vegetable oils, in which the stripping is accomplished using water vapor under vacuum conditions and elevated temperatures (Heldman and Hartel 1998). 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.

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 solvent. During the 19th century, several solvents were applied, and patents about the process and its main extraction were registered in several countries of Europe. Gradually, the method attracted the attention of the manufacturers, and large-scale experiments were conducted independently by industry workers.

Volatile oils are extracted preferentially with nonpolar solvents, such as ether, petroleum ether, or dichloromethane. However, these solvents extract other lipophilic compounds besides the volatile oils, which decrease the commercial value of the oils. The undesired extraction of other compounds is difficult to prevent.

The principle of the 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 into 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 the lowest possible temperature. After the solvent is completely driven off, the 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, in general, has a flavor that better represents the original flavor present in raw materials.

Despite the 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 and fruits. The products of solvent extraction possess one main advantage—their true-to-nature odor.

Two factors must be investigated and carefully defined to guarantee the success of solvent extraction: the selection of the solvent and the extraction unit. Nowadays, 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 organoleptic quality, and the extraction yield (Danisco 2001).

Apps and Tock (2005) proposed the use of continuous liquid–liquid extraction for the concentration of flavor fractions presented in guava pulp. A magnetic stirrer was used to prevent channel formation by the solvent through the sample. This modification improves the efficiency from 2.8 to 17.6.

Extraction with Supercritical Carbon Dioxide In this extraction, the solvent, carbon dioxide, is pressurized at least to 73 bar (critical pressure) at a temperature higher than 31°C (critical temperature). Above these critical values, the carbon dioxide reaches the "supercritical state." At this state, the viscosity is 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 then to loose its high capacity of dissolution. The product can be completely separated from the solvent, which is again compressed and recycled into the process.

This technique allows the recovery of different kinds of natural flavors and volatile oils with high efficiency. Nowadays, 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 the ones obtained by other techniques.

Solvent extraction from solids typically consists of two main stages: extraction and the separation. A schematic description of a typical supercritical extraction process, a particular example of solvent extraction process, can be seen in Figure 12.1.

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 (mainly density, viscosity, and mass diffusivity), which near the critical point changes significantly with small changes in temperature and pressure. This behavior causes changes in the solvent dissolving capacity, which makes it possible to define a specific set of compounds to be extracted.

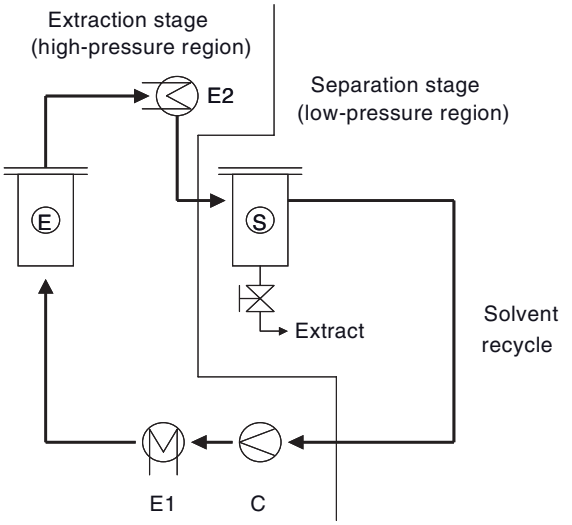

Figure 12.1. Typical supercritical extraction process.

On the other hand, it becomes difficult to operationally control the extraction unit. The typical values of these operational parameters are 1.01–1.10 for reduced temperature (ratio of operational to critical temperature) and 1.01–1.50 for reduced pressure (ratio of operational to critical pressure).

The solid material will be depleted from the extractable material in the direction of the flow. Therefore, the concentration of the extract components increases in the direction of flow in the supercritical solvent and in the solid material. This behavior is a function of the operational conditions, the kinetic extraction properties in the solid matrix, and 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 (Ayala and Luque de Castro 2001; Bernardo-Gil et al. 2002; Hawthorne et al. 1993; Moyler 1993).

Some solvents, such as water (critical temperature and pressure of 374°C and 220 bar, respectively), have higher critical pressure and temperature compared with carbon dioxide. Thus, the extraction operation in a supercritical condition would need 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, even with carbon dioxide, to adopt operational conditions under the solvent critical point. An example is the continuous subcritical water extraction (CSWE) process, which was tested by Jiménez-Carmona and others (1999) in the recovery of flavor compounds from marjoram essential oils.

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 pericarps 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 obtained by gravity, centrifugation, or fractionated distillation.

Solid Phase Microextraction (SPME) SPME is a new technique that started in the 1990s, with applications not consolidated yet, even in the theoretical point of view. The initial studies were on the applications in laboratories exploiting fundamental aspects of the method.

SPME is a process with multiphase equilibrium. It is a microtechnique, in which the process of extraction of the analytes takes place in an unusual scale. The basic device consists of a 10-mm thin slapstick made of melted silica optical fiber and one of its ends recovered with a thin polymeric film. 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 analytic instrument (gas chromatography). The sample consists of an aqueous solution with solid particles in suspension having several interactions of adsorption with the analytes besides the gas phase. The desorption time depends on several parameters, and the most important are the volatility of the chemical compounds, the kind of injector, and the temperature (Cai et al. 2001; Tellez et al. 2004; Zhu et al. 1999). The great advantage of the method is the extraction with the direct quantification of flavor compounds present in different matrices, gas, liquid, and solid.

Enhancement Techniques A procedure that can be used to increase the extraction potential is the previous freezing of the raw material. Freezing causes rupture of the cell wall, easing the action of the solvent on the products of cellular metabolism. Moreover, the conventional extraction methods can have their yield enhanced by ultrasonic waves that promote a better interaction between the solvent and the analyte molecules.

Extraction using microwave 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 shown good efficiency. Solvent extraction enhanced by microwave radiation has been used in a large scale to obtain essential oils from various vegetal raw materials. The advantages are the 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 using it.

According to Lacerda (1999), microwave is used to improve the extraction of organic substances from solid matrices, such as soil, seeds, and food. A conventional microwave oven is applied to irradiate seven times, for 30 s each time, the solid matrix with the solvent. The extraction assisted by microwave was more efficient than the extraction using the Soxhlet apparatus in relation to polar substances.

Comparative measurements were performed using Soxhlet (conventional extraction technique) and extractions assisted by sonic waves and microwave. The results indicated that the extraction with microwave was a more feasible alternative than

Soxhlet and the sonic waves. Extraction with microwave used a lower amount of organic solvent, and the process efficiency was increased by reducing extraction time to less than 10 min, and due to the possibility to extract several samples simultaneously (Lacerda 1999).

Extract Treatment Often, it is necessary to whiten, 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 from the oil. The use of chromatographic techniques, in particular exclusion chromatography, allows the separation of the volatile oils from other lipophilic nonvolatile compounds. A partial fractionation allows the separation of the original solution in a fraction rich in monoterpenes and another rich in sesquiterpenes.

GENERAL COMMENTS

Extraction processes can be applied to selectively obtain a more specific and standard product, with a higher degree of purity. Nowadays, extraction of volatile substances is still done 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 of higher extraction temperatures, aiming better yields. After extraction, the process to separate the solvent from the final product is another point of difficulty. For example, when using hydrodistillation, besides heating, there is the inconvenience of the direct contact of the material with hot water, which can cause hydrolysis of some substances. Extracts obtained from organic solvent extraction often contain 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 becomes.

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 presented below.

Blanch and others (1993a,b) investigated the 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, which worked as a second cooling system.

Zabetakis and Gramshaw (1998) identified 1,2-propanediol in strawberries, after extraction with dichloromethane, as precursor of flavor. Strawberry flavor consists of approximately 350 aromatic compounds, but two of them has the typical flavor and is considered the key aroma components of the fruit. It was concluded that 1,2-propanediol functions as a key intermediate in the metabolism of other flavor components.

Morales and others (1998) proposed the extraction of volatile components present in the virgin olive oil by supercritical fluid extraction–high-resolution gas chromatography–mass spectrometry. Jiménez and others (2007) applied high-power ultrasound and sonication to improve the yield of the virgin oil extraction. The high-power ultrasound did not change the quality parameters, and the oil showed lower bitterness and higher content of tocopherols, chlorophylls, and carotenoids. The yield varied from 7.23% to 16.1% depending on the treatment of the olive paste. Kalua and others (2007) also observed that the unique and delicate flavor of olive oil is attributed to the volatile compounds during and after oil extraction from the olive fruit.

Jiménez-Carmona and others (1999) compared the CSWE with hydrodistillation in the recovery of flavor compounds from marjoram essential oil. The CSWE method was faster (15 min compared with 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 natural products and compared its performance with hydrodistillation. The following advantages of CSWE were observed: shorter extraction time (50 min instead of 4 h of hydrodistillation), fairly higher energy cost for performing hydrodistillation, and possibility of tuning the composition of the oil by changing 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, among them were supercritical CO_2 extraction, 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 extracted, followed by methylene chloride extraction. About two-thirds of the flavor was recovered in the solvent extraction, and approximately 5% was lost in the concentration step.

Padukka and others (2000) studied five different extraction methods, namely 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. The continuous operation with 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 compared with uniform temperature along the column.

Diaz-Maroto and others (2002) compared hydrodistillation and solvent extraction with supercritical CO_2 to recover 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.

Barbe and others (1998) investigated the retention of volatile organic flavor/fragrance components in the concentration of Gordo grape and Valencia orange juice by osmotic distillation. The results suggested that there is a correlation between the degree of retention of organic volatile flavor/fragrance components and membrane surface pore size when their aqueous solutions are subjected to osmotic distillation. Jiao and others (2004) used the membrane processes like membrane distillation and osmotic distillation, for the concentration of fruit juices to reduce the storage and shipping costs and to achieve longer storage. The promising alternative is the reverse osmosis concentration, but it cannot reach concentrations larger than 25–30°Brix. The processes using membrane had achieved a maximum concentration of 60–70°Brix. Vaillant and others (2005) clarified using microfiltration melon juice obtained from fruits discarded by exporters and concentrated it by osmotic evaporation. The integrated membrane process permitted two valuable products to be obtained: a clarified concentrate of melon juice that had not undergone any thermal treatment, and a glowing-orange retentate that was enriched in provitamin A. Cassano and others (2007) also studied the use of membranes for the clarification and concentration of the cactus pear juice, with interest because of their nutritional and antioxidant properties due to the presence of ascorbic acid, fibers, and amino acids. The fresh juice containing about 11°Brix was previously clarified by ultrafiltration and concentrated using the osmotic distillation, achieving 61°Brix at 28°C. Babu and others (2008) also used the osmotic membrane distillation for the concentration of pineapple juice that reached a total soluble solids of 62°Brix at ambient conditions.

Kahle and others (2005), to extract the volatiles present in the pear fruit, prepared the sample by simultaneous distillation extraction for 2 h using a mixture of pentane–diethyl ether, and the extracts obtained were dried. The method helped to identify more than 150 volatiles in pear fruit.

Silva and others (2005) investigated the influence of different hydrodistillation procedures (with water immersion, with water immersion and vapor injection, and with direct vapor injection) on the deodorization of turmeric, a plant that has a yellow and flavorful powder when dried and ground. It has a pungent flavor that resembles pepper and is desirable in mustard and spices. As it cannot be used in sweets and food with a mild flavor, turmeric is not widely used in the food industry. The flavor components of turmeric are mainly ketones and sesquiterpene alcohols. There are three major turmeric products available commercially: turmeric powder, turmeric oleoresin, and curcumin. Turmeric oleoresin, obtained by organic solvent extraction of turmeric, is a viscous oily product containing 30–55% of curcuminoid pigments and 15–25% of volatile oil. Several methods were not effective and viable like Kjeldahl, high vacuum, and rotary evaporation; only the Soxhlet apparatus provided good results.

Rout and others (2007) compared the subcritical CO_2 and organic solvents in the extraction of *Zanthoxylum rhesta* oil and volatiles. The essential oil from the fruits

and fruit pericarp has the linalool as the major constituent. The yields varied from 1.8 (hydrodistillation) to 6.4 (extraction with methanol-diethyl ether). Higher yields were obtained with subcritical CO_2 and subcritical CO_2 and methanol.

Zellner and others (2008) studied the application of gas chromatography–olfactometry to analyze food flavors like milk, cheese, coffee, meat, and fruits. They defined fruit flavors as a subtle blend of characterizing volatile compounds, combined with carbohydrates (glucose, fructose, and sucrose), organic acids, and non-characterizing volatile esters. An individual fruit may have well over a hundred different volatile compounds present in higher concentrations (more than 30 ppm).

DISTILLATION

General Aspects

Distillation is the most common and mature separation technique, and it is widely used in the chemical processing industries for separating components with different volatilities. It utilizes the creation of two or more coexisting phases, generally two phases, vapor and liquid phases at the same temperature and pressure, which differ in composition, and this is the driven force explored for the separation of the species. Its costs may be more than 50% of plant operating costs.

According to Seader and Henley (1998), the art of distillation dates back at least the first century AD, when it was used to produce alcoholic beverages. As cited by Heldman and Hartel (1998), distillation is used in the food industry for the separation of volatile components like flavors from different kinds of natural matrices. For example, in processing of orange juice, the vapors coming off an evaporator contain a significant amount of volatile flavors and aromas, which are separated from the condensed vapors in a distillation column.

The principles of distillation are important to optimize and control distillation columns, to reduce the operating costs by decreasing the consumption of energy, and to improve their efficiency of separation. By the 11th century, distillation consisted of a process in which a liquid to be separated was placed in a vessel to which heat was applied, causing part of the liquid to evaporate. The vapor rising from the liquid passed out of the heating vessel and was condensed in another chamber producing the distillate (Seader and Henley 1998). Distillation can be defined as an operation in which a mixture of substances, liquid and/or vapor, called feed, is separated into its component of desired quality using heat as the separation agent. This operation is based on the difference of boiling point of the substances where the lighter, lower boiling, components tend to concentrate in the vapor phase, while the heavier, higher boiling, components tend to concentrate in the liquid phase. Thus, the vapor phase becomes richer in the components that have lower boiling points, the light components, and the liquid phase will be richer in the components that have higher boiling points, the heavy components. In the literature, many texts about distillation and its principles and operation mathematical modeling can be found (Coulson and Richardson 1991 and McCabe et al. 1993 for a first reading, and King 1980 for a more detailed reading).

Distillation columns and/or distillation operation can be classified according to many aspects. Focusing on the operation mode, one can have a batch or continuous

operation. In batch operations, the column is charged with an amount of feed, and when the desired task is achieved, the column is opened and cleaned, and another amount of feed is charged to start a new operational cycle. Each operational cycle is called a batch. In a continuous operation (continuous columns), the feed stream flows continuously into the column, and also the products are continuously withdrawn. In the continuous operation mode, distillation columns can process high amount of materials, while in the batch operation mode, lower amount of materials is admitted. As a function of the number of components in the feed, one has the binary columns, when the feed contains two components, and multicomponent columns, when the feed contains more than two components. Multiproduct columns have more than two product streams: extractive distillation, when an extra feed appears in the bottom product stream; and azeotropic distillation, when an extra feed appears at the top product stream. The type of column internals to promote the phases' contact divides the columns into two major types: tray columns and packed columns. In tray columns, the liquid–vapor contact takes place 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 enhanced typically by specially designed small objects that are randomly dumped into the column or by structured packing with 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.

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 higher boiling point and more 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 causing a higher concentration of this component in the top stream. The stream containing the third component is an independent feed of the column. 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 up 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 carried out; column internals such as trays/plates and/or packing, 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 leaving 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 column internals and, together with the condenser and reboiler, constitutes a distillation column (Tham 1997). Figure 12.2 shows a schematic representation of a conventional distillation column.

The feed material, which is to be separated into fractions, is introduced usually somewhere near the middle of the column to a tray known as the feed tray or in another points along the column shell. The feed tray divides the column into a top

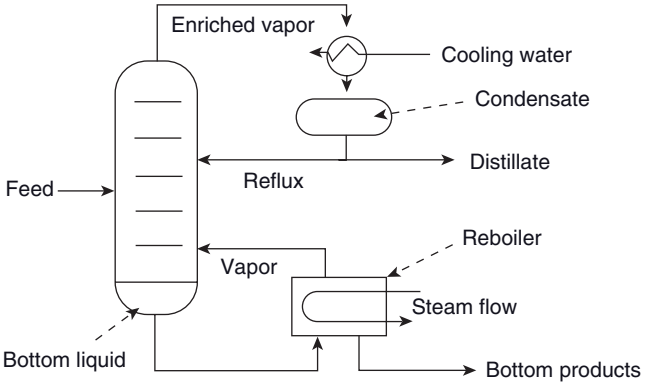

Figure 12.2. Typical distillation column.

(enriching 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, this is normally steam. The vapor raised 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 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 this 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, and the separation of components from a feed mixture via distillation depends on the differences in the boiling points of the individual components. Also, depending on the concentration of its components, the feed mixture will have different boiling point characteristics. Therefore, distillation processes depend on the vapor pressure characteristics of liquid mixtures.

Distillation columns are designed based on the boiling point properties of the components to be separated. Thus, the sizes, particularly the height, of distillation columns are determined by the vapor–liquid equilibrium (VLE) data for the mixture external flows of feeds and product streams, into and out of the column.

Distillation involves the transfer of material from one to another phase. 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 on 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

that can be called column efficiency, which relates actual product compositions with the compositions based on the phase equilibrium.

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 can occur if direct distillation is employed. The liquid is put in the still, and the steam is passed through it. The solubility of steam in the liquid must be very low. One can use superheated steam that provides sufficient heat to vaporize the material concerned, without it condensing or some of the steam may condense, producing a liquid water phase, as cited by Coulson and Richardson (1991).

Steam distillation of plant materials at reduced pressure may be subdivided in two types: steam distillation at slightly reduced pressure and vacuum steam distillation. The processes using higher pressures are faster than the ones under vacuum conditions. Using vacuum pressures, the temperatures must remain just enough above that of the cooling water to allow sufficient condensation of the obtained steam/oil vapor mixture. The principal advantage in vacuum steam distillation is that the more pure odor of the volatile oil is thereby obtained, and it is normally free from any off-odor caused by decomposition, which accompanies most oils distilled above 70°C.

The majority of essential oils has always been obtained by steam distillation, or, in the more general sense, by hydrodistillation (first used by von Rechenberg as referring to distillation with water vapors). 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, which vary widely in regard to their concentrations and boiling points. They are present in the interstice of vegetable tissues and can be extracted by hydrodistillation or by pressing. If the extraction by pressing is used, the obtained liquid mixture normally has to pass by a separation process in order to remove undesired substances. At present, the most common process employed in this case is again hydrodistillation. Normally, these substances have a very low vapor pressure because they are high boiling point substances. So, one may consider the intensity of the odor as a manifestation of the volatility (related to the boiling point and vapor pressure) of the substance. Of course, there are many exceptions.

About the general methods of distillation, little investigation has been undertaken of the process by which steam actually isolates the flavors present in the essential oils. It is often assumed that the steam penetrates the plant tissue and vaporizes all volatile substances. If this 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. Therefore, this model sometimes is not able to describe the extraction step in the process, and then the complete prediction of the hydrodistillation process mainly from solid matrix by mathematical models is not possible even in a context of many available theoretical models for phase equilibrium of the involved substances.

Considering the manner of promoting the contact between the water and the original matrix, a terminology was developed that distinguishes three types of hydro-

distillation: 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, while in the second method, the material is supported on a perforated grid or screen inserted 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 in contact with steam only and not with boiling water. The last type of hydrodistillation, direct steam distillation, resembles the proceeding one except that no water is kept in the bottom of the still but live steam, saturated or superheated, and the process is held frequently at pressures higher than atmospheric.

Hydrolysis of certain components of the essential oils and decomposition caused by heat are always present in the hydrodistillation process. Although it is an unavoidable reaction, typically the intensity of hydrolysis is low under the conditions normally used. The process temperature is determined entirely by operating pressure, and its value is around 100°C using pressures near the atmospheric. In this temperature level, decomposition by degradation of some substances is present and can cause interference in the obtained oil odor. Considering theses previous aspects, hydrodistillation to extract essential oils should be used, keeping the temperature as low as possible, and the contact between water and the original matrix should be minimized.

Hydrodistillation at high and reduced pressure and with superheated steam for certain plant materials may be used. Orris root, sandalwood, cloves, caraway seed, and pine needles, for example, are occasionally distilled with steam at a pressure higher than atmospheric in order to obtain a more suitable 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 it is necessary, because for this purpose, superheated steam gives better results. Distill plant material with high-pressure steam should not be made a general practice, as this will increase the quantity of decomposition products in the plant material and in the oil. The degree of decomposition is influenced by the level of the pressure applied resulting from the rising of the temperature and the length of the distillation. Synthesizing water distillation of plant material at high pressure is not recommended, because the resulting higher temperature gives rise to decomposition products, imparting an unpleasant odor of the oil. Neither is there any appreciable gain in the ratio of oil to water in the distillate, except perhaps in cases where previous distillation under atmospheric pressure has been carried out inefficiently.

Some oils cannot be processed by hydrodistillation, because boiling water and steam have a deteriorating influence on the rather delicate odoriferous constituents. Certain raw materials yield no oil at all when distilled and hence must be processed by methods other than distillation.

Steam distillation of plant material at reduced pressure may be subdivided in two types: steam distillation at slightly reduced pressure, which often shortens the length of the distillation, and vacuum steam distillation at such a low pressure that the temperature remains just enough above that of the cooling water to allow sufficient condensation of the steam/oil vapors. The principal advantage in vacuum steam distillation consists in the pure odor of the volatile oil thereby obtained. It will be free from any off-odor 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 types of vapor–liquid contacting devices (Henley and Seader 1981). This effort can also be utilized to other more recent separation processes, like supercritical fluid extraction.

There are some advantages and disadvantages of the methods for processing natural essential oils to concentrate aromatic components and for steam distillation of volatile oils.

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 on the delicate flavor substances. Moreover, certain raw materials yield no oil during hydrodistillation.
- Solvent extraction—it 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 concretes and alcohol soluble absolutes.
- Supercritical solvent extraction—this technology can be applied to different raw materials with 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. Nowadays, its main disadvantage is the high production costs due to the pressures used.

REFERENCES

Apps P, Tock MLA. 2005. Enhanced flavour extraction in continuous liquid-liquid extractors. *J Chromatogr A* 1083:215–8.

Ayala RS, Luque de Castro MD. 2001. Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food Chem* 75:109–13.

Babu BR, Rastogi NK, Raghavarao KSMS. 2008. Concentration and temperature polarization effects during osmotic membrane distillation. *J Memb Sci* 322:146–53.

Barbe AM, Bartley JP, Jacobs AL, Johnson RA. 1998. Retention of volatile organic flavor/fragrance components in the concentration of liquid foods by osmotic distillation. *J Memb Sci* 145:67–75.

Bernardo-Gil MG, Ribeiro MA, Esquível MM. 2002. Produção de extratos para a indústria alimentar: Uso de fluidos supercríticos. *Bol Biotecnol* 73:24–30.

Blanch GP, Herraiz M, Reglero G, Tabera J. 1993a. Preconcentration of samples by steam distillation—Solvent extraction at low temperature. *J Chromatogr A* 655:141–9.

Blanch GP, Herraiz M, Reglero G, Tabera J. 1993b. Preconcentration of volatile components of foods: Optimization of the steam distillation-solvent extraction at normal pressure. *J Chromatogr A* 628:261–8.

Brunner G. 1994. *Gas Extraction—An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*. New York: Springer.

Cai J, Liu B, Su Q. 2001. Comparison of simultaneous distillation extraction and solid-phase micro extraction for the determination of volatile flavor components. *J Chromatogr A* 930:1–7.

Cassano A, Conidi C, Timpone R, D'Avella M, Drioli E. 2007. A membrane-based process for the clarification and concentration of the cactus pear juice. *J Food Eng* 80:914–21.

Coulson JM, Richardson JM. 1991. *Particle Technology and Separation Processes*, Vol. 2, 4th Ed. Oxford: Butterworth Heinemann.

Danisco PP. 2001. *Comparing Extraction by Traditional Solvents with Supercritical Extraction from an Economic and Environmental Standpoint*. Versailles: ISASF. CD-ROM.

Diaz-Maroto MC, Pérez-Coello MS, Cabezudo MD. 2002. Supercritical carbon dioxide extraction of volatiles from spices. Comparison with simultaneous distillation-extraction. *J Chromatogr A* 947:23–9.

Gámiz-Garcia L, Luque de Castro MD. 2000. Continuous subcritical water extraction of medicinal plant essential oil: Comparison with conventional techniques. *Talanta* 51:1179–85.

Hawthorne SB, Riekkola ML, Serenius K, Holm Y, Hiltunen R, Hartonen K. 1993. Comparison of hydrodistillation and supercritical fluid extraction for the determination of essential oils in aromatic plants. *J Chromatogr* 634:297–308.

Heldman DR, Hartel RW. 1998. *Principles of Food Processing*. Gaithersburg, MD: Chapman & Hall Food Science Book, Aspen Publishers.

Henley EJ, Seader JD. 1981. *Equilibrium-Stage Separation Operations in Chemical Engineering*. New York: John Wiley & Sons.

Jiao B, Cassano A, Drioli E. 2004. Recent advances on membrane processes for the concentration of fruit juices: A review. *J Food Eng* 63:303–24.

Jiménez A, Beltrán G, Uceda M. 2007. High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. *Ultrason Sonochem* 14:725–31.

Jiménez-Carmona MM, Ubera JL, Luque de Castro MD. 1999. Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil. *J Chromatogr A* 855:625–32.

Kahle K, Preston C, Richling E, Heckel F, Schreier P. 2005. On-line gas chromatography combustion/pyrolysis isotope ratio mass spectrometry (HRGC-C/P-IRMS) of major volatiles from pear fruit (*Pyrus communis*) and pear products. *Food Chem* 91:449–55.

Kalua CM, Allen MS, Bedgood DR Jr., Bishop AG, Prenzler PD, Robards K. 2007. Olive oil volative compounds, flavour development and quality: A critical review. *Food Chem* 100(1):273–86.

King CJ. 1980. *Separation Process*, 2nd Ed. New York: McGraw-Hill.

Kondo M, Akgun N, Goto M, Kodama A, Hirose T. 2002. Semi-batch operation and countercurrent extraction by supercriti$_c$al CO2 for the fractionation of lemon oil. *J Supercrit Fluids* 23:21–7.

Lacerda MEG. 1999. Extratos Vegetais: Qualidade e Monitoramento. DSc thesis, NPPN, UFRJ.

McCabe WL, Smith JC, Harriot P. 1993. *Unit Operations of Chemical Engineering*, 5th Ed. New York: McGraw-Hill.

Morales MT, Berry AJ, McIntyre PS, Aparicio R. 1998. Tentative analysis of virgin olive oil aroma by supercritical fluid extraction-high resolution gas chromatography-mass spectrometry. *J Chromatogr A* 819:267–75.

Moyler DA. 1993. Extraction of essential oils with carbon dioxide. *Flav Frag J* 8:235–47.

Padukka I, Bhandari B, D'Arcy B. 2000. Evaluation of various extraction methods of encapsulated oil from cyclodextrin-lemon oil complex powder. *J Food Compos Anal* 13:59–70.

Rizvi SSH, Mittal GS. 1992. *Experimental Methods in Food Engineering*. New York: Van Nostrand Reinhold.

Rout PK, Naik SN, Rao YR, Jadeja G, Maheshwari RC. 2007. Extraction and composition of volatiles from *Zanthoxylum rhesta*: Comparison of subcriti¸al CO2 and traditional processes. *J Supercrit Fluids* 42:334–41.

Sarrazin C, Le Queré JL, Gretsch C, Liardon R. 2000. Representativeness of coffee aroma extracts: A comparison of different extraction methods. *Food Chem* 70:99–106.

Seader JD, Henley EJ. 1998. *Separation Process Principles*. New York: John Wiley & Sons.

Sides A, Robards K, Helliwell S. 2000. Developments in extraction techniques and their application to analysis of volatiles in foods. *Trends Analyt Chem* 19(5):322–9.

Silva LV, Nelson DL, Drummond MFB, Dufossé L, Glória MBA. 2005. Comparison of hydrodistillation methods for the deodorization of turmeric. *Food Res Int* 38:1087–96.

Simões CMO, Spitzer V. 2003. Óleos Voláteis. In Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (eds.), *Farmacognosia: da Planta ao Medicamento,* 5th Ed. Porto Alegre: Editora da UFRGS, pp. 467–95.

Tham MT. 1997. Introduction to distillation. http://lorien.ncl.ac.uk/ming/distil/distil0.htm

Tellez MR, Khan IA, Schaneberg BT, Crockett SL, Rimando AM, Kobaisy M. 2004. Steam distillation-solid-phase microextraction for the detection of *Ephedra sinica* in herbal preparations. *J Chromatogr A* 1025(1):51–6.

Vaillant F, Cisse M, Chaverri M, Perez A, Dornier M, Viquez F, Dhuique-Mayer C. 2005. Clarification and concentration of melon juice using membrane processes. *Innov Food Sci Emerg Technol* 6:213–20.

Zabetakis I, Gramshaw JW. 1998. 1,2-Propanediol in strawberries and its role as a flavour precursor. *Food Chem* 61(3):351–4.

Zellner BA, Dugo P, Dugo G, Mondello L. 2008. Gas chromatography-olfactometry in food flavour analysis. *J Chromatogr A* 1186:123–43.

Zhu M, Aviles FJ, Conte ED, Miller DW, Perschbacher PW. 1999. Microwave mediated distillation with solid-phase microextraction: Determination of off-flavors, geosmin and methylisoborneol, in catfish tissue. *J Chromatogr A* 833:223–30.

CHAPTER 13

Flavor Extraction: Headspace, SDE, or SFE

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INTRODUCTION

The identification and quantification of the volatile and semivolatile flavor compounds can be done using several extraction and concentration methods, including headspace (HS), simultaneous distillation–extraction (SDE), and supercritical fluid extraction (SFE). The above approaches were also discussed in view of the application to new fields on reviewed articles. Sides and others (2000) dealt with the developments in analysis of aroma components in foods and some advances in sampling. Augusto and others (2003) reviewed advances and applications in sampling and sample preparation for analysis of aromas and fragrances. More recently, Bicchi and others (2008) reviewed, over the period 1996–2007, the evolution of vapor phase sampling of the volatile fraction of vegetable matrices, or of products directly related to them.

The aim of extraction is to obtain a concentrated sample containing mainly the volatile components and to improve detection limits of specific compounds. The different sampling techniques offer a number of individual advantages but also suffer from specific limitations. Problems common to all techniques are the potential destruction of aroma components and/or production of aroma artifacts.

HS METHODS

The merits of HS sampling for recovery of volatile compounds associated with aroma have long been recognized. In spite of its continuous evolution, it is only over the last two decades that HS sampling has enjoyed a remarkable development; this came about at the same time as the ever-increasing success of solvent-free sample preparation techniques, that is, techniques in which the analyte(s) is isolated from

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a matrix without using a liquid solvent. HS sampling meets this definition perfectly, since its main aim is to sample the gaseous or vapor phase in equilibrium (*or not*) with a solid or liquid matrix, in order to characterize its composition.

The original HS procedure involved static (static headspace [SHS]) recovery in which sample was equilibrated in a sealed container at a controlled temperature and the HS sample was withdrawn via septum. The simplest way to assess the chemical composition of the aroma is direct analysis (by gas chromatography [GC] or another convenient technique) of a portion of the air in contact with the odor source, without any other sample-treatment step. However, since little or no preconcentration of the analytes is involved and considering the typical trace or ultratrace levels of the components, it is not usually feasible to apply SHS to "flavor" analysis. Applications of SHS sampling are limited by a number of factors including low sensitivities.

A large number of reports describing the use of dynamic headspace (DHS) methods can be found in the literature regarding the chemical characterization of flavors. DHS is probably still the most widely used vapor phase sampling approach, because of flexibility in terms both of sampled even for trace components, and of the number of possible trapping approaches and materials, allowing sampling systems to be especially optimized for the problem under study. The dynamic procedure (termed purge and trap) involves passing an inert gas through the sample and collecting the stripped volatile constituents in a trap. The equilibrium between the sample and HS is constantly removed resulting in improved sensitivity. The details differ in the type of trap, loading and unloading of the trap with methods including cryogenic trapping, absorption on a sorbent bed, on-column vapor traps, and whole-column cryo-trapping. Cold traps have the disadvantage that water is collected with the volatile material. Closed loop stripping and binding of water with excess sodium sulfate increases the effectiveness of high flow DHS for isolation of water-soluble volatiles. Sorbent traps using charcoal or porous polymers are common. Desorption of trapped analytes for subsequent analysis can be performed either with small volumes of adequate solvents or using on-line automated thermal desorption (ATD) devices, the latter being eminently suitable for routine procedures.

Traditionally, HS sampling operates either in static (SHS) or in dynamic mode (DHS): paradoxically, the principles of these two approaches were defined so clearly that they limited the development of HS techniques until the end of the 1980s. A vacuum headspace (VHS) isolation technique (cryogenic vacuum trapping) was introduced by Joulain (1986) as a convenient method for trapping the volatile compounds emitted by fragrant flowers. To date, this method has been applied successfully by other authors to a large number of floral species (Brunke et al. 1993; Joulain 1993; Surburg et al. 1993) as well as to the flavor analysis of strawberries, peaches, and cupuacu fruits (Fischer et al. 1993, 1995) (Fig. 13.1).

The renewed interest in the HS technique coincided with the introduction, in the early 1990s, of an additional approach, which acts as a bridge between SHS and DHS: high-concentration capacity headspace techniques (HCC-HSs). These techniques are based on either the static or the dynamic accumulation of volatile(s) on polymers operating in sorption and/or adsorption modes, or, more seldom, on solvents. One of several successful techniques based on the HCC-HS approach is nowadays widely used in addition to conventional SHS and DHS sampling: HS–solid phase microextraction (HS-SPME).

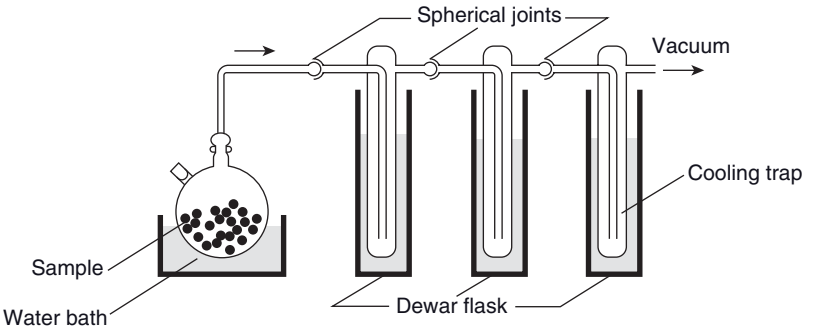

Figure 13.1. Scheme of vacuum headspace sampling system. Reproduced from Werkhoff and others (1998).

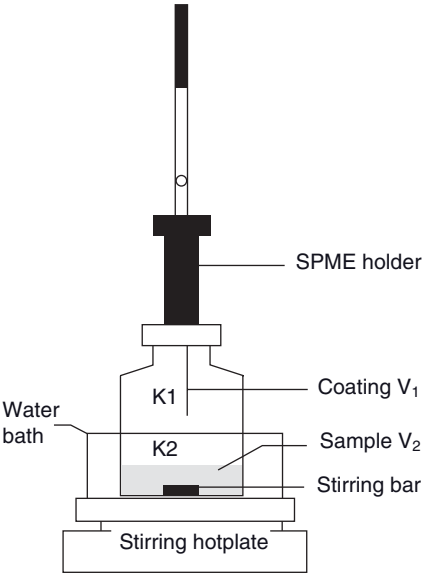

Figure 13.2. Diagram for the isolation of headspace flavor compounds by SPME. Reproduced from Jia and others (1998).

Solid phase microextraction (SPME) is a relatively new and simple adsorption technique for the isolation of HS flavor compounds (Arthur and Pawliszyn 1990; Arthur et al. 1992; Zhang and Pawliszyn 1993). SPME HS sampling requires neither solvent extraction and purification steps nor a complicated purge-and-trap apparatus. The SPME can be inserted into a gas chromatograph injection port to separate the isolated volatile flavor compounds. An SPME unit consists of a holder and a fused silica fiber, which is coated with a layer of stationary phase such as nonpolar polydimethylsiloxane (PDMS) or polar polyacrylate (PA). When an SPME coating is exposed in the HS of an airtightly sealed sample bottle, an equilibrium partition process occurs between the sample and the SPME coating. The diagram for the isolation of HS flavor compounds by SPME is shown in Figure 13.2.

The analyte recovery from HS by a fiber depends on two closely related but distinct equilibria: the first is the matrix/HS equilibrium responsible for the HS composition (measured by its distribution coefficient K_2); the second is the HS/polymeric fiber coating equilibrium (measured by its distribution coefficient K_1). The equilibrium partition of flavor compounds between the HS of the sample bottle and the SPME coating mainly depends on the heating time, temperature, sample volume, and sample concentration of the bottle. Although this technique was developed mainly for the analysis of environmental samples in the beginning, the interest in using SPME for food flavor analysis has increased during the past few years.

Two types of fiber SPME techniques can be used to extract analytes: HS-SPME and direct immersion (DI)-SPME. In HS-SPME, the fiber is exposed in the vapor phase above a gaseous, liquid, or solid sample. In DI-SPME, the fiber is directly immersed in liquid samples. Agitation of the sample is often carried out with a small stirring bar to increase the rate of equilibration. After a suitable extraction time, the fiber is withdrawn into the needle, the needle is removed from the septum and is then inserted directly into the injection port of the gas chromatograph or the desorption chamber of the SPME–high-performance liquid chromatography (HPLC) interface. HS- and DI-SPME techniques can be used in combination with any GC, GC–mass spectrometry (MS), HPLC, and HPLC-MS system. The desorption of analyte from the fiber coating is performed by heating the fiber in the injection port of a gas chromatograph or GC-MS, or by loading solvent into the desorption chamber of the SPME-HPLC interface, and then the analyte are transferred directly to the column for analysis (Kataoka et al. 2000).

SDE

One of the principal methods for separating the volatile substances from foods, beverages, and other agricultural products is that of steam distillation (SD), frequently followed by extraction with an organic solvent. A simple and effective means for performing these two operations simultaneously was published by Nickerson and Likens (1966) with a drawing of their distillation–extraction apparatus. Schultz and others (1977) presented a detailed description of a modification of this apparatus, and proposed for this isolation method and the apparatus the name simultaneous distillation and extraction (SDE) also known as the Likens–Nickerson method.

One of the modified distillation–extraction apparatus is shown in Figure 13.3. As with the earlier apparatus, a relatively large flask, for the sample (with added water if necessary), is coupled at the lower right joint, and a smaller vessel for the organic solvent (less dense than water) and extract is attached at the left.

As SDE proceeds, the two liquid phases of the condensate continually return to their respective flasks; an interface between the two phases forms in the separatory tube (central tube below the condenser) a little below the lower end of the solvent-return arm. In this operation, aroma analytes are removed from the matrix by the water vapor and transferred to the organic phase when the liquids condense together on the cold tube. Both water and solvent are collected in the extractor body after their condensation and return to the corresponding flask, allowing continuous reflux.

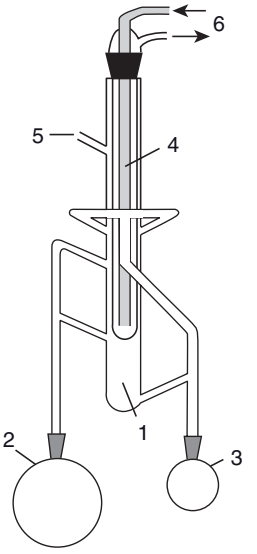

Figure 13.3. Modified Likens–Nickerson simultaneous distillation–extraction apparatus. Reproduced from Augusto and others (2003). 1, body; 2, sample flask; 3, extracting solvent flask; 4, cold tube; 5, inlet for purge gas; 6, cold water inlet and outlet.

A modified apparatus allows the use of solvents lighter than water, such as pentane or ethyl acetate as extractors.

SDE is a well-known technique used for the analysis of essential oil. The main disadvantage of SDE lies in its relatively high extraction temperature that might result in certain chemical changes of some temperature-sensitive compounds of the extracted samples. A vacuum SD can also be conducted in an SDE apparatus as an extraction solvent. The simultaneous distillation–extraction at reduced pressure (SDEV) has been shown to be a successful technique.

SFE

In recent years, the use of supercritical fluid (SF) for extraction has been considered one of the most promising new methods of sample preparation. SFs have received a great deal of attention because of the ease with which their chemical potential can be adjusted with pressure. That is, their physical properties can be turned while the inherent molecular structure of the solvent is maintained. As a consequence, supercritical solvents have been used extensively for extractions. The miscibility of essential oil with carbon dioxide leads to a number of applications of SFE on the study of essential oil, as well as of flavor and fragrance compounds in the food industry and other fields. For this purpose, both offline SFE and SFE coupled with GC were used. Recently, numerous industrial and academic research and development laboratories have investigated the fundamentals and process applications of SF solvents, that is, gases or liquids above their critical points.

When an SF is used as an extraction solvent, it is possible to separate a multicomponent mixture by capitalizing on both the differences in component volatilities (i.e., the salient feature of distillation) and the differences in the specific interactions

between the mixture components and the SF solvent (i.e., the salient feature of liquid extraction). The application of SF solvents is based on the experimental observation that many gases exhibit enhanced solvating power when compressed to conditions above the critical point. The critical region has its origin at the critical point. At this point, we can define an SF as any substance that is above its critical temperature (T_c) and critical pressure (P_c). The critical temperature is therefore the highest temperature at which a gas can be converted to a liquid by an increase in pressure. The critical pressure is the highest pressure at which a liquid can be converted to a traditional gas by an increase in the liquid temperature. In the so-called critical region, there is only one phase and possesses some of the properties of both a gas and liquid.

The essential components of a supercritical fluid chromatography (SFC) instrument consist of a mobile phase container, which is usually a pressurized gas cylinder, a pump, an injector, a column in a thermostated compartment, a restrictor, and a detector. The components are similar to the components of a GC or an HPLC instrument, with the exception of the restrictor, which is needed to maintain the pressure above the critical point. Cylinders with carbon dioxide are equipped with a dip tube to allow the liquid phase to be withdrawn. With gas phase detectors working at atmospheric pressure, the restrictor is connected prior to the detector (Fig. 13.4a). If the detection is performed under supercritical conditions, the restrictor is the last component in the sequence (Fig. 13.4b).

Traditionally, SFE was used for the industrial processing of plant matrices such as coffee beans, hops, and spices. Using CO_2 as the SF, extractions can be performed under mild conditions, thus reducing both the risks of thermal degradation and the poor collection efficiencies of volatile analytes that sometimes occur during the SD or solvent extraction of essential oils and fragrances.

Supercritical and liquid CO_2 can both be used as solvents. In contrast to subcritical CO_2 (i.e., liquid), the solvating power of the SF is highly dependent on its temperature and pressure.

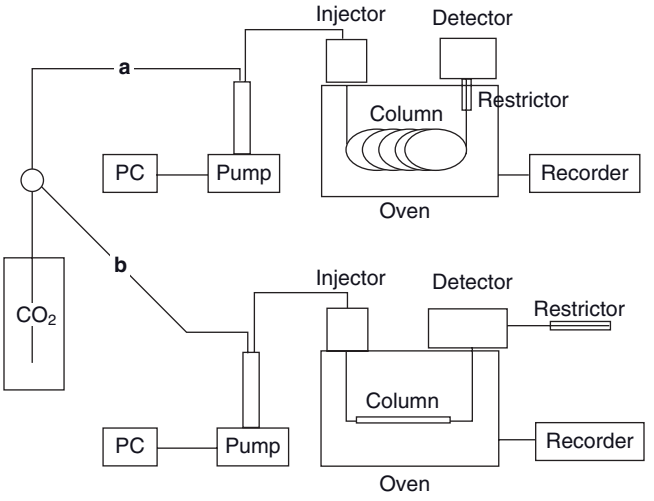

Figure 13.4. Schematic diagram of SFC instrument with gas phase detector (a) and with the detector cell under pressure (b). Reproduced from Greibrokk (1993).

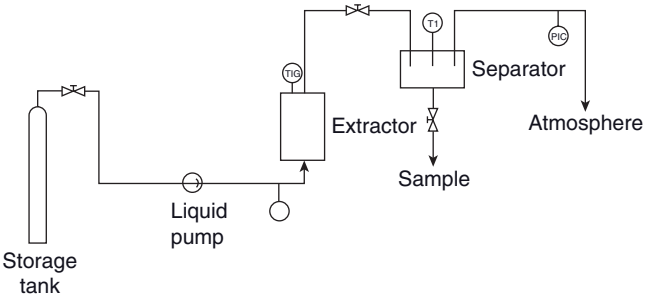

Figure 13.5. Schematic presentation of the Autoclave Engineers Screening System. Reproduced from Glisic and others (2007).

Compared with the direct extraction methods discussed above, SFE presents some advantages for flavor analysis. For example, since the critical point for CO_2 (the most popular and most convenient fluid for SFE) is 31.1°C at 73.8 bar, extractions can be carried out under milder temperature conditions and without the need of further aggressive procedure, such as distillation of excess solvent, as is common in liquid–liquid extraction (LLE) and solid phase extraction (SPE).

Extractions with supercritical carbon dioxide (SC CO_2) can be carried out in the Autoclave Engineers Screening System shown in Figure 13.5. The supercritical extraction screening system is designed for small batch research runs, using CO_2 as the supercritical medium. Liquid CO_2 is supplied from a CO_2 cylinder by a siphon tube. The CO_2 is pumped into the system by the liquid metering pump until the required pressure is obtained. Back pressure regulators are used to set the system pressure (in extractor and separator). The extractor vessel is filled with the material from which a substance is to be extracted. Heaters are supplied on the extractor vessel for temperature elevation. The SC CO_2 flows through the extractor and enters the separator vessel. Samples of the extracted substance can be taken by opening the ball valve located at the bottom of the vessel. A flowmeter is provided to indicate the flow rate of CO_2 being passed through the system and the flow can be adjusted by a micrometering valve. The CO_2 continues to flow out of the separator through the flowmeter/totalizer and out to the atmosphere.

Currently, CO_2 is generally recognized as safe and is regulated by the U.S. Food and Drug Administration, as a direct human food ingredient. The use of traditional organic solvents in food processing requires the removal of residual solvent to permitted levels. Furthermore, the chances of off-flavors resulting from residual solvent or processing can be minimized when extracting with SC CO_2. Extraction with SC CO_2 has several advantages such as shorter extraction time, smaller solvent consumption, and lower working temperature.

APPLICATION OF EXTRACTION METHODS AND SAMPLING TECHNIQUES

To obtain aroma concentrates that are truly representative of tropical passion fruit flavor, Werkhoff and others (1998) applied four different flavor isolation techniques: VHS method, DHS method, SDE at atmospheric pressure, and SDEV.

Yellow passion fruit (*Passiflora edulis f. flavicarpa*) is one of the most popular and best known tropical fruits having a floral, estery aroma with an exotic tropical sulfury note. The attractive tropical flavor note of ripe yellow passion fruits has been shown to be associated with trace levels of sulfur volatiles. Volatile sulfur components are important trace constituents of natural products and play an important role in the sensory properties of food flavors. Flavor extracts were organoleptically evaluated and the aroma composition, of different flavor concentrates was investigated by GC-MS.

Significant differences were found not only in the chemical composition of the resultant extracts but also in their sensory properties. Table 13.1 shows the sensory assessment of the four passion fruit extracts by a panel of experienced flavorists.

The comparison of the four isolation methods showed that the VHS procedure resulted in the most powerful and typical flavor concentrate representing the true natural delicate and characteristic aroma notes reminiscent in a very typical way of the original fruit flesh. The flavor of the VHS sample was noted to be of particular sensory interest and was generally recognized as superior to those obtained by classical isolation techniques. Only the odor of the VHS extract was representative of the fruit and was described as fresh, juicy, tropical, grassy, fruity, green, sulfury, and estery and possessing odor notes reminiscent of honey, pineapples, melons, and grapefruits.

Table 13.2 lists the compounds identified in different passion fruit flavor concentrates along with their GC peak area percents. Each compound listed was identified by its GC retention index and by its mass spectrum obtained during a GC-MS run.

Significant differences were found in both the qualitative and quantitative compositions of extracts obtained by different isolation procedures. The chromatograms of both HS samples are shown in Figure 13.6. The VHS concentrate contains more high boiling components.

The SDE technique involves the possibility of forming thermally induced artifacts. Therefore, atmospheric SDE produced a falsified aroma, with as light, cooked jam-like nuance. Consequently, the SDE sample contained, for example, some furan derivatives or hexadecanoic acid, neither of which was present in the HS extracts in comparable concentrations. Furthermore, losses of high volatility components have been observed using the SDE technique. Vacuum SDE appears to be a valuable alternative that avoids thermal degradation and formation of thermal artifacts

TABLE 13.1 Organoleptic Evaluation of Different Passion Fruit Flavor Concentrates

Sample	Comments
Vacuum head space concentrate (VHS)	Tropical, strongly fruity, estery, green, juicy, fresh, sulfury, honey like, typical passion fruit
Dynamic head space concentrate (DHS)	Herbaceous, faintly tropical, very weak aroma, atypical
Simultaneous distillation–extraction at atmospheric pressure (SDE)	Fruity, sulfury, faintly tropical, acidic background, burnt, cooked aroma
Simultaneous distillation–extraction at reduced pressure (SDEV)	Tropical, sulfury, sweet, fruity, somewhat passion fruit like

Reproduced from Werkhoff and others (1998).

TABLE 13.2 Flavor Constituents of Yellow Passion Fruits/Comparison of Sample Preparation Techniques

	Area %					Area %			
Compound[a]	VHS	DHS	SDE	SDEV	Compound[a]	VHS	DHS	SDE	SDEV
Ethyl acetate	0.7	3.0	1.4	2.0	(*E*)-2-hexenyl acetate	<0.1			
2-Butanone	0.3	1.3	0.3		1-Hexanol	8.0	3.3	2.5	5.4
2- and 3-Methylbutanal			0.4		2-Cyclopenten-1-one	<0.1	<0.1	0.3	<0.1
Ethyl propanoate	<0.1	<0.1	0.1	<0.1	3-Nonanone	<0.1	<0.1		<0.1
2-Pentanethiol			<0.1		(*E*)-3-hexen-1-ol	0.6	0.1	0.2	0.3
2-Ethylfuran			<0.1		(*Z*)-3-hexen-1-ol	2.5	0.6	1.0	1.7
2,3-Butanedione			<0.1		2-Methyl-3(2*H*)-furanone			<0.1	
Propyl acetate	<0.1	<0.1		<0.1	Butyl hexanoate	<0.1	<0.1	0.1	<0.1
2- and 3-Pentanone	<0.1	0.1	0.1	0.1	Hexyl butanoate	8.9	15.5	2.9	8.7
Pentanal			<0.1		α-Angelica lactone			<0.1	
Methyl butanoate	<0.1	<0.1	<0.1	<0.1	Hexyl 2-methylbutanoate	0.1	0.1	<0.1	0.1
2-Methyl-2-butanol			<0.1		(*E*)-2-octenal				<0.1
Isobutyl acetate	<0.1	0.1	<0.1	<0.1	1-Isopropenyl-4-methylbenzene	<0.1		<0.1	<0.1
2-Butanol	<0.1	0.1	<0.1	<0.1	Ethyl octanoate	0.3	0.1	0.1	0.3
α-Pinene	<0.1	0.3	<0.1	<0.1	*trans*-Linalool oxide (f)	0.2		1.2	0.4
Ethyl butanoate	4.7	23.0	7.9	9.1	Acetic acid	<0.1	<0.1	<0.1	<0.1
1-Propanol	<0.1	<0.1	<0.1	<0.1	Methional			<0.1	
(*E*)-2-butenal			<0.1	<0.1	Ethyl (methylthio)acetate	<0.1			
2,3-Pentanedione	<0.1	<0.1	<0.1	<0.1	Hexyl 3-methylbutanoate				<0.1
Ethyl 2-methylbutanoate	<0.1	<0.1	<0.1	<0.1	(*E*)-3-hexenyl butanoate	0.3	0.3	0.1	0.2
S-methyl acetothioate			<0.1		Furfural	<0.1	<0.1	2.3	<0.1
Butyl acetate	0.1	0.6	<0.1	0.1	1-Heptanol	<0.1			<0.1
Hexanal	0.1	0.1	0.1	0.2	(*Z*)-3-hexenyl butanoate	2.1	2.2	0.5	1.3
2-Methyl-1-propanol	0.1	0.2	<0.1	<0.1	Nerol oxide			<0.1	
1-Hexen-3-one			<0.1	<0.1	*cis*-Linalool oxide (f)	<0.1		0.6	0.1
2-Methyl-(*E*)-2-butenal			<0.1		2-Acetylfuran and 2,5-dimethyl-3(2*H*)-furanone			0.4	

TABLE 13.2 ***Continued***

Compound[a]	Area %				Compound[a]	Area %			
	VHS	DHS	SDE	SDEV		VHS	DHS	SDE	SDEV
2,6,6-Trimethyl-2-vinyltetrahydropyran			0.4		Theaspirane A	<0.1		<0.1	<0.1
3-Pentanol	<0.1	<0.1		<0.1	Benzaldehyde	1.9	<0.1	1.9	2.9
2-Pentanol	<0.1			<0.1	Ethyl 3-hydroxybutanoate	1.6	<0.1	0.2	<0.1
β-Pinene		<0.1	<0.1	<0.1	*cis*-2-Methyl-4-propyl-1,3-oxathiane	<0.1		<0.1	<0.1
2- and 3-Methylbutyl acetate	<0.1	0.3	<0.1	<0.1	Theaspirane B	<0.1			<0.1
Propyl butanoate	<0.1	<0.1	<0.1	<0.1	Linalool	0.5	0.3	4.6	4.9
3-Penten-2-one			<0.1	<0.1	Hexyl 2-butenoate	<0.1	<0.1		<0.1
Ethyl pentanoate	<0.1	<0.1	<0.1	<0.1	*trans*-2-Methyl-4-propyl-1,3-Oxathiane	<0.1	<0.1	<0.1	<0.1
4-Methyl-3-penten-2-one	<0.1		<0.1	<0.1	1-Octanol	2.3	0.4	0.3	0.8
(*Z*)-3-hexenal			<0.1	<0.1	Ethyl 3-(methylthio)propanoate	<0.1	<0.1	<0.1	<0.1
1-Butanol	0.2	0.1	0.2	0.3	5-Methylfurfural			0.1	
(*E*)-3-hexenal	<0.1		<0.1	<0.1	4-Terpineol	0.3		0.1	0.2
3-Heptanone	<0.1	<0.1	<0.1	<0.1	Hotrienol	<0.1		<0.1	<0.1
Δ-3-Carene	<0.1	<0.1		<0.1	*p*-1-Menthenal-9 P1			<0.1	<0.1
Myrcene	0.3	4.4	0.7	1.2	*p*-1-Menthenal-9 P2			<0.1	<0.1
Ethyl 2-butenoate	<0.1	<0.1	<0.1	<0.1	Hexyl hexanoate	26.7	7.6	10.3	24.4
Isobutyl butanoate	<0.1	<0.1		<0.1	Octyl butanoate	<0.1			0.2
α-Phellandrene		<0.1	<0.1	0.1	Phenylacetaldehyde			0.8	<0.1
α-Terpinene		0.1	<0.1	0.2	Butanoic acid			<0.1	<0.1
2-Heptanone	<0.1	0.1	<0.1	<0.1	(*E*)-3-hexenyl hexanoate			0.2	
Cyclopentanone	0.2	0.1	<0.1	0.1	Riesling acetal			<0.1	
Methyl hexanoate	<0.1	<0.1	<0.1		Furfuryl alcohol			0.1	
Limonene	0.2	1.8	0.4	0.7	(*Z*)-ocimenol			0.1	
3-Methyl-2-butenal	<0.1			<0.1	(*Z*)-3-hexenyl hexanoate	4.7	1.2	1.6	3.7
2- and 3-Methyl-1-butanol	0.2	<0.1	0.1	0.2	Diethyl succinate	0.3		<0.1	0.2
trans-Anhydrolinalool oxide			0.3		(*E*)-ocimenol			0.4	

β-Phellandrene		<0.1		0.2	2- and 3-Methylbutanoic acid			<0.1	
1,8-Cineole	<0.1	<0.1	<0.1	<0.1	Ethyl 3-hydroxyhexanoate	0.2		0.1	0.1
(*E*)-2-hexenal	0.1		0.4	0.4	α-Terpineol	0.5	<0.1	2.0	1.7
Butyl butanoate	<0.1	<0.1		<0.1	γ-Terpineol			<0.1	
Ethyl hexanoate	5.2	7.2	2.0	5.8	3-Mercaptohexyl acetate			<0.1	
cis-Anhydrolinalool oxide			0.2		Benzyl acetate	1.0	0.1	0.2	0.3
cis-β-Ocimene		<0.1	<0.1		(*Z*)-3, (*E*)-6-farnesene	<0.1			0.1
γ-Terpinene	0.1	0.3	0.1	0.2	Ethyl 3-(methylthio)-(*E*)-2-propenoate	<0.1		<0.1	0.1
trans-β-Ocimene	0.3	4.1	0.7	1.0	(*E*)-3, (*E*)-6-farnesene	0.1		<0.1	0.5
1-Pentanol			<0.1		1-Decanol	0.1		<0.1	0.1
2- and 3-Methylbutyl butanoate	<0.1	<0.1			β-Citronellol	0.1			0.1
Hexyl acetate	2.0	7.9	0.7	1.6	Methyl 2-hydroxybenzoate	0.2	<0.1	<0.1	0.1
3-Hydroxy-2-butanone and terpinolene	0.4	1.3	0.4	0.7	Neryl 2-methylpropanoate	<0.1			<0.1
Octanal	<0.1		<0.1	<0.1	Nerol	0.1		0.3	0.2
Ethyl (*E*)-3-hexenoate	<0.1	<0.1	<0.1	0.1	Ethyl 2-hydroxybenzoate	<0.1		<0.1	
Cyclopentanol	<0.1	<0.1	<0.1	<0.1	Phenethyl acetate	0.1		0.1	0.1
(*E*)-4,8-dimethyl-1,3,7-nonatriene and (*E*)-3-hexenyl acetate	0.2	1.1	0.2	0.3	Hexyl octanoate	0.3	<0.1	0.4	0.6
(*Z*)-3-hexenyl acetate	1.6	4.2	0.5	1.3	3-[(*E*)-1-propenyl]-α-terpineol P1	<0.1			
3-Methyl-2-buten-1-ol	<0.1	<0.1	0.7	<0.1	Octyl hexanoate	<0.1	<0.1	<0.1	<0.1
(*Z*)-2-penten-1-ol	<0.1		<0.1	<0.1	β-Damascenone	<0.1		<0.1	<0.1
Propyl hexanoate	<0.1	<0.1	<0.1	<0.1	7,8-Dihydro-β-ionone	0.2		0.1	0.2
2-Heptanol	<0.1	<0.1		<0.1	3-Mercaptohexanol	0.1		0.2	0.2
Hexyl propanoate		<0.1		<0.1	Hexanoic acid	<0.1		0.1	<0.1
Ethyl (*E*)-2-hexenoate	<0.1	<0.1		<0.1	*p*-Cymenol-8	<0.1		<0.1	<0.1

TABLE 13.2 ***Continued***

Compound[a]	Area % VHS	DHS	SDE	SDEV	Compound[a]	Area % VHS	DHS	SDE	SDEV
Hexyl 2-methylpropanoate	<0.1	<0.1		<0.1	Geraniol	0.3		0.8	0.8
Ethyl heptanoate	<0.1				3-[(*E*)-1-propenyl]-α-terpineol P2	0.2			
(*Z*)-3-hexenyl octanoate	0.1			<0.1	Benzyl hexanoate	0.3		0.1	0.1
Benzyl butanoate	0.3		0.1	0.1	Hexyl benzoate	0.1			
Benzyl alcohol	2.8	<0.1	0.3	0.2	3-Phenylpropyl butanoate	0.1			
Ethyl 5-hydroxyhexanoate	<0.1				(2-Nitroethyl)benzene	0.1			
Ethyl 3-phenylpropanoate	0.5				Ethyl cinnamate	0.8		0.3	0.3
Geranyl butanoate	<0.1				3-(Methylthio)hexyl hexanoate	<0.1			<0.1
3-(Methylthio)hexanol			<0.1		(*E*)-3, (*E*)-5-pseudoionone	0.2		<0.1	<0.1
2-Phenylethanol	<0.1		0.1	<0.1	γ-Decalactone	0.2		0.1	<0.1
3-(Methylthio)hexyl butanoate	<0.1			<0.1	Eugenol	<0.1		0.1	
β-Ionone	0.1		0.1	0.2	2-Methoxy-4-vinylphenol			0.1	
Nerylacetol	0.1				Phenethyl hexanoate	0.1			<0.1
3-Phenylpropyl acetate	<0.1				Nonanoic acid	<0.1			
Phenethyl butanoate	<0.1			<0.1	*cis*-γ-Jasmin lactone	<0.1			
Phenethyl butanoate	<0.1			<0.1	*cis*-γ-Jasmin lactone	<0.1			
Phenethyl butanoate	<0.1			<0.1	*cis*-γ-Jasmin lactone	<0.1			
Geranylacetol	0.1				*trans*-γ-Jasmin lactone	<0.1			
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	0.1			0.1	*trans*-Isoeugenol			<0.1	
3-Phenyl-1-propanol	0.2				γ-Dodecalactone	<0.1		0.1	<0.1
3-Mercaptohexyl hexanoate	<0.1			0.1	Hexadecanoic acid			14.6	
Octanoic acid			0.2						

Reproduced from Werkhoff and others (1998).

[a]Identifications based on mass spectral data and linear retention indices on DB-Wax.

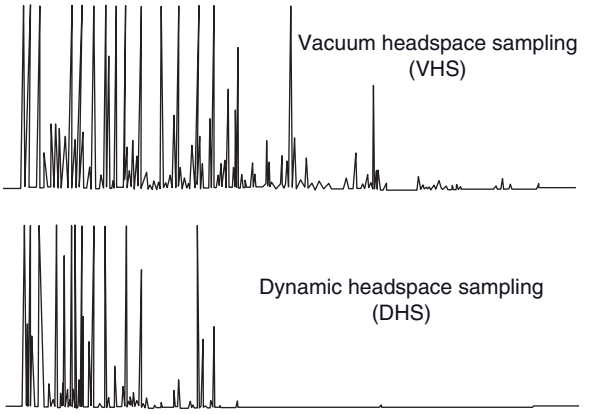

Figure 13.6. Capillary gas chromatograms of yellow passion fruit volatiles obtained by VHS and DHS. Reproduced from Werkhoff and others (1998).

(Maignial et al. 1992; Pollien and Chaintreau 1997). Furthermore, the well-known browning reactions observed during the atmospheric extraction are largely suppressed under vacuum. Consequently, the concentrate obtained by vacuum SDE showed fruitier and more exotic flavor characteristics compared with the atmospheric SDE sample.

Most analytic approaches to flavor analysis require that the flavor components be separated from nonflavor components in the sample prior to their quantitative determination (Teranishi and Kint 1993). This presumes that there is some way to discard the irrelevant chemicals without risking the loss of significant flavor factors.

Off-flavor analysis typically also requires careful, often laborious sample preparation for the same purpose. The analytic task may be complicated by not knowing in advance the chemicals causing the problem or, for quality assessment of frozen or packaged food products, even whether there is a problem. Off-flavors taint food by several different mechanisms, which require different sample-handling strategies (Wilkes et al. 2000).

Compared with solvent extraction, purge-and-trap, and direct injection GC methods, equilibrium HS GC dramatically reduces sample preparation time for analysis of volatile flavor and odor components (Paik and Venables 1991). Commercial instrumentation with autosampler capability exists. However, the method suffers volatility-based discrimination, which necessitates experimentally developed or theoretically calculated calibration curves for each component. The required data manipulations can consume all the time saved by easier sample preparation. Also, without trapping and concentration steps, SHS analysis often has insufficient sensitivity for trace components. With all these limitations, the method has been used for certain food applications.

Equilibrium (static) HS GC has been used to analyze packed orange juice for the volatile flavor components a-pinene, octanal, and d-limonene, which are absorbed into polymeric packing materials during storage (Paik and Venables 1991).

Shaw and others (1993) used HS GC with pattern recognition to classify 60 samples of commercial orange juice into four categories: (1) fresh-squeezed, not pasteurized; (2) pasteurized, not from concentrate; (3) reconstituted, from concentrate; and (4) single-strength, aseptically packaged from concentrate.

Page and Lacroix (1993) described application of the SPME-GC system for the analysis of exogenous volatiles (environmental pollutants, but not necessarily off-flavors, that could be found in food) in fruit juices (pear, orange, apple, grapefruit), soft drinks (orange), and fruit drinks (citrus, cranberry/raspberry, lemonade).

Zhang and others (2008) used a combination sampling method, including HS-SPME, SDE, and SD, to study the volatile compositional characteristics of common tomatoes (*Lycopersicon esculentum* var. commune) and cherry tomatoes (*Lycopersicon esculentum* var. cerasiforme) during storage, followed by GC-MS detection. Twenty-one and 14 volatile compounds of fresh and stale common tomatoes and 27 and 17 volatile compounds of fresh and stale cherry tomatoes were identified. C6-aldehydes and alcohols were the main volatile components of both common and cherry tomatoes. The volatile compositional characteristics obtained from HS-SPME sampling of fresh and stale samples was subjected to principal component analysis (PCA) in the original "chromatographic data processing system." Potential biomarkers were looked for, based on volatile compositional characteristics, by a common model strategy. Of the characteristic volatile compounds of tomato, saturated hexanal increased gradually, whereas unsaturated (*E*)-2-hexenal decreased during storage of common tomato. The combination of HS-SPME and conventional methods provided the most representative information on tomato volatiles during storage.

SPME was evaluated for use in the quantification of aroma volatile production by Granny Smith apples during cool storage (Matich et al. 1996). The quantification of higher-molecular-weight (MW) volatiles by SPME was hindered by the slow transport of analytes into the gaseous phase, which results in long equilibration times and HS depletion of analyte during sampling, and by adsorptive losses onto walls of containers. These difficulties are not exhibited by all volatiles and for lower MW compounds that equilibrate rapidly between fruit and fiber. SPME offers possibilities for rapid, nonintrusive quantitative sampling of apple volatiles.

Verhoeven and others (1997) used a PA-coated fiber for the extraction of fruit (strawberry and apple) flavors by DI-SPME. Ibanez and others (1998) developed a method based on HS-SPME for the analysis of volatile compounds in fruits. Conditions of sampling have been tuned. Repeatability and recoveries obtained with this method have been determined by using a mixture of typical components of fruit aroma. Several fruits, such as raspberries, strawberries, blackberries, banana, and mango have been analyzed by the proposed method (Fig. 13.7).

The patterns obtained include compounds typically found in fresh fruits and compounds formed during processing or storage. The results showed the use of the technique for fruit characterization and its potential as a routine method for analyzing changes in key flavor compounds during different fruit processing regimes.

Isolation of carrot fruit (*Daucus carrota* L., cultivar "Chanteney") essential oil by SC CO_2 was investigated by Glisic and others (2007). Carrot fruit essential oil is widely used as a flavor ingredient in most major food categories, and as a fragrance component in perfumes, cosmetics, and soaps. It is the source of sesquiterpenic alcohols, carotol and daucol, and the sesquiterpene β-caryophyllene. The conventional method for carrot essential oil isolation is SD of dried fruits. Claimed properties of the oil include antibacterial.

In the SFE process of carrot fruit essential oil, particle size had no influence on the extraction rate in the two outermost cases: fine milled material and coarsely

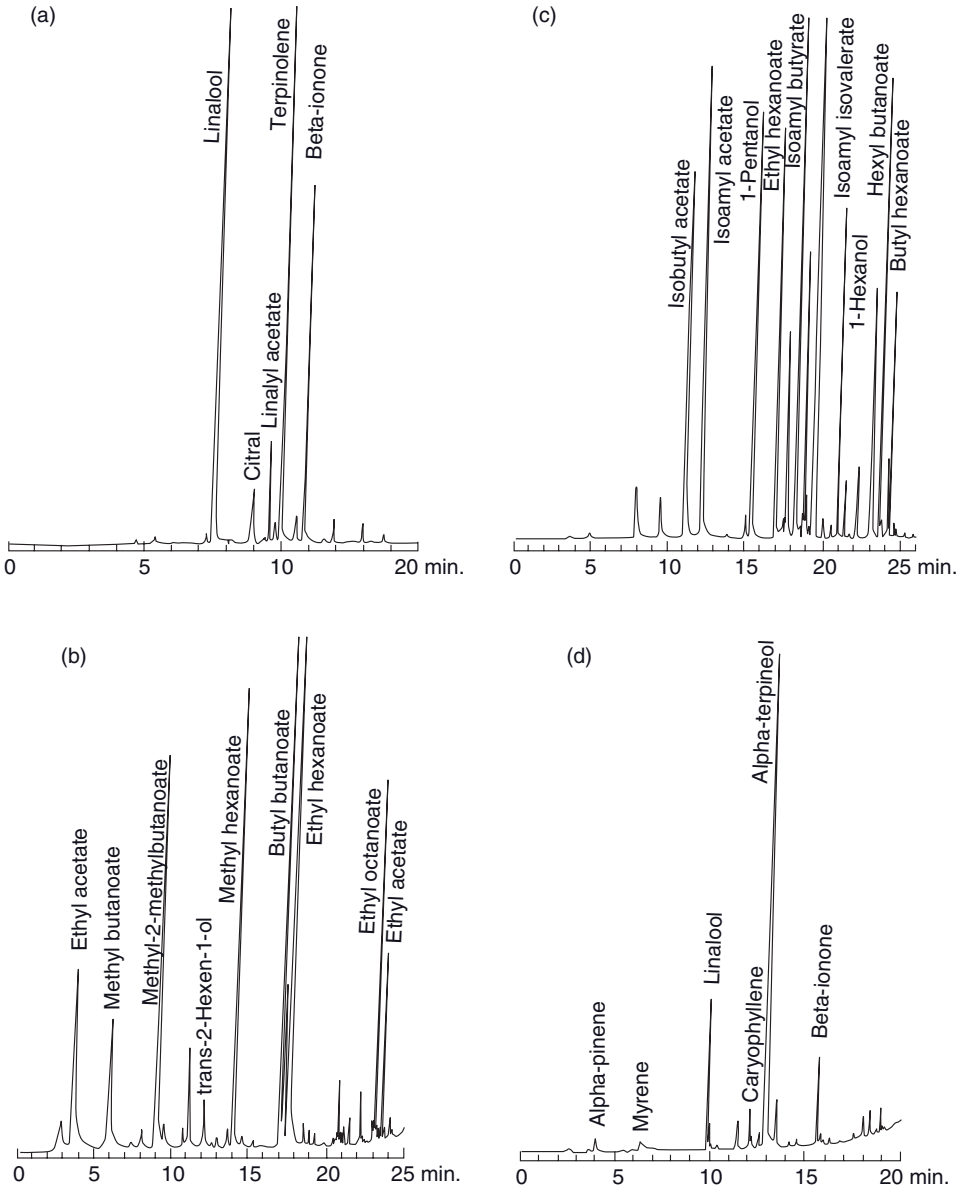

Figure 13.7. Gas chromatographic patterns of the SPME extracts of (A) raspberry, (B) strawberry, (C) banana, and (D) mango. SPME conditions: fiber, 100 µm PDMS; extraction mode, headspace; sample, solid (1 g); extraction, 60°C for 30 min; desorption, 200°C for 15 min. GC conditions: column, CP-Sil-5CB (50 m × 0.25 mm i.d., 0.25-µm film thickness); column temperature, program from 50°C (3-min hold) to 250°C at 5°C/min and hold at 250°C for 17 min; injection temperature, 200°C; detector temperature, 250°C; detection, flame ionization detector (FID). Reproduced from Ibanez and others (1998).

ground plant material, which is important on the industrial scale. The highest extraction yield in the SFE process (1.17%) was obtained at 40°C and 10 MPa. This value was higher than the yield obtained by hydrodistillation, as analytic analyses showed, due to coextraction of heavier weight compounds during the SFE process. The SFE extract, as well as the essential oil obtained by hydrodistillation, were the most effective against gram-positive bacteria and fungi investigated.

Xylopia aromatica (Lamarck) Martius (Annonaceae family) is a small tree (4–5 m tall) commonly found in open savannas in Central and South America, which produces white-yellowish flowers and small red cylindrical fruits along its long hanging branches. Due to its charming scent, the ground fruit from this Annonaceae is used in food products, perfumes, and cosmetics. It is reported, that there are between 100 and 150 species of *Xylopia* distributed throughout the tropical regions of the world, particularly Africa, among them, *Xylopia aethiopica*, *Xylopia brasiliensis*, *Xylopia frutenscens*, *Xylopia grandiflora*, which have been studied more completely, than *X. aromatica*. The various extracts from *Xylopia* spp. have been shown to possess antiseptic and analgesic properties and insecticidal activity against adult mosquito, several leaf-eating insects, and houseflies.

Colombian *X. aromatica* fruit essential oils and the various volatile fractions, obtained by different HS techniques (SHS, purge and trap [P&T], HS-SPME), were rich in β-phellandrene (up to 65%), a component of interest to the perfume industry and a useful starting material for fine organic synthesis. The relative chemical compositions of oils and extract, obtained by HD and SDE, were similar, but differed from volatile fractions, isolated by SHS, P&T, and HS-SPME. SFE isolated a larger amount of heavier compounds (sesquiterpenoids, benzenoids, and hydrocarbons). SDE was particularly effective for monoterpene hydrocarbon isolation. The relative amounts of volatiles from *X. aromatica* dry fruits, extracted by HS-SPME or P&T methods, depended on fiber exposure or purging times (Stashenko et al. 2004).

REFERENCES

Arthur C, Killam L, Buchholz K, Pawliszyn J. 1992. Automation and optimization of solid phase microextraction. *Anal Chem* 64:1960–6.

Arthur C, Pawliszyn J. 1990. Solid phase microextraction with thermal desorption fused silica optical fibres. *Anal Chem* 62:2145–8.

Augusto F, Lopes AL, Zini CA. 2003. Sampling and sample preparation for analysis of aromas and fragrances. *Trends Anal Chem* 22:160–9.

Bicchi C, Cordero C, Liberto E, Sgorbini B, Rubiolo P. 2008. Headspace sampling of the volatile fraction of vegetable matrices. *J Chromatogr* 1184:220–33.

Brunke EJ, Hammerschmidt FJ, Schmaus G. 1993. Headspace analysis of selected European medicinal plants. *Riv Ital Eppos* Numero Speciale:142–64.

Fischer N, Hammerschmidt FJ, Brunke EJ. 1993. Occurrence of γ-jasmolactone as a constituent of peach flavour. In Schreier P, Winterhalter P (eds.), *Progress in Flavor Precursor Studies*. Carol Stream, IL: Allured Publishing, pp. 287–94.

Fischer N, Hammerschmidt FJ, Brunke EJ. 1995. Analytical investigation of the flavor of cupuacu (*Theobroma gradiflorum Spreng.*). In Rouseff RL, Leahy MM (eds.), *Fruit Flavors, Biogenesis, Characterization, and Authentication*. Washington, DC: American Chemical Society, pp. 8–20.

Glisic SB, Misic DR, Stamenic MD, Zizovic IT, Asanin RM, Skala DU. 2007. Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity. *Food Chem* 105:346–52.

Greibrokk T. 1993. Instrumentation for supercritical fluid chromatography. In Dean JR (ed.), *Applications of Supercritical Fluids in Industrial Analysis*. Glasgow: Blackie Academic & Professional, pp. 13–45.

Ibanez E, Lopez-Sebastian S, Ramos E, Tabera J, Reglero G. 1998. Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem* 63:281–6.

Jia M, Zhang QH, Min DB. 1998. Optimization of solid phase micro extraction analysis for headspace flavor compounds of orange juice. *J Agric Food Chem* 46:2744–7.

Joulain D. 1986. Study of the fragrance given off by certain spring time flowers. In Brunke EJ (ed.), *Progress in Essential Oil Research*. Berlin: de Gruyter, pp. 57–67.

Joulain D. 1993. Cryogenic vacuum trapping of scents from temperate and tropical flowers. In Teranishi R, Buttery RG, Sugisawa H (eds.), *Bioactive Volatile Compounds from Plants*. Washington, DC: American Chemical Society, pp. 187–204.

Kataoka H, Lord HL, Pawliszyn J. 2000. Applications of solid-phase micro extraction in food analysis. *J Chromatogr* 880:35–62.

Maignial L, Pibarot P, Bonetti G, Chaintreau A, Marion JP. 1992. Simultaneous distillation-extraction under static vacuum: Isolation of volatile compounds at room temperature. *J Chromatogr* 606:87–94.

Matich AJ, Rowan DD, Banks NH. 1996. Solid phase microextraction for quantitative headspace sampling of apple volatiles. *Anal Chem* 68:4114–8.

Nickerson GB, Likens ST. 1966. Gas chromatographic evidence for the occurrence of hop oil components in beer. *J Chromatogr* 21:1–5.

Page BD, Lacroix G. 1993. Application of solid-phase microextraction to the headspace gas chromatographic analysis of halogenated volatiles in selected foods. *J Chromatogr* 648:199–211.

Paik JS, Venables AC. 1991. Analysis of packaged orange juice volatiles using headspace gas chromatography. *J Chromatogr* 540:456–63.

Pollien P, Chaintreau A. 1997. Simultaneous distillation-extraction: Theoretical model and development of a preparative unit. *Anal Chem* 69:3285–92.

Schultz TH, Flath RA, Mon TR, Eggling SB, Teranishi R. 1977. Isolation of volatile components from a model system. *J Agric Food Chem* 25:446–9.

Shaw PE, Buslig BS, Moshonas MG. 1993. Classification of commercial orange juice types by pattern recognition involving volatile constituents quantified by gas chromatography. *J Agric Food Chem* 41:809–13.

Sides A, Robards K, Helliwell S. 2000. Development in extraction techniques and their application to analysis of volatiles in foods. *Trends Anal Chem* 19:322–9.

Stashenko EE, Jaramillo BE, Martinez JR. 2004. Analysis of volatile secondary metabolites from Colombian *Xylopia aromatica* (Lamarck) by different extraction and head space methods and gas chromatography. *J Chromatogr* 1025:105–13.

Surburg H, Güntert M, Harder H. 1993. Investigation of volatiles from flowers. Analytical and olfactory aspects. In Hopp R, Mori K (eds.), *Recent Developments in Flavor and Fragrance Chemistry*. Weinheim: VCH Verlagsgesellschaft, pp. 103–21.

Teranishi R, Kint S. 1993. Chapter 5. In Acree TE, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: American Chemical Society, p. 137.

Verhoeven H, Beuerle T, Schwab W. 1997. Solid-phase microextraction: Artifact formation and its avoidance. *Chromatographia* 46:63–6.

Werkhoff P, Güntert M, Krammer G, Sommer H, Kaulen J. 1998. Vacuum headspace method in aroma research: Flavor chemistry of yellow passion fruits. *J Agric Food Chem* 46:1076–93.

Wilkes JG, Conte ED, Kim Y, Holcomb M, Sutherland JB, Miller DW. 2000. Sample preparation for the analysis of flavors and off-flavors in foods. *J Chromatogr* 880:3–33.

Zhang Z, Pawliszyn J. 1993. Headspace solid phase microextraction. *Anal Chem* 65:1843–52.

Zhang ZM, Zeng DD, Li GK. 2008. Study of the volatile composition of tomato during storage by a combination sampling method coupled with gas chromatography/mass spectrometry. *J Sci Food Agric* 88:116–24.

PART IV

FLAVORS FOR FRUIT COMMODITIES

CHAPTER 14

Annona Fruits

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INTRODUCTION

Tropical fruits have attracted people with their exotic appearance and unique flavors. Several hundred species of edible tropical fruits are known to grow over the world, but only a few, such as banana, pineapple, mango, and papaya, have been commonly consumed outside of their growing areas. In addition to these major tropical fruits, certain minor ones, such as guava, lychee, passion fruit, and annona fruits, are also popular in some regions. In recent years, economic values of some of these minor fruits have increased due to their nutritional value and increased production. However, the flavors of these minor tropical fruits have received less attention than those of the major fruits.

A commercially desirable and acceptable fruit depends on factors such as nutritional value, flavor, appearance, size, easy cultivation, conservation, transportation, industrial processing, and market. In addition, historical factors, artificial selection, and the development of specific technologies have also been determinant, as shown by citrus fruits (selection and adaptation to subtropical conditions and the canning industry), banana (maturation control), and pineapple (adaptation to the canning industry). Undoubtedly, the progress in postharvest technology has also benefited many tropical fruits.

Native fruits present unique opportunities to widen or reconquer domestic markets, diversify agricultural production, and engage in sustainable development of specific areas. On the other hand, responding to the need of urban markets, the crops must be adapted to the requirements of new consumers and new growing conditions. For example, controlling pests and diseases is an important aspect when the plant is grown in the traditional home garden. In addition, much attention has been paid to some other studies, for example, reproductive biology, heredity of main traits, and the existence of a gene pool suitable for breeding, including cultivars as well as related species, wild or at an intermediate stage of domestication. These data are important not only for the genetic improvement of new crops, but also for the

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safe conservation of their diversity, safeguarding our ability to respond to future needs.

In this chapter, the flavor composition of annona fruits is summarized, but this contribution is not limited to a compilation of reported compounds. Each section presents an overview of important publications and a description of the characteristic features of the volatile composition of the fruits. Reviews of published analytic studies on the volatile compounds of these fruits include those of Ekundayo (1989), Nijssen and others (1996), and Pino (1997).

The overall flavor impression is determined by odor and taste. Color and texture of a product may also modify the overall subjective assessment of a particular flavor. However, the aroma is the single most important contributor to the characteristic flavor of most foods. This review will concentrate on volatile compounds in two aspects, reflecting recent and future trends and developments in flavor research: the analytic methodology for the determination of volatile compounds and the contribution of individual components to the characteristic flavor.

Annonaceae is a family of flowering plants consisting of trees, shrubs, or lianas. The number of genera and species of this botanical family is still debated. According to Bailey (1949), this family had 46 genera and 500–600 species, while Fries (1959) described the family as having 119 genera and over 2000 species. Later, Popenoe (1974) asserted that it contained 40–50 genera and more than 500 species. The family is concentrated in the tropics, with few species found in temperate regions. About 900 species are neotropical, 450 African, and the rest Asian. Only five genera are cultivated: *Annona*, *Asimina*, *Rollinia*, *Artabrotys*, and *Cananga* (Campbell 1996; Koek-Noorman et al. 1990; Murrillo 2001).

Members of the Annonaceae family have simple, alternate, petiolate leaves with smooth, entire margins. The leaves are arranged in two rows along the stems. The flowers are radially symmetrical and often bisexual. In most species, the three sepals are united at the base. There are six brown to yellow petals, many stamens in a spiral, and many pistils, each with a one-chambered ovary containing many ovules. Flowers are sometimes borne directly on large branches or on the trunk. They are receptacle convex to globose or elongated, elevated; sepals are deciduous, smaller than outer petals, and valvate in bud. Fruits have fleshy syncarps, one per flower, usually ovoid to nearly globose, of which its surface is variable depending on orientation, structure, and relative connation of pistil apices (Chatrou 1998; Kessler 1993; Koek-Noorman et al. 1990).

The large, pulpy fruits of some members are edible, including species of *Annona* (cherimoya, soursop, custard apple, sugar apple, atemoya, and ilama), *Asimina* (papaw), and *Rollinia* (biribá). Besides bearing edible fruits, some members also have aromatic oils that are used for perfumes or spices. The strong bark is used for carrying burdens in Amazonia. The wood is valued as firewood. The bark, leaves, and roots of some species are used in folk medicines and some species are also cultivated as ornamental plants.

As a matter of fact, the genus *Annona* is the most important source of edible fruits in Annonaceae. The name *annona* derives from the Latin "annual harvest" (Lizana and Reginato 1990). *Annona* species are shrubs or small trees, with height from 5 to 11 m; they are erect or somewhat spreading and possess gray-brown bark, often rough and corrugated. With few exceptions, the species are deciduous, even the tropical species, especially when cultivated in areas with dry or cool seasons and

without irrigation. The flowers are hermaphrodite, usually somewhat fragrant, solitary, or in fascicles with two or four flowers, with three green sepals and six petals arranged in two verticils. The flowers have several conglomerated and spirally arranged stamens below and around an upper globose-shaped dome of numerous united carpels, which have one ovule each. After fertilization, the united carpels will form a syncarp or composite fruit (Pinto et al. 2005). According to Geurts (1981), of the 119 species described in the genus, 109 are native to tropical America and 10 to tropical Africa.

All of the domesticated species are American, while one African species (*Annona chrysophylla syn. Annona senegalensis*) is probably in the process of domestication. The fruits of these species are delicately flavored and are marketed mainly in local, regional, or national trade, only rarely in international trade. Most of the species are found in the tropics, with only a few genera present in the temperate zone. These species are not difficult to cultivate and require comparatively little care. Depending on the species, the commercial life of a healthy tree can span 15 years, with yields varying from 8 to 25 t/ha (Pinto 2006).

The most common *Annona* species dealt with in this monograph are cherimoya (*Annona cherimola*), soursop (*Annona muricata*), sugar apple (*Annona squamosa*), custard apple (*Annona reticulata*), pond apple (*Annona glabra*), wild soursop (*Annona montana*), atemoya (*A. cherimola* × *A. squamosa*), and wild custard apple (*A. chrysophylla*) (Table 14.1). These species have several common names, and they need to be used with caution because some names may be applied to two or more species in different countries, or even in different regions of the same country. Therefore, the botanical descriptions are essential to distinguish among them. Key botanical literature includes Ochse and others (1974), León (1987), and Pinto and others (2005).

CHERIMOYA

A. cherimola Miller is thought to originate from cold but frost-free valleys of the Andes at an altitude of between 700 and 2500 m. The fruit is conical or somewhat heart shaped, 10–20 cm long and up to 10 cm in width, weighing on the average 0.1–0.5 kg, but the largest fruits may reach 2 kg in weight. The skin, thin or thick, may be smooth with fingerprint-like markings or covered with conical or rounded protuberances. The sweet, juicy, white flesh is melting, subacid, and very fragrant. The fruit is of a primitive form with spirally arranged carpels, resembling a raspberry. Each segment of flesh surrounds a single hard black bean-like seed. The fruit size is generally proportional to the number of seeds within. The fruit is fleshy and soft, sweet, white in color, with a custard-like texture, which gives it its secondary name, custard apple. Some characterize the flavor as a blend of pineapple, mango, and strawberry. Others describe it as tasting like commercial bubble gum. Regardless of its unusual appearance, cherimoya is readily accepted by the Western consumers due to its fine taste and flavor and has become a favorite tropical fruit. The pulp can be eaten either fresh, prepared in drinks and sherbets, or used to produce an alcoholic beverage (Morton 1987).

The fruit is cultivated mainly in the Mediterranean (Anderson and Richardson 1990; Sanewski 1991). Spain leads the world in cherimoya production, with some

TABLE 14.1. Botanical and Common Names of the Annona Species

Botanical Name	Common Names
Annona cherimola Miller	Cherimoya, cherimolia, custard apple (United States), chirimorrinon (Venezuela), graveola (Brazil), chirimoya (Colombia, Ecuador, Cuba), anona poshte (Salvador), cachiman la Chine, corossol du Pérou (Haiti), cabeça de negro, anona do Chile (Portugal), anona (Costa Rica), chirimoyo (España, Chile), custard apple (Australia), srikaya (Indonesia), nona (Malay)
Annona muricata L.	Soursop (United States), guanábana (Cuba, España, Honduras, Costa Rica), guanábana o cabeza de negro (Mexico), guanaba (El Salvador), huanaba (Guatemala), graviola, araticum do grande, anona, curassol, curaçao do rainha (Brazil), sirsak (Indonesia), durian belanda (Malay)
Annona reticulata L.	Custard apple, bullock's heart (United States), anona, anona colorada (Mexico), corazón (Venezuela), anón (Panama, Venezuela), anona roja, anona colorada (Guatemala), anona rosada (El Salvador), anona de redecilla (Nicaragua, Honduras), mamón (Cuba), anón pelón (Colombia), anona corazón (Peru), chirimoya roja (Bolivia), araticum ape, araticum do mato (Brazil), sarikaya (Philippines)
Annona squamosa L.	Sugar apple, sweetsop, custard apple (United States), saramuyo (Mexico), ata, ati, pinha, fruto do conde (Brazil), anón (Cuba, Costa Rica, Bolivia, Panama), anón blanco (Cuba, Honduras, Guatemala), riñón (Venezuela), anón de azúcar, anón doméstico (Colombia), anona de castilla (El Salvador), anona de Guatemala (Nicaragua), chirimoya (Guatemala, Ecuador), cachiman (Argentina), sweetsop (Bahamas, Jamaica), atis (Phillipines), sitaphal, sharifa (India)
Annona glabra L.	Pond apple, alligator apple, monkey apple, corkwood, bobwood (United States), bagá (Cuba), araticum do brejo, araticum do Rio (Brazil)
Annona diversifolia Safford	Ilama (United States, Mexico), anona blanca, papauce (Guatemala), anona blanca (El Salvador)
Annona cherimola × *Annona squamosa*	Atemoya (United States, Cuba), chirimorinon (Venezuela)
Annona chrysophylla Boj.	Wild custard apple, wild soursop (United States), mavulu, mugosa, mbokwe, makulo, mlamote (Kenya), mtopetope (Zanzibar), mabengeya, elipo, obwolo, ovolo (Uganda), dilolo, iolo, malolo (Angola), araticum da areia (Brazil)
Annona scleroderma Safford	Cawesh (United States), anona del monte (Guatemala)
Annona montana Macfad.	Wild soursop, mountain soursop, wild custard apple (United States), guanábana cimarrona (Cuba), araticum açú, araticum apé, araticum do brejo, araticum caca (Brazil)

3600 ha cultivated in the southern part of the country, which yielded 28,000 t of fruit (Pérez de Oteyza 2002). The export market is low, only 8–10% is sold to other European countries. Nevertheless, the European market is interested in this fruit (Lüdders 2002). Cherimoya is considered an important crop in Chile, where it is grown on approximately 1000 ha for national and international markets, primarily the United States, Japan, and a number of Latin-American countries. The fruit is also produced on a limited commercial scale in Argentina, Bolivia, Ecuador, Mexico, and Peru (Sanewski 1991), and production has recently begun in Colombia and Brazil. Outside Europe and the Americas, cherimoya is cultivated in Central Africa (and on an experimental basis, in South Africa), Thailand, Indonesia, Australia, and, most recently, New Zealand (Rasai et al. 1995). In general, the fruit commands high wholesale and retail prices, but costs are also high due to the high labor costs that include pruning, pollination, ant control and irrigation (Mahdeem 1994), and fruit deterioration, for example, major crop losses from frost and fruit splitting. In South America, the fruit fly is an additional menace. These instances make commercial cultivation notwithstanding, resulting in most of the cherimoyas are consumed or sold from plants growing in home gardens or in the wild (Gardiazabal 1993). In particular, the cherimoya is an important backyard crop in Bolivia, Colombia, Ecuador, Peru, and Venezuela. True cherimoya plantations are found only in Spain, Chile, and the United States. In the United States, only California is planting the fruit, and most of the products never leave the state though its demand in all U.S. markets greatly exceeds the supply.

Excellent cultivars are known, all produced by vegetative propagation, which are planted on a commercial scale in Spain, Chile, Australia, Israel, the United States, and the island of Madeira. The fruit is sold in the supermarkets of many countries and is highly regarded. According to Pinto (2006), the most important commercial cultivars are White, Bays, Golden Russet, Libby, and Lisa in the United States; Whaley, Pink's Mammoth, and Mosman in Australia; Concha Lisa and Bronceada in Chile; and Kabri and Malalai in Israel. In Spain, the most important cultivars are Fino de Jete and Campas (Guirardo et al. 2003; Pinto 2006), while in New Zealand is Reretai and in Portugal are Funchal and Mateus I.

In the regions where the cherimoya is still a marginal crop, new methods must be applied: artificial pollination, grafting of superior cultivars either on to stock of the same species or on to stock of *A. squamosa* or *A. glabra*; the control of anthracnosis and seed-boring insects; the control of green leafhoppers; and fruit handling and packaging (Mahdeem 1994).

The volatile compounds of cherimoya were early studied by Herres and others (1983) with the introduction of high resolution gas chromatography-Fourier transform infrared (HRGC-FTIR) analysis, and as a result, 26 volatile chemicals were identified, including 10 esters, 13 alcohols, 2 acids, and a phenol derivative. Additionally, Idstein and others (1984) analyzed a Chilean cultivar using high-vacuum distillation, solvent extraction, and column chromatography combined with gas chromatography–mass spectrometry (GC-MS) and GC-FTIR. In total, 208 volatiles were identified, including 58 esters, 54 alcohols, 47 carbonyls, 23 hydrocarbons, and 26 volatiles of miscellaneous structures. Quantitatively, alcohols such as 1-butanol, 3-methyl-1-butanol, 1-hexanol, and linalool and a series of butanoates and 3-methylbutyl esters comprised the major part of the constituents. Although sensory evaluation was not carried out, the authors stated that esters should play an important role in the character aroma of this fruit.

A year later, Toulemonde and Beauverd (1985) analyzed the volatiles by headspace method with charcoal traps. Fifty-six compounds were identified, mainly methyl and ethyl esters from aliphatic acids, especially from butanoic acid. Although the presence of butyrates was important, it was not sufficient to explain the characteristic flavor of cherimoya.

Regarding the volatile acids of a Chilean cultivar, Idstein and others (1985) reported 47 compounds, among which the major acids were hexanoic (3 mg/kg) and octanoic (1 mg/kg).

The volatile composition of the Cuban cherimoya was investigated by capillary GC and GC-MS following isolation by simultaneous steam distillation–solvent extraction (Pino 2000). A total of 47 constituents were identified; major compounds were α-pinene (23 mg/kg), terpene-4-ol (19.8 mg/kg), α-thujene (18.7 mg/kg), and germacrene D (17.6 mg/kg). Sensory evaluation was not carried out in this investigation.

SOURSOP

A. muricata L. is possibly native to the Antilles and to the northern part of South America. Its altitude limit for growth is 1000 m. Its commercial production has been developed in Brazil, Venezuela, Costa Rica, and other countries for local consumption and export. Cultivation practices have been established in the production areas mentioned; they include the control of insects and diseases and protection of the fruit in plastic bags. There is a great deal of variation in fruit size and sugar content (Mahdeem 1994). According to Pinto (2006), the most important commercial cultivars are Giant of Alagoas, Selection of Ibimirim, and Cerradina in Brazil; and Morada, Lisa, and Blanca in Colombia.

The fruit is an ovoid syncarp, more or less oval or heart shaped, sometimes irregular, lopsided, or curved due to improper carper development or insect injury. The size ranges from 10 to 30 cm long and up to 15 cm in width and the weight may be up to 4.5–6.8 kg. The fruit is compound and covered with a reticulated, leathery-appearing but tender, inedible, bitter skin from which there is a few or many protrudable, stubby, or more elongated and curved, soft, pliable "spines." The skin is dark green in the immature fruit, becoming slightly yellowish green before the mature fruit is soft to the touch. Its inner surface is cream colored and granular and separates easily from the mass of snow-white, fibrous, juicy segments surrounding the central, soft-pithy core. When ripened, the pulp is juicy and has a rather cottony texture and a pleasant aroma. Comparisons of its flavor range from strawberry and pineapple mixed together to sour citrus flavor notes contrasting with an underlying creamy roundness of flavor reminiscent of coconut or banana. Pulp is eaten fresh or made into sherbets and jams. Other uses also include making creamy juice (called champola in Cuba and Brazil; carato in Puerto Rico) as well as candies, sorbets, and ice cream flavorings. Immature soursops are cooked as vegetables or used in soup in Indonesia, and they are roasted or fried in northeastern Brazil. The fruits are considered to be diuretic, and the juice is considered to be a tonic and a vermifuge (Morton 1987).

The soursop juice is not ready to be oxidized as that of other *Annona* species. It can be exported in its fresh form because it keeps its flavor even after deep-freezing

(Satney 1994). The pulp is treated at 70°C for 20 min, with the addition of ascorbic acid (0.5%) as antioxidant, and packed in high-density polyethylene bags, which can be stored up to 1 month at 5°C. The production of frozen pulp and nectar for small companies is relatively easy, not requiring big investments. The nectar produced and packed at the small enterprise level has as average composition: 17.8% fruit pulp, 10.7% sugar, 0.02% sodium benzoate, and 0.02% sodium metabisulfite. It is held at 100°C for 15 min. In this case, 330 kg of fruit can produce 2000 bottles of nectar (500 mL each). Jellies can also be prepared, with a pH between 3.1 and 3.3, 60% syrup, and 31% sugar added in relation to the total sugars (Alix 1999). Peters and others (2001) also claimed that nectars that were produced from pasteurized 8°Brix pulp, with no intermediate storage, and subsequently adjusted to 15°Brix, were highly ranked after 12 weeks of storage.

The first investigation into soursop flavor was performed by MacLeod and Pieris (1981). Volatile compounds of the fruit from Sri Lanka were obtained by means of simultaneous steam distillation–solvent extraction using 2-methylbutane. Compounds were identified by GC-MS using both electronic ionization (EI) and chemical ionization (CI) MS. Most volatile components were esters (~80% of the sample), and they constituted a chemically closely related series. Methyl hexanoate (0.38 mg/kg) and methyl hex-2-enoate (0.33 mg/kg) were the two most abundant components and together amounted to ~0.7 mg/kg of fruit. The odor qualities were unremarkable, and no GC peak seemed to represent any specific element of the characteristic soursop flavor. The obvious deduction must be drawn that soursop flavor is basically a blend of the 15 esters, together with at least *trans*-β-farnesene, in the correct proportions. The authors concluded that it would certainly be important in any processed product to retain all these components as far as possible to maintain the characteristic fresh fruit flavor.

In examining the physiological changes during fruit maturation, Paull (1982) and Paull and others (1983) found that ester production increased at the third harvest day, with a maximum 2–3 days later, followed by a drastic decrease and a detection of numerous unidentified GC peaks. Ten years later, Iwaoka and others (1993) identified soursop volatiles and described their changing profiles during ripening. The volatiles were isolated by liquid–liquid continuous extraction using methylene chloride combined with capillary GC and GC-MS, identifying 17 compounds. (*Z*)-3-hexenol was the major compound in mature green fruit, while methyl (*E*)-2-hexenoate, methyl (*E*)-2-butenoate, methyl butanoate, and methyl hexanoate were the major volatiles present in ripe fruit. Concentrations of these compounds decreased, and several other unidentified volatiles appeared when the fruit became overripe.

In the only known research using headspace techniques, Franco and Rodriguez-Amaya (1983) investigated the trapped volatiles of the soursop juice on Porapak Q by suction. Using low vacuum and fixed operating conditions, very high reproducibility was obtained (relative errors lower than 0.10%). Unfortunately, the 52 GC peaks detected were not identified.

The volatile constituents of Malaysian soursop were investigated by Wong and Khoo (1993), who isolated the volatiles by simultaneous steam distillation–solvent extraction using pentane as solvent in combination with capillary GC and GC-MS, identifying 51 constituents. The yield of total volatiles was estimated to be 7 mg/kg, and the presence of the various classes of compounds was as follows: aliphatic esters, 57.2%; lactones, 0.6%; aliphatic carbonyls, 1.6%; aliphatic alcohols, 11.2%;

terpenoids, 11.2%; nitrogen- and sulfur-containing compounds, 5.6%; and miscellaneous compounds, 10.5%. A comparison of the Malaysian and Sri Lankan soursop volatiles (MacLeod and Pieris 1981) indicated some similarities, but significant differences were observed. Methyl (*E*)-2-butenoate, reported to be the most abundant constituent (19.7% of GC area), was present at a concentration of 4.8% in the Sri Lankan fruit. The terpenoids in the Malaysian fruit comprised six monoterpenes, of which the most abundant was linalool (9.3%), whereas in the Sri Lankan fruit, only *trans*-β-farnesene (6.5%) was found.

In 1994, Pélissier and others analyzed the leaf, peel, and fruit pulp oils of soursop from Côte d'Ivoire. The volatile compounds were isolated by hydrodistillation using an oil trap. However, this is not a recommended way to collect volatiles from fruits, since many of them should be dissolved in the steam distillate. The oil yield for the pulp was 0.15% (v/m). The fruit oil contained 40 constituents, essentially aliphatic acids and esters, in particular, methyl (*E*)-2-hexenoate (39.8% of GC area).

Jirovetz and others (1998) isolated the essential oil of a Cameroonian fruit pulp by steam distillation. It is not clear if the authors used an oil trap or extracted the distillate with a solvent to collect the essential oil, but they reported a yield of 0.08% (m/m). In total, 53 constituents were identified by capillary GC and GC-MS. Esters of aliphatic acids were especially dominant (total amount ~51% of GC area), with methyl 2-hexenoate (23.9%), ethyl 2-hexenoate (8.6%), methyl 2-octenoate (5.4%), and methyl 2-butenoate (2.4%) as main compounds. Additional mono- and sesquiterpenes were highly concentrated in the essential oil. In comparison with previous published data, it seems noteworthy that aliphatic esters are the main constituents regardless of the fruit's origin. The olfactory data of the essential oil can be correlated as follows: the exotic-fruity odor impression is produced by the esters and the monoterpenes linalool and α-terpineol; the fatty notes are brought about by nonane, tetradecane, and esters of the hexadecanoic acid, respectively, while the green-sour-herbal notes are contributed by hexane derivatives.

Studying the flavor composition of a Cuban cultivar, Pino and others (2001) were able to determine 41 volatile constituents isolated by simultaneous steam distillation–solvent extraction using diethyl ether combined with capillary GC and GC-MS. The yield of total volatiles was estimated to be 94 mg/kg, and the major compounds were methyl cinnamate (10.6% of GC area), methyl (*E*)-2-hexenoate (8.8%), and methyl 2-hydroxy-4-methylvalerate (7.2%). Methyl cinnamate and 2-hydroxy-4-methylvalerate were not previously reported in soursop.

SUGAR APPLE

A. squamosa L. seems to be native to southeastern Mexico, in dry areas and until 1000 m, although it grows well in regions of medium humidity. It has spread throughout the tropics and displays great variability in India. The fruit is nearly round, ovoid, or conical, 6–10 cm in diameter, and weighs 100–230 g; its thick rind is composed of knobby segments, pale green, gray-green, bluish green, or, in one form, dull, deep pink externally (nearly always with a bloom); separating when the fruit is ripe and revealing the mass of conically segmented, white to light yellow, delightfully fragrant, juicy, sweet, delicious flesh (Morton 1987). It resembles and tastes like custard. Pulp is eaten fresh or made into sherbets and jams.

Sugar apple is propagated by seed with satisfactory results; however, commercial cultivars are grafted. According to Pinto (2006), the most important commercial cultivars are Red in United States; IPA Selections in Brazil; Noi in Thailand; Molate and Lobo in the Philippines; Cuban Seedless in Cuba; and Balanagar and Red Sitaphal in India. The main problems are seed-boring insects, the green leafhopper, the tendency toward mummification of the fruit, and harvesting and packaging difficulties caused by the fruit's lack of firmness (Mahdeem 1994).

Wong and Khoo (1993) were the first to investigate sugar apple flavor. Volatile compounds of the fruit from Malaysia were obtained by means of simultaneous steam distillation–solvent extraction using pentane. Compounds were analyzed by capillary GC and GC-MS. The amount of volatile material was estimated to be 6 mg/kg fruit pulp. Thirty-two volatiles were identified (95.4% of GC area), of which terpenoids constituted 88.5% of the fruit volatiles. Of the 13 monoterpenoids found, the most abundant were α-terpineol (5.1%), bornyl acetate (1.8%), 1,8-cineole (1.4%), and β-pinene (1.3%), whereas the sesquiterpene alcohols T-cadinol (37.0%), T-muurolol (12.4%), spathulenol (6.8%), and globulol (4.0%) showed the highest concentrations in this class.

In 1994, Pélissier and others analyzed the leaf, peel, and fruit pulp oils of sugar apple from Côte d'Ivoire. The volatile compounds were isolated by hydrodistillation using an oil trap and by maceration in dichloromethane. The fruit oil contained spathulenol (17.8% and 21.8% using hydrodistillation and solvent extraction, respectively) and germacrene D (9.6% and 2.1%, respectively) as major constituents.

After simultaneous steam distillation–solvent extraction with pentane of the fruit pulp from Brazil, the volatile compounds were subjected to GC-MS analysis (Andrade et al. 2001). A total of 27 monoterpenoids (75.5% of GC area) and sesquiterpenoids (21.6%) were identified. The major monoterpenoids were α-pinene (25.3%), sabinene (22.7%), limonene (10.1%), and (*E*)-β-ocimene (7.2%), whereas spathulenol (6.3%), germacrene D (6.0%), and bicyclogermacrene (3.5%) showed the highest concentrations in the sesquiterpenoid compounds. A comparison of the fruit aroma results with those obtained for a Malaysian specimen of sugar apple (Wong and Khoo 1993) indicated similarities, because terpenoids predominated in both specimens. On the other hand, the quantitative values are quite distinguishable. According to Wong and Khoo (1993), the constituents found in the Malaysian specimen were mainly sesquiterpenes (65.6%), including epi-α-cadinol that accounted for 37.0%. In the Amazonian specimen, epi-α-cadinol occurred only with the low content of 1.4%, contrasting with the high value of 25.3% of α-pinene. In the Malaysian specimen, this monoterpene occurs only with 0.8%. The reason for the quantitative differences is, in our opinion, based on the methodologies used to obtain the volatile compounds. In the case of the Malaysian specimen, the authors concentrated the extract previous to the analysis, while in the Amazonian specimen, the extract was not concentrated.

CUSTARD APPLE

Although it is said that *A. reticulata* L. is a native of the Antilles, the presence in Guatemala and Belize of a wild variety, *A. reticulata* var. primigenia, and also of a

very wide variability of cultivars suggests that this zone is the species' area of origin. It has been introduced in other regions of the American tropics and Southeast Asia, without achieving a level of importance comparable to that of *A. cherimola* or *A. squamosa* (Mahdeem 1994). Its altitude limit in Central America is 1500 m.

The fruit is heart shaped or spherical and 8–15 cm in diameter, and the weight may be up to 0.5 kg. According to the cultivar, the flesh varies from juicy and very aromatic to hard with a repulsive taste. The flavor is sweet and agreeable though without the distinct character of the cherimoya, sugar apple, or atemoya (Morton 1987). Both the outside and inside colors vary according to the cultivar. Pulp is eaten fresh or made into sherbets and jams. Superior cultivars in Florida (United States) have been selected, especially from Belize and Guatemala: Tikal, Canul, Sartenaya, San Pablo, Benque, Caledonia, and Chonox. In Central America, important cultivars are *primigenia* and *lutescens*. Two probable causes seem to be the reason for the marginalization of this species: reproduction by seed, which results in many trees producing much smaller fruit; and the attack of the seed weevil that lays its eggs in the young fruit. When the adult insect develops, it bores tunnels through the flesh, causing mycotic infections and subsequent deterioration of the fruit (Mahdeem 1994).

Wong and Khoo (1993) were the first to examine custard apple volatiles and identified 47 constituents (92.3% of the GC area) in fruits from Malaysia. Volatiles were isolated by simultaneous steam distillation–solvent extraction using pentane as solvent in combination with capillary GC and GC-MS. The yield of total volatiles was estimated as 9 mg/kg fruit pulp. Like the sugar apple or sweetsop (*A. squamosa*) volatiles, the compounds of custard apple contained mainly terpenoids (98.3%). While sesquiterpenoids predominated in the sugar apple, monoterpenoids accounted for 91.2% of the total volatiles of the custard apple. The most abundant volatiles were terpinen-4-ol (70.5%) and α-terpineol (10%).

Studying the flavor composition of an unknown Cuban cultivar, Pino (2000) found 49 volatile compounds at a total level of 109 mg/kg fruit pulp, 27 of them were reported for the first time. Mono- and sesquiterpenoid hydrocarbons comprised the largest class of compounds (51.7% and 19.9%, respectively). The composition of other classes of compounds was as follows: aliphatic esters, 0.2%; oxygenated monoterpenoids, 4.9%; oxygenated sesquiterpenoids, 1.4%; acids, 1.7%; and diterpenes, 5.9%. The detection of diterpenes is of interest, because kauren-19-ol was reported in the previous study (Wong and Khoo 1993), and they were not reported in other *Annona* fruits.

Augusto and others (2000) identified 21 volatile compounds in a frozen integral pulp of Brazilian custard apple. Compounds were isolated by solid phase microextraction using different types of fibers. The authors claimed that the prevailing compounds in this fruit were α-unsaturated methyl esters of the type $R–CH=CH–COOCH_3$ (R = ethyl, butyl, or hexyl), as methyl crotonate (R = ethyl), methyl 2-hexenoate (R = butyl), and methyl 2-octenoate (R = hexyl), as well as aliphatic esters of butyric and hexanoic acids and ethanol. Earlier studies of the volatile fraction did not report these compounds.

Recently, Pino and others (2003) studied the flavor composition of four important commercial Cuban cultivars (Cenizo, Rojo, Verde, and De Ojo). Volatile compounds were isolated by simultaneous steam distillation–solvent extraction using diethyl ether as solvent and identified by capillary GC and GC-MS. One hundred and eighty

compounds were reported in the fruit pulp, of which α-pinene (50–100 mg/kg), β-pinene (71–97 mg/kg), myrcene (12–20 mg/kg), limonene (14–20 mg/kg), terpinen-4-ol (1–22 mg/kg), and germacrene D (10–27 mg/kg) were the major constituents. Fruits from cv. De Ojo containing the highest concentration of total volatiles and the highest terpenoid content had the highest custard-like and overall fruity aroma intensity. The presence of many terpenoids is thought to contribute to the unique flavor of custard apple.

POND APPLE

A. glabra L. is native to the West Indies and Florida and is common in the Everglades. It grows in swamps, is tolerant of salt water, and cannot grow in dry soil. The fruit is oblong to spherical and apple sized or larger, 7–15 cm long and up to 9 cm in diameter, and falls when it is green or ripening yellow. It is edible for humans and can be made into jam, although the taste is usually not preferred to soursop's and other related fruits. The flesh is sweet scented and agreeable in flavor, but so strongly narcotic that it has never attained general popular use.

The volatile compounds of the fruits in three different maturity stages, growing wild in North Brazil, were isolated by simultaneous steam distillation–solvent extraction using pentane and analyzed by GC-MS (Santos et al. 1998). A total of 61 compounds were identified, from which the most abundant were terpenoids. The major components found were α-pinene (unripe, 18.5%; half-ripe, 11.3%; ripe, 15.0%), limonene (unripe, 20.0%; half-ripe, 20.7%; ripe, 20.6%), α-phellandrene (unripe, 21.0%; half-ripe, 1.2%; ripe, 2.0%) and (*E*)-β-ocimene (unripe, 15.8%; half-ripe, 19.7%; ripe, 18.3%). The half-ripe and ripe fruit specimens compared with the unripe fruit specimen showed a significant difference in the content of α-phellandrene. The identified alcohols, ketones, and esters occurred only in ripe and half-ripe fruits, and the authors concluded that they play an important role in the characteristic custard-like flavor of this fruit.

Volatile compounds of pond apple from Cuba were isolated by steam distillation–solvent extraction using diethyl ether (Pino et al. 2002a). The yield of total volatiles was estimated as 887.9 mg/kg fruit pulp. Seventy-eight compounds were identified for the first time as pond apple volatiles. Mono- and sesquiterpene hydrocarbons comprised the largest class of volatiles. Some 2-hydroxy and 3-hydroxy esters were also present but in lesser quantities. Major constituents were myrcene (47.1% of GC area), (*Z*)-β-ocimene (16.3%), limonene (11.2%), and α-pinene (9.5%).

WILD SOURSOP

A. montana Macfad. is said to be native to Tropical America (Roig 1988). It grows wild in the Amazon, Central America, and West Indies. It is a small tree, common in muddy lands, very similar to the common soursop (*A. muricata* L.), but with larger leaves and smaller, round fruits, 10–15 cm in diameter that turn yellow when ripe. The whitish to reddish pulp is somewhat tasteless and astringent. It is edible for humans and can be made into jam, although the taste is usually not preferred to that of soursop and other related fruits.

In the volatile extract obtained by simultaneous steam distillation–solvent extraction using diethyl ether, 58 compounds were identified (Pino et al. 2002b). The yield of total volatiles was estimated to be 137.7 mg/kg fruit pulp. Aliphatic ester comprised the largest class of constituents, with major representatives of 3-methyl-2-butenyl acetate (16.7% of GC area), methyl octanoate (15.6%), and methyl hexanoate (7.5%). An aliphatic alcohol, 3-methyl-3-butenol, was also present in a large amount (9.6%).

ATEMOYA

The name atemoya, derived from "ata" (in Portuguese) and "cherimoya," is given to hybrids of the species *A. squamosa* and *A. cherimola*. The fruit is conical or heart shaped, generally 10 cm long and 9.5 cm wide, some weighing as much as 2.25 kg; pale bluish green or pea green, and slightly yellowish between the areoles. The fragrant flesh is snowy white, of fine texture, almost solid, not conspicuously divided into segments, with fewer seeds than the sugar apple, and sweet and subacid at the same time, resembling the cherimoya in flavor (Morton 1987). Pulp is eaten fresh or used in the preparation of nectars, drinks, sherbets, ice cream, syrups, and cakes.

Known are several cultivars that are grown commercially in the United States (Florida), Australia, Israel, South Africa, the Philippines, and numerous parts of Central and South America. According to Nakasone and Paull (1998), the most important commercial cultivars are African Pride (South Africa, Israel, and Australia), Bradley and Page (California and Florida, respectively), Jennifer, Kabri, and Malalai (Israel); and finally, Nielsen, Island Gem, and Pink's Mammoth (Australia). The best atemoyas combine adaptation to low altitudes and hot climate and the high productivity and good flavor of *A. squamosa* with the firm skin, low flesh/seed ratio, and flavor of *A. cherimola* so that, from the standpoint of quality and packaging, the product is comparable to the best cherimoyas, although it has higher sugar content (Mahdeem 1994).

The volatiles of fresh custard apple (*A. atemoya* cv. African Pride) were separated by simultaneous steam distillation–solvent extraction and analyzed by capillary GC and GC-MS (Wyllie et al. 1987). All of the 37 compounds identified were mono- or sesquiterpenoids with α-pinene (25.6% of GC area), β-pinene (21.0%), bicyclogermacrene (14.6%), and germacrene D (10.9%) constituting the major components. No marked change in the composition of the volatiles was observed during ripening.

In the same year, volatile compounds of Australian atemoya were isolated by both trapping on Tenax GC followed by thermal desorption and simultaneous steam distillation–solvent extraction using pentane (simultaneous distillation–extraction [SDE]) (Bartley 1987). Analysis by capillary GC led to the characterization of 40 components. Twenty components were identified in the headspace sample, and 94% of the GC peak area consisted of esters. The major components were methyl butanoate (36.5%), ethyl butanoate (27.3%), and methyl hexanoate (12.1%). All 25 components detected in the SDE extract were terpenes, major components being germacrene D (18.2%), α-pinene (14.4%), (*E*)-β-ocimene (17.7%), and β-pinene (11.6%). The reason for the quantitative differences is, in our opinion, based on the methodologies used to obtain the volatiles.

Pino and Rosado (1999) evaluated the volatile components of Cuban atemoya using simultaneous steam distillation–solvent extraction with diethyl ether. Fifty-three compounds were identified by means of GC-MS, 29 of them reported for the first time. The yield of total volatiles was estimated as 240.6 mg/kg fruit pulp. Mono- and sesquiterpene hydrocarbons comprised the largest class of compounds and accounted for approximately 77% of the total volatiles. The major components were α-pinene (28.9%), limonene (18.6%), and β-pinene (11.7%). In general terms, though not in detailed composition, percentage figures of volatiles of the Cuban specimen studied were similar to those found in specimens grown in Australia (Bartley 1987; Wyllie et al. 1987). While aliphatic esters were reported in significant amounts in one Australian specimen (Bartley 1987), no evidence of this class of compounds was found, with the exception of the new ones, ethyl acetate and 2-methylbutyl acetate.

WILD CUSTARD APPLE

A. chrysophylla Boj. (syn. *A. senegalensis* Auct. non Pers., often cited erroneously as *A. senegalensis* Pers.) is a spreading shrub or small, semideciduous tree, commonly found in the savanna region of West Africa. There is no precise information on the origin and diversity of wild custard apple. The compound fruit is pineapple scented and smooth, but with the carpers distinctly outlined on the surface. It has an ovate, globose, or subglobose form, measuring 2.5–5 cm in length and 2.5–4 cm in width. The fruit is yellow or orange when ripe. The fruit pulp is edible and said to have an apricot-like flavor (Morton 1987; Pinto et al. 2005). Fruits are sold in local markets in Africa.

The chemical composition of the fruit oil isolated by steam distillation was determined by capillary GC and GC-MS (Ekundayo and Oguntimein 1986). The oil yield was estimated to be 0.06% fruit pulp. Nineteen compounds were identified, mainly terpenoids, of which δ-3-carene (22.1%) and sabinene (11.1%) were the most abundant.

CONCLUDING REMARKS

Several of the annona fruits discussed here, especially cherimoya, soursop, and sugar apple, have great potential for expanded participation in the world market of fresh fruit and industrially processed products. The lesser known fruits, such as custard apple, wild soursop, wild custard apple, and other species not discussed, have limited importance for consumption as fresh fruit. However, the expansion of the cultivated areas of *Annona* species is still limited, except for cherimoya in China, Taiwan, Spain, and Chile; soursop in Brazil and Mexico; and sugar apple in India.

It has been observed that tropical fruits can be classified into two broad categories determined by whether esters or terpenoids predominate in the volatiles (MacLeod and Ames 1990). On this basis, therefore, the cherimoya, soursop, and wild soursop belong to the first category, whereas the sweetsop, sugar apple, custard apple, pond apple, atemoya, and wild custard apple fit into the second category.

The full natural flavor of *Annona* fruits still awaits elucidation. An important step toward this goal is the identification of sensorially active compounds by capillary

GC–sniffing techniques and/or the application of the concept of odor units (Plutowska and Wardencki 2007; Schieberle 1995). Further systematic studies of the sensory properties of the volatile constituents of these fruits are needed.

REFERENCES

Alix C. 1999. *Frutales y condimentarias del trópico*. La Ceiba, Honduras: UNAH/CURLA-PROFORFITH.

Anderson P, Richardson A. 1990. Which cherimoya cultivar is best? *Orchardist N Z* 63(11):17–9.

Andrade EHA, Zoghbi MGB, Maia JGS, Fabricius H, Marx F. 2001. Chemical characterization of the fruit of *Annona squamosa* L. occurring in the Amazon. *J Food Compos Anal* 14:227–32.

Augusto F, Valente ALP, Tada EDS, Rivellino SR. 2000. Screening of Brazilian fruit aromas using solid-phase microextraction–gas chromatography–mass spectrometry. *J Chromatogr A* 873:117–27.

Bailey LH. 1949. *Manual of Cultivated Plants*. New York: Macmillan.

Bartley JP. 1987. Volatile constituents of custard apple. *Chromatographia* 23:129–31.

Campbell RJ. 1996. South American fruits deserving further attention. In Janic J (ed.), *Progress in New Crops*. Arlington, VA: ASHS Press, pp. 431–9.

Chatrou LW. 1998. Changing genera: Systematic studies in Neotropical and West African Annonaceae. PhD dissertation, Utrecht University, Utrecht.

Ekundayo O. 1989. A review of the volatiles of the Annonaceae. *J Essent Oil Res* 1(5):223–45.

Ekundayo O, Oguntimein B. 1986. Composition of the essential oils of *Annona senegalensis* var. senegalensis. *Planta Med* 52:202–4.

Franco MRB, Rodriguez-Amaya DB. 1983. Trapping of coursop (*Annona muricata*) juice volatiles on Porapak Q by suction. *J Sci Food Agric* 34:293–9.

Fries RE. 1959. Annonaceae. In Engler A, Prantl K (eds.), *Die Natürlichen Pflanzenfamilien*, 2nd Ed., Band 17a II. Berlin: Duncker und Humblot, pp. 1–171.

Gardiazabal I. 1993. *El cultivo del chirimoyo*. Valparaíso, Chile: Ediciones Universitarias de Valparaíso, pp. 42–3.

Geurts F. 1981. *Annonaceous Fruits*. Amsterdam: Royal Tropical Institute.

Guirado E, Hermoso JM, Pérez de Oteyza MA, Farré JM. 2003. *Introducción al cultivo del chirimoyo*. Granada, Spain: Caja Rural de Granada.

Herres W, Idstein H, Schreier P. 1983. Analysis by HRGC/FT-IR: Cherimoya (*Annona cherimola* Mill.) fruit volatiles. *J High Resolut Chromatogr Chromatogr Commun* 6:590–4.

Idstein H, Bauer C, Schreier P. 1985. Flüchtige Säuren in Tropenfrüchten: Cherimoya (*Annona cherimolia*, Mill.), guava (*Psidium guajava*, L.), mango (*Mangifera indica*, L., var. Alphonso), papaya (*Carica papaya*, L.). *Z Lebensm Unters Forsch* 180:394–7.

Idstein H, Herres W, Schreier P. 1984. High resolution gas chromatography-mass spectrometry and Fourier transform infrared analysis of cherimoya (*Annona cherimolia* Mill.) volatiles. *J Agric Food Chem* 32:383–9.

Iwaoka WT, Zhang X, Hamilton RA, Chia CL, Tang CS. 1993. Identifying volatiles in soursop and comparing their changing profiles during ripening. *HortScience* 28(8):817–9.

Jirovetz L, Buchbauer G, Ngassoum MB. 1998. Essential oil compounds of the *Annona muricata* fresh fruit pulp from Cameroon. *J Agric Food Chem* 46:3719–20.

Kessler PJA. 1993. Annonaceae. In Kubitzki K, Rohwer JG, Bittrich V (eds.), *The Families and Genera of Vascular Plants. II. Flowering Plants—Dicotyledons*. Berlin: Springer-Verlag, pp. 93–104.

Koek-Noorman J, Westra LYTH, Maas PJM. 1990. Studies in Annonaceae. XIII. The role of morphological characters in subsequent classifications of Annonaceae: A comparative survey. *Taxon* 39(1):16–32.

León J. 1987. *Botánica de los Cultivos Tropicales*. San José, Costa Rica: IICA.

Lizana LA, Reginato G. 1990. Cherimoya. In Nagy S, Shaw PE, Wardowski WF (eds.), *Fruits of Tropical and Subtropical Origin: Composition, Properties and Uses*. Lake Alfred, FL: Florida Science Source, pp. 131–48.

Lüdders P. 2002. Cherimoya (*Annona cherimola* Mill.)—Botany, cultivation, storage and uses of a tropical-subtropical fruit. *Erwerbsobstbau* 44(4):122–6.

MacLeod AJ, Pieris NM. 1981. Volatile flavor components of soursop (*Annona muricata*). *J Agric Food Chem* 29:488–90.

MacLeod G, Ames JM. 1990. Volatile components of starfruit. *Phytochemistry* 29:165–72.

Mahdeem H. 1994. Custard apples. In Hernándo Bermejo JE, León J (eds.), *Neglected Crops: 1492 from a Different Perspective*. Rome: FAO, pp. 85–92.

Morton J. 1987. *Fruits of Warm Climates*. Winterville: NC: Media, pp. 65–9.

Murrillo J. 2001. Las Annonaceae de *Colombia. Biota Colombiana* 2(1):49–58.

Nakasone HY, Paull RE. 1998. Annona. In Nakasone HY, Paull RE (eds.), *Tropical Fruits*. London: CAB International, pp. 45–75.

Nijssen LM, Visscher CA, Maarse H, Willemsens L, Boelens M. 1996. *Volatile Compounds in Food. Qualitative and Quantitative Data*, Vol. 1. Zeist, The Netherlands: TNO-CIVO Food Analysis Institute.

Ochse JJ, Soule MJ Jr., Dijkman MJ, Wehlburg C. 1974. Otros cultivos frutales. In *Cultivo y Mejoramiento de Plantas*. México DF: Editorial Limusa, pp. 587–818.

Paull RE. 1982. Postarvest variation in composition of soursop (*A. muricata* L.) fruit in relation to respiration and ethylene production. *J Am Soc Hortic Sci* 107:582–5.

Paull RE, Deputy I, Chen NJ. 1983. Changes in organic acids, sugars, and headspace volatiles during fruit ripening of soursop (*A. muricata* L.). *J Am Soc Hortic Sci* 108:931–4.

Pélissier Y, Marion CH, Kone D, Lamaty G, Menut CH, Bessière J-M. 1994. Volatile components of *Annona muricata* L. *J Essent Oil Res* 6(4):411–4.

Pérez de Oteyza MA. 2002. El banco español de germoplasma de chirimoyo. Un poco de historia y situación actual. Resúmenes Tercer Congreso Internacional de Anonáceas, Quillota, Chile.

Peters M, Badrie N, Comissiong E. 2001. Processing and quality evaluation of soursop (*Annona muricata* L.) nectar. *J Food Qual* 24:361–74.

Pino JA. 1997. Los constituyentes volátiles de las frutas tropicales. I. Frutas de las especies de *Annona*. *Alimentaria* (286):19–26.

Pino JA. 2000. Volatile components of Cuban *Annona* fruits. *J Essent Oil Res* 11(5):613–6.

Pino JA, Agüero J, Marbot R. 2001. Volatile components of soursop (*Annona muricata* L.). *J Essent Oil Res* 13:140–1.

Pino JA, Marbot R, Agüero J. 2002a. Volatile components of baga (*Annona glabra* L.) fruit. *J Essent Oil Res* 14:252–3.

Pino JA, Marbot R, Agüero J. 2002b. Volatile components of wild soursop (*Annona montana* Macf.) fruit. *J Essent Oil Res* 14:257–8.

Pino JA, Marbot R, Fuentes V. 2003. Characterization of volatiles in bullock's heart (*Annona reticulata* L.) fruit cultivars from Cuba. *J Agric Food Chem* 51:3836–9.

Pino JA, Rosado A. 1999. Volatile constituents of custard apple (*Annona atemoya*). *J Essent Oil Res* 11:303–5.

Pinto AC de Q. 2006. *Annona. Field Manual for Extension Workers and Farmers*. Southampton, UK: International Centre for Underutilised Crops, University of Southampton.

Pinto AC de Q, Cordeiro MCR, de Andrade SRM, Ferreira FR, Filgueiras HA de C, Alves RE, Kinpara DI. 2005. *Annona Species*. Southampton, UK: International Centre for Underutilised Crops, University of Southampton.

Plutowska B, Wardencki W. 2007. Aromagrams—Aromatic profiles in the appreciation of food quality. *Food Chem* 101:845–72.

Popenoe W. 1974. The annonaceous fruits: The cherimoya. In *Manual of Tropical and Subtropical Fruits*, Facsimile of the 1920 edition. London: Hafner Press, pp. 161–89.

Rasai S, George AP, Kantharajah AS. 1995. Tissue culture of *Annona* spp. (cherimoya, atemoya, sugar apple and soursop): A review. *Sci Hortic* 62:1–14.

Roig JT. 1988. *Diccionario Botánico de Nombres Vulgares Cubanos*. La Habana: Editorial Cientifico-Técnica.

Sanewski G. 1991. *Custard Apples, Cultivation and Crop Protection*. Brisbane, Australia: Queensland Department of Primary Industries.

Santos AS, Andrade EHA, Zoghbi MGB, Maia JGS. 1998. Volatile constituents of fruits of *Annona glabra* L. from Brazil. *Flav Frag J* 13:148–50.

Satney A. 1994. Marketing of selected minor fruit crops. In *Third Regional Workshop on Tropical Fruits in Grenada*. Port-of-Spain, Trinidad and Tobago: Inter-American Institute for Cooperation on Agriculture, IICA Office, pp. 120–2.

Schieberle P. 1995. Recent developments in methods for analysis of flavor compounds and their precursors. In Goankar A (ed.), *Characterization of Food: Emerging Methods*. Amsterdam: Elsevier, pp. 403–31.

Toulemonde B, Beauverd D. 1985. Headspace analysis: Trap desorption by microwave energy. Application to the volatile components of some tropical fruits. In Adda J (ed.), *Progress in Flavor Research*. Amsterdam: Elsevier Publishers B.V., pp. 533–48.

Wong KC, Khoo KH. 1993. Volatile constituents of Malaysian *Annona* fruits. *Flav Frag J* 8:5–10.

Wyllie SG, Cook D, Brophy JJ, Richter KM. 1987. Volatile flavor components of *Annona atemoya* (custard apple). *J Agric Food Chem* 35:770–4.

CHAPTER 15

Apple (*Malus* × *domestica* Borkh.)

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INTRODUCTION

Apple is one of the major fruit crops produced in the world, with a total production of more than 40 million metric tons worldwide. The annual per capita consumption of apples throughout the world is estimated to be about 10 kg. More than 7500 varieties of apples are grown throughout the world, with the top apple cultivars being "Red Delicious," "Golden Delicious," "Granny Smith," "Gala," and "Fuji" (USDA 2004).

Controlled atmosphere (CA) storage of apples has become very popular throughout the world as it offers greater guarantees for maintaining fruit quality (Dixon and Hewett 2001). Consumer demand for high-quality stored apples has increased in recent decades, and this is a continuing trend (Van Zeebroeck et al. 2007). Fresh apple quality involves many aspects, including appearance, color, texture, flavor, and nutritional value (Harb et al. 2008). Numerous developments in preharvest and postharvest technologies have extended the availability of fresh apples by improving their appearance and standard quality, but they have often neglected flavor. A clearer understanding of how cultivar, maturity stage, and postharvest technologies affect apple flavor would therefore provide new opportunities for improving overall quality and thereby meeting consumer demands (Kader 2004).

The importance of flavor cannot be overstressed; flavor is lost long before visual qualities deteriorate to undesirable levels (Pelayo et al. 2003). This largely explains why success in extending storage life has hitherto failed to include extending the life of flavor. However, flavor components, and especially aroma, play a key role when producers want their products to offer extra value. The main reason why aroma has not previously been included in standard descriptions of postharvest quality—and indeed in assessments of quality in general—is because of the complexity of measuring this parameter.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

This chapter focuses on research work carried out in recent years in areas associated with the quality of postharvest flavor and examines the influence of different factors that affect apple flavor.

COMPONENTS INFLUENCING APPLE FLAVOR

Flavor is one of the most important quality traits in apple. Although the initial selection of a fruit by the consumer is principally determined by the visual appearance of the product, satisfaction and repeat purchase are mainly determined by flavor (Schotsmans and Prange 2006). Flavor is derived from a collective mosaic involving numerous compounds that influence odor and taste (Kays and Wang 2000).

Human perception of flavor is exceedingly complex. During oral processing and after swallowing, volatile aroma compounds are released from the food matrix and into the air, thus permitting their flow to the olfactory epithelium where they interact with olfactory receptors. During this process, the different senses interact in a nonlinear way and several cross-modal phenomena take place, with texture and taste influencing the perception of the aroma of the food product, and vice versa (Hornung and Enns 1994; Negoias et al. 2008; Noble et al. 1991).

Taste implies the detection of nonvolatile compounds (in concentrations of parts per hundred) using several types of receptors that are mainly on the tongue, but are also located on the cheeks, soft palate, and throat. Only five basic sensations determine taste: sweet, sour, salty, bitter, and umami, whereas up to 10,000 odors can be discriminated by the human olfactory system (Kays and Wang 2000). The perception of sweetness, for example of sugars, one of the most important components of apple flavor, is modified by sourness or acidity levels, and aroma compounds (Durán and Costell 1999).

Aroma volatile compounds can be detected in parts per trillion (ppt) concentrations, and aroma stimuli usually reach the olfactory epithelium via two separate pathways: the nose, during sniffing, and the mouth, during eating (Negoias et al. 2008). Three major chemosensory systems are involved in the perception of these stimuli: smell, taste, and trigeminally mediated sensations. The volatile compounds of fruits only rarely exceed concentrations of 10 ppm, and the intensity and quality of aroma are not necessarily determined by the compounds that are present in the highest proportions. The contribution of a specific aroma compound to flavor is therefore expressed in odor units (aroma value), which is the ratio of this concentration to its detection threshold (Takeoka et al. 1992). Volatile compounds with positive odor units are assumed to contribute to the flavor of a food, while those with negative units may still contribute to overall food flavor as background notes (Buttery 1993).

Gas chromatography (GC) and mass spectrometry have made it possible to identify more than 300 volatile compounds present in different apple cultivars (Paillard 1990) including alcohols, aldehydes, esters, ketones, and ethers (Dimick and Hoskin 1983), but the most important of these are esters (78–92 g/100 g) and alcohols (6–16 g/100 g) (Dixon and Hewett 2001). Several studies have sought to quantify detection thresholds and describe the odor of volatile compounds using GC–olfactometry and about 15–20 compounds have been identified as the principal contributors to apple aroma in different cultivars (Lo Scalzo et al. 2003; Mehinagic et al. 2006; Plotto et al. 2000; Rizzolo et al. 1989; Wang et al. 2005). Table 15.1 shows

TABLE 15.1. Volatile Compounds Emitted by Six Apple Varieties at Commercial Harvest Date

Volatile Compounds		Odor Detection Threshold	Starking Delicious 170 Days after Full Bloom		Golden Delicious 160 Days after Full Bloom		Granny Smith 183 Days after Full Bloom		Fuji Nagafu 6 195 Days after Full Bloom		Mondial Gala 120 Days after Full Bloom		Pink Lady 215 Days after Full Bloom	
N[0] Esters	Odor Descriptor	(µg/kg)	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit
1 Ethyl acetate	Ethereal-fruity[a]	13,500[j]	655.8	0.049	42.2	0.003	15.1	0.001	4.8	0.0003	17.8	0.001	2.8	0.00003
2 Propyl acetate	Pear-raspberry[b]	2000[j]	36.6	0.018	31.2	0.016	53.8	0.027	2.3	0.001	12.1	0.005	1.5	0.001
3 2-Methylpropyl acetate	fruity[b]	65[k]	23.6	0.363	9.4	0.145	24.8	0.382	2.9	0.045	21.9	0.337	3.0	0.046
4 Butyl acetate	Red apple[c]	66[j]	39.8	0.603	9.6	0.145	30.2	0.458	9	0.136	577.8	8.754	21.2	0.321
5 2-Methylbutyl acetate	Banana-apple[a]	11[k]	76.3	6.936	8.4	0.764	5.1	0.464	197.1	17.918	284.9	25.900	50.3	4.573
6 Pentyl acetate	Apple, fruity[d]	43[j]	ND		5.2	0.121	13	0.302	2.4	0.056	64.3	1.495	3.3	0.077
7 Hexyl acetate	Fruity[a]	2[l]	50	25	8.3	4.150	9.7	4.850	12.9	6.450	342.5	171.250	68.8	34.400
8 Ethyl propanoate	Sweet-apple[e]	10[k]	97.9	9.790	37.5	3.750	64.9	6.490	ND		ND		ND	
9 *tert*-Butyl propanoate		19[m]	385.9	20.310	3.8	0.200	10.9	0.574	0.8	0.042	6.9	0.363	0.9	0.047
10 Butyl propanoate	Faintly sweet[b]	25[g]	ND		ND		ND		11.7	0.468	17.9	0.716	9.4	0.376
11 2-Methylbutyl propanoate		19[m]	ND		ND		ND		2.8	0.147	5.0	0.263	6.8	0.358
12 Hexyl propanoate	Apple[f]	8[n]	ND		ND		ND		4.5	0.563	21.0	2.625	26	3.250
13 Ethyl butanoate	Fruity[d]	1[o]	500.7	500.700	<0.5		ND		<0.5		4.5	4.500	1.6	1.600
14 Butyl butanoate	Rotten Apple[f]	100[p]	7.2	0.072	<0.5		ND		2.9	0.029	24.8	0.248	6.1	0.061

TABLE 15.1. ***Continued***

Volatile Compounds		Odor Detection Threshold	Starking Delicious 170 Days after Full Bloom		Golden Delicious 160 Days after Full Bloom		Granny Smith 183 Days after Full Bloom		Fuji Nagafu 6 195 Days after Full Bloom		Mondial Gala 120 Days after Full Bloom		Pink Lady 215 Days after Full Bloom	
N[0] Esters	Odor Descriptor	(µg/kg)	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit	(µg/kg)	Odor Unit
15 Hexyl butanoate	Apple[f]	250[k]	3.8	0.015	ND		ND		3.4	0.014	64.0	0.256	35.9	0.144
16 Ethyl 2-methylbutanoate	Ripe Apple[e]	0.006[k]	928.9	154,816.6	8.6	1433.33	10.7	178.333	121.2	20,200	4.4	733.300	2.8	466.600
17 Butyl 2-methylbutanoate	Apple, fruity[f]	17[p]	ND		ND		ND		4.9	0.288	8.6	0.506	9.8	0.576
18 2-Methylbutyl 2-methylbutanoate	Fruity[b]	24[b]	8.5	0.354	ND		ND		ND		ND		ND	
19 Hexyl 2-methylbutanoate	Flesh-green Fruity[a]	6[p]	5.1	0.850	3.7	0.617	8.3	1.383	7.6	1.267	21.7	3.616	59.7	9.95
20 Ethyl hexanoate	Fruity[h]	1[k]	116.6	116.6	4.4	4.400	ND		1.2	1.200	ND		1.7	1.700
21 Butyl hexanoate	Green Apple[f]	700[p]	ND		ND		ND		4.6	0.007	43.5	0.062	16	0.023
22 Hexyl hexanoate	Apple[f]	6400[b]	ND		ND		ND		ND		39.7	0.006	70.0	0.011
Alcohols														
23 Ethanol	Slight[b]	100,000[g]	14.6	0.00015	ND		4.3	0.00004	4.2	0.00004	14.1	0.0001	ND	
24 1-Propanol	Sweet[a]	9000[g]	10.2	0.001	6.6	0.001	9.2	0.001	1.2	0.00013	10.4	0.001	0.5	0.00006
25 2-Methyl-1-propanol	Chemical[i]	250[l]	6.5	0.026	6.2	0.025	18.9	0.076	1	0.004	18.5	0.074	0.9	0.004
26 1-Butanol	Sweet aroma[d]	500[g]	ND		<0.5		ND		2.4	0.005	238.6	0.477	1.4	0.003

27	4-Methyl-2-pentanol			8.1		<0.5		ND		0.6		ND		<0.5	
28	2-Methyl-1-butanol	Pleasant[d]	250[o]	5.2	0.021	ND		ND		29	0.116	40.4	0.161	2.0	0.008
29	1-Pentanol	Fusel-like sweet[b]	4000[l]	ND		ND		ND		0.5	0.00012	9.3	0.002	0.5	0.0001
30	1-Hexanol	Grassy[a]	500[l]	8.3	0.017	ND		ND		3.3	0.007	26.9	0.053	1.7	0.003
31	2-Hexen-l-ol	Green[b]	6700[n]	ND		ND		ND		ND		ND		ND	
Aldehydes, terpenes, and terpenoids															
32	Hexanal	Green[c,d]	10.5[o]	ND		ND		ND		0.5	0.048	ND		ND	
33	D-limonene	citruslike[h]	34[o]	ND		ND		ND		0.8	0.024	1.5	0.044	0.5	0.015
34	Linalool	Floral[b]	4[b]	4.9	1.255	ND		ND		ND		ND		ND	
35	α-Terpineol	Lilac odor[b]	280[b]	6.7	0.024	ND		ND		ND		ND		ND	
36	Nerol	Rose fragrance	680[b]	10.6	0.016	ND		13.4	0.020	ND		ND		ND	
Total				3011.8	639,921.6	185.1	13,735.4	292.3	191,058.3	440.4	47037.0	1943.0	951,443.6	405.1	512,135.0

[a]Dimick and Hoskin (1983).
[b]Burdock (2002).
[c]Young and others (1996).
[d]Rizzolo and others (1989).
[e]Komthong and others (2006).
[f]Plotto (1998).
[g]Flath and others (1967).
[h]Buettner and Schieberle (2001).
[i]Rizzolo and others (1997).
[j]Takeoka and others (1996).
[k]Takeoka and others (1992).
[l]Buttery (1993).
[m]Schnabel and others (1988).
[n]Van Gemert and Nettenbreijer (1977).
[o]Rychlik and others (1998)
[p]Takeoka and others (1990).
ND, not detected.

the thirty-six volatile compounds emitted by six apple varieties at the commercial harvest date. Ester compounds respectively represented more than 81%, 85%, 90%, 93%, 98%, and 98% of total volatile compounds in "Mondial Gala," "Granny Smith," "Fuji," "Golden Delicious," "Starking Delicious," and "Pink Lady" cultivars.

Esters have been identified as being responsible for overall aroma in apple extracts by analyses involving dynamic headspace (Plotto et al. 1999) and vacuum hydrodistillation (Mehinagic et al. 2006). Acetates, propionates, butanoates, and hexanoates are the most important esters contributing to apple odor in such cultivars as "Golden Delicious" and "Granny Smith" (López et al. 1998a), "Starking Delicious" (López et al. 1998b), "Royal Gala" and "Red Gala" (Lo Scalzo et al. 2003), and "Fuji" (Echeverría et al. 2004a). Ethyl 2-methylbutanoate has been identified as a key aroma impact compound in several fruits, including apples (Flath et al. 1967; Kollmannsberger and Berger 1992; Paillard 1990), and also in synthetic apple juices (Diirr and Schobinger 1981) and apple essences (Paillard 1990). Ethyl 2-methylbutanoate has an aroma threshold of 0.006 μg/kg with an intense odor that has been characterized as "apple like," "fruity," and "giving an impression of ripeness" (Paillard 1990). 2-Methylbutyl acetate has been identified as the major ester in the flesh of "Rome" (Fellman et al. 1993) and "Fuji" (Echeverría et al. 2004a,b) apples.

Alcohols are the second most important group of organic compounds in terms of contribution to apple flavor. They may even become the most important group if distillation methods are selected for the extraction of apple essences. Knee and Hatfield (1976) observed that alcohols, and particularly 1-butanol, were found at higher concentrations outside rather than inside apples. Although, according to Henry's and Fick's laws, this cannot be explained in physical terms, it is known that esterase activity takes place within the fruit peel. Alcohols are probably enzymatically produced from esters passing from the cortex through to the peel and then out of the apple. As well as the aliphatic alcohols, straight- and branched-C-chain alcohols with two to six C atoms and with double bonds have also been identified; examples include (*Z*)-3-hexenol and (*E*)-2-hexenol (Brackmann et al. 1993). These two alcohols are important constituents of apple juice (Mannheim and Passy 1975). Short-chained alcohols like 1-butanol, which possesses a sweet aroma, are considered desirable for obtaining the characteristic flavor of apples (Mehinagic et al. 2006).

The total number of volatile components in a given apple is cultivar specific and depends on its enzymatic activity (Fellman et al. 1993), on how permeable its membrane is to the organic chemicals in question (Kakiuchi et al. 1986), and on their concentrations at a specific time, bearing in mind that this relationship constantly varies throughout the pre- and postharvest periods (Dirinck and Schamp 1989). Volatile compounds are synthesized from amino acids, membrane lipids, and carbohydrates (Sanz et al. 1997). Fatty acids supply straight-chain alcohols and acyl-CoAs through β-oxidation. Branched-chain volatiles are formed by the metabolism of amino acids, and in particular that of isoleucine, leucine, and valine (Heath and Reineccius 1986). Alcohols are formed from fatty acids and amino acids by the reduction of aldehydes catalyzed by alcohol dehydrogenase (ADH, EC 1.1.1.1) (Sanz et al. 1997). Esters are produced by combining alcohols and CoA derivatives of carboxylic acids in an oxygen-dependent reaction catalyzed by alcohol acyl-CoA transferase (AAT, EC 2.3.1.84) (Harada et al. 1985). As acetyl-CoA is the most

abundant CoA present in fruit tissue, the majority of esters are acetate esters (Dixon and Hewett 2001).

The aroma of apples is mainly due to several volatile compounds that are present in their peel and flesh (Fellman et al. 1993; Holland et al. 2005), though the flavor profile changes as apple fruits progress through maturation, harvest, and subsequent storage (Echeverría et al. 2004a; Rizzolo et al. 2006; Song and Bangerth 1994, 1996; Vanoli et al. 1995).

CHANGES IN APPLE FLAVOR PROFILE

Season-to-season variability is an important factor in flavor development. Climatic factors have been shown to play an important role in the subsequent ripening of apple fruits. "Golden Delicious," "Granny Smith" (López et al. 1998a), and "Fuji" (Echeverría et al. 2002, 2004a) apples displayed different aroma production characteristics, both qualitatively and quantitatively, in two consecutive seasons, in spite of receiving the same pre- and postharvest treatments. A number of other factors also influence volatile emissions after apple storage. These include cultural practices before harvest, postharvest handling practices, maturity stage (Echeverría et al. 2004a; Fellman et al. 2003), and processing procedures; the latter may include the storage and shelf-life periods and storage atmospheres.

The Influence of Cultivars and Maturity Stages on the Flavor of Apple Fruits

The abundance of volatile compounds varies greatly, largely depending on the cultivar in question (Dixon and Hewett 2000; Fellman et al. 2000). Most of the variable aroma compounds are present in volatile emissions from the majority of apple cultivars, and there is apparently no key reference compound for any specific cultivar (Paillard 1990). Even so, there tend to be quite significant sensory differences in flavor and aroma between different cultivars (Cunningham et al. 1986).

Apple volatile production has been categorized according to the type and quantity of esters or alcohols (Dirinck and Schamp 1989; Paillard 1990), aroma production pattern (Dirinck and Schamp 1989), skin color (Paillard 1979), and relative presence of C6-aldehydes (Paillard 1990). Ester-type cultivars are categorized according to the relative predominance of these volatile compounds in the aroma profile: acetate ester types ("Calville Blanc," "Gala," "Golden Delicious," "Granny Smith," "Red Delicious"); butanoate ester types ("Belle de Boskoop," "Canada Blanc," "Gravenstein," "Richared," "Starking"); and propanoate ester types ("Reinette du Mans," "Richared") (Aaby et al. 2002; Fellman et al. 2003; López et al. 1998a,b; Paillard 1990; Young et al. 1996). Yellow-skinned cultivars have been reported to produce mainly acetic acid esters, while red-skinned cultivars mostly produce butyric acid esters (Paillard 1979). Concentrations of C6-aldehydes in "Cox's Orange Pippin" and "Jonathan" apples were four to five times greater than in "Golden Delicious" for hexanal, and 100 times greater for *trans*-2-hexenal (Paillard 1990).

Monitoring and controlling ripeness has become a very important issue in fruit management, because the state of maturity at harvest determines the quality of the

final product as measured in terms of customer satisfaction. Ripening is a genetically regulated process in which both molecular and biochemical changes cause physical modifications in the fruit, including color changes, softening, and the development of characteristic aroma and taste traits (Seymour et al. 1993). The synthesis of aroma volatile compounds is associated with fruit ripening and will therefore depend on developmental stage at harvest (Mattheis et al. 1995; Song and Bangerth 1994). For two consecutive seasons, concentrations of ethanol in "Fuji" apples harvested 195 days after full bloom (dafb) were lower than for 185 dafb fruits (Echeverría et al. 2004a); this lower level of ethanol emission in more mature fruit has also been reported by Fellman and others (2000) for "Gala" apples.

The postharvest ripening period had an effect on both total volatile emission and the individual volatile compounds of different apple cultivars (Echeverría et al. 2004a; Rizzolo et al. 2006; Song and Bangerth 1996; Vanoli et al. 1995). For early-harvested apples, greater ester emissions were reported throughout postharvest ripening by both Fellman and others (2000) and Echeverría and others (2004c) for "Fuji" apples. In the case of late-harvested apples, total volatile emission decreased after 1, 5, and 10 days of ripening at 20°C (Echeverría et al. 2004c). This observed decrease in the concentration of volatile compounds could perhaps indicate that these late-harvested apples were less able to produce volatile compounds during ripening. The proportion of linolenic acid present in lipids from postclimacteric apples was lower than in those from preclimacteric apples (Dixon and Hewett 2000). This could explain the reduced number of straight-chain esters in postclimacteric apples, because one of the biosynthetic pathways in straight-chain esters is the β-oxidation of fatty acids such as linoleic acid (Brackmann et al. 1993).

Ideally, fruit should be harvested at its optimal eating quality. However, storage and handling considerations, especially for fruit subjected to long-term CA storage, require immature fruit to be harvested in order to maximize its storage life and minimize physical damage. Although immature fruits can be more successfully stored and transported, they often lack flavor (Kader 2004). Immature fruits produce fewer volatile compounds at harvest and lose their capacity for volatile production during storage, and especially during long-term CA or ultralow CA storage (Fellman et al. 2003; Mattheis et al. 1991; Song and Bangerth 1996). Harvest maturity influences not only volatile formation at harvest but also determines the regeneration of aroma volatiles after CA storage. As harvest maturity advances, the time required to regenerate aroma volatiles to optimal levels after CA storage decreases (Fellman et al. 2003).

Effects of Storage Technology on Apple Fruit Flavor

Nowadays, only a small percentage of apple fruits are marketed fresh; the majority are put into cold storage to keep fruit available to the market for an extended period (Knee 1993). CAs are generally considered beneficial for apple storage, as fruits maintain their color and firmness longer than when cold stored in air (Dixon and Hewett 2001), but the influence effect of cold storage on fruit aroma is not clear. CA effects are largely dependent on O_2 and CO_2 concentrations in the storage atmosphere and on the storage and ripening periods (Aaby et al. 2002; Echeverría et al. 2004a; Harb et al. 2000; López et al. 2007; Lo Scalzo et al. 2003; Plotto et al. 1999).

Figure 15.1 shows the total volatile emissions in two consecutive seasons for "Starking Delicious," "Golden Delicious," "Granny Smith," and "Fuji" apples under cold storage in air (AIR) and three different CAs in which oxygen and carbon dioxide were held at concentrations of 1%, 2%, and 3 % (ULO, LO, and SCA, respectively) followed by 10 days of ripening at 20°C (Echeverría 2003; Lavilla 1998).

Lower O_2 and higher CO_2 levels and longer storage periods resulted from the suppression of volatile emissions in apples (Ampun and Hewett 1997; Brackmann et al. 1993; Echeverría et al. 2002; Fellman et al. 2000; Yahia et al. 1990). However, conditions of 2% O_2/CO_2 resulted in a higher production of aroma volatiles than with 3% and 1% O_2/CO_2 (López et al. 1998b, 1999).

Apples stored for 3 months in a CA (3% O_2/CO_2) produced smaller amounts of volatile compounds than those stored in an air atmosphere in tests conducted with "Gravenstein" (Aaby et al. 2002), "Bisbee Delicious" (Mattheis et al. 1995), and "Jonagold" (Hansen et al. 1992) cultivars. However, atmosphere did not seem to have any significant effect on the production of total alcohols and esters for "Golden Delicious" and "Starking Delicious" (López et al. 1998b, 1999) or "Macintosh" and "Cortland" (Yahia et al. 1991) apples. The greatest emission of volatile compounds was obtained after 5 months of cold storage of "Starking Delicious," "Golden Delicious," and "Granny Smith" apples. After 5 months of cold storage, the total volatile emission in CAs decreased more than in air atmospheres (Lavilla 1998).

Furthermore, due to a residual effect, the production of certain aroma compounds may decrease when apples are returned to normal environmental conditions (Yahia et al. 1991). The production of straight-chain esters (originating from lipid metabolism) decreased after long-term CA storage (Brackmann et al. 1993); however, no residual effect was detected for branched-chain esters (derived from amino acids) due to CA storage (Fellman et al. 2003; Mattheis et al. 1995; Seong-Jin and Jee Eun 1999). Substrate availability is an important factor in the recovery of flavor in apples stored for long periods under CA conditions (Fellman et al. 1993). Since their amino acid content is relatively constant during storage (Ackermann et al. 1992) and they are the precursors of branched-chain esters, this could explain why 2-methylbutyl acetate and ethyl 2-methylbutanoate contents were not affected by CA storage in "Gala" (Mattheis et al. 1998) and "Starking Delicious" apples (López et al. 1998b).

However, sensory analysis revealed similar aroma profiles for apples stored in air and CA. This does not contradict the fact that apples stored in air tend to have higher concentrations of volatile compounds than those stored in CA, since the concentrations of the compounds contributing to aroma were approximately the same. Aroma values of ethyl 2-methylbutanoate and ethyl hexanoate were equal in air and CA apples, while those of ethyl butanoate were 1.5 times higher in air than in CA (Aaby et al. 2002).

SENSORY CHARACTERIZATION OF APPLE FLAVOR

The importance of sensory evaluation in apple production and processing is obvious if a consistent high-quality product is desired (Dimick and Hoskin 1983).

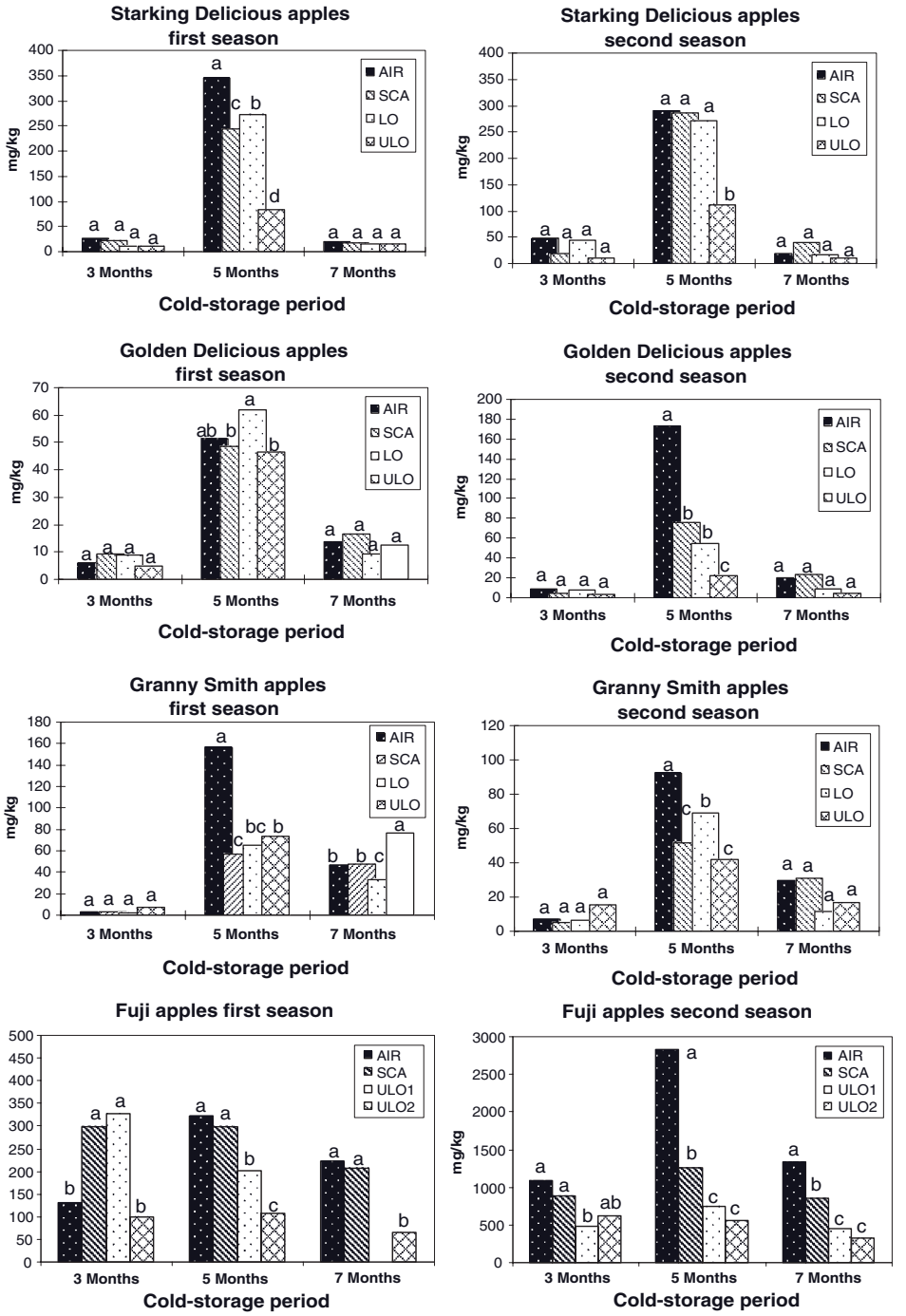

Figure 15.1. Total volatile emission after cold storage plus 10 days of ripening at 20°C. Bars with the same letter each cold-storage period are not significantly different ($p \leq 0.05$) according to the least significant difference (LSD) test.

Sensory evaluation methods offer a way of collecting information about the sensory attributes of food samples as perceived by the human senses. Stow (1995) determined texture and flavor to be the most important attributes for consumer acceptance. Sensory analysis is also used to measure the components of taste, texture, and aroma, and instrumental measurements are often used to predict eating quality. Research correlating analytic and sensory measurements in apples has so far been limited. Bourne (1979) outlined the difficulties associated with correlating analytic and sensory measurements due to high fruit-to-fruit variation. When constructing sensory models based on measurements of aroma components by GC, a conventional multiple linear regression approach is not advisable due to numerous problems of multicolinearity. Researchers have thus tended to turn to bilinear multivariate procedures, of which the most common are principal component analysis and partial least-squares regression (Brockhoff et al. 1993; Martens and Naes 1989).

Instrumental texture measurements, volatile composition, and sensory analysis have been correlated by Karlsen and others (1999) for 13 apple cultivars. Sour, bitter, and grassy flavors were best explained when sensory odor and flavor attributes were correlated with texture measurements and volatile composition data at the same time.

The importance of some volatile compounds for consumer acceptability was also reported in some early works. Young and others (1996) reported that hexyl and 2-methylacetates were identified by a tasting panel as having the greatest impact on determining the attractiveness of ripe "Royal Gala" fruit. López and others (2000) showed that "Golden Delicious" apples that had been stored in ULO were less well accepted after 5 months of storage. Theses apples have greater firmness, higher acidity and, lower aroma content than apples kept under air storage conditions. Regression analysis was used to correlate aroma compounds, quality parameters, and sensory evaluation for "Granny Smith" apples after cold storage: the highest acceptability score was positively correlated with ethyl 2-methylbutanoate, *tert*-butyl propionate, pentyl acetate, and 1-butanol. Soluble solid concentration was one of the quality parameters that best correlated with the sensory score (Lavilla et al. 1999).

Echeverría and others (2004b) reported that the most accepted "Fuji" apples had greater concentrations of ethanol, *tert*-butyl propanoate, ethyl butanoate, hexyl acetate, (*E*)-2-hexenol, and butyl hexanoate (Fig. 15.2). These results should be understood in conjunction with sensorial descriptors (Visai et al. 1993), such as sweet (ethanol and hexyl acetate), slightly caramel like, and fruity (E-3-hexenol) (Dimick and Hoskin 1983), and the low odor thresholds of ethyl butanoate (1 μg/L) and hexyl acetate (2 μg/L).

In the case of "Pink Lady" apples, López and others (2007) reported that, together with hexyl 2-methylbutanoate and hexyl hexanoate content, soluble solid content and titratable acidity were the instrumental measurements that most influenced consumer acceptability (Fig. 15.3). As regards to "Mondial Gala®" apples, Echeverría and others (2008) reported that the best accepted fruits showed the highest emissions of ethyl 2-methylbutanoate, ethyl hexanoate, *tert*-butyl propanoate, and ethyl acetate, with the first two of the these compounds being considered to make a significant contribution to the aroma profile of "Mondial Gala®" apples based on their respective odor units. These fruits also exhibited the highest titratable acidity and firmness values. This important contribution of acidity and firmness to acceptability

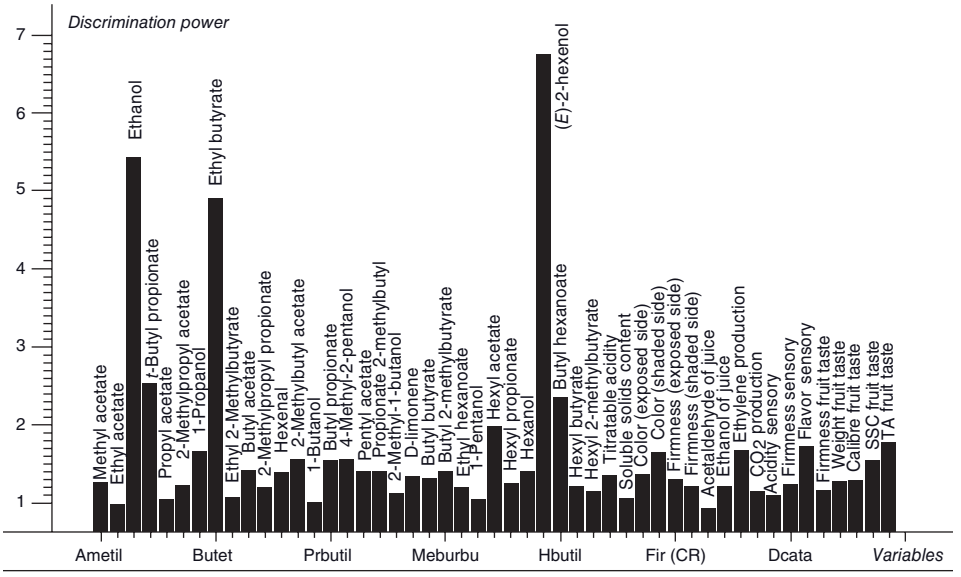

Figure 15.2. Discrimination power plot from the principal component analysis of sensory data of "Fuji" apples cold stored in different atmospheres. SSC, soluble solids content; TA, titratable acidity.

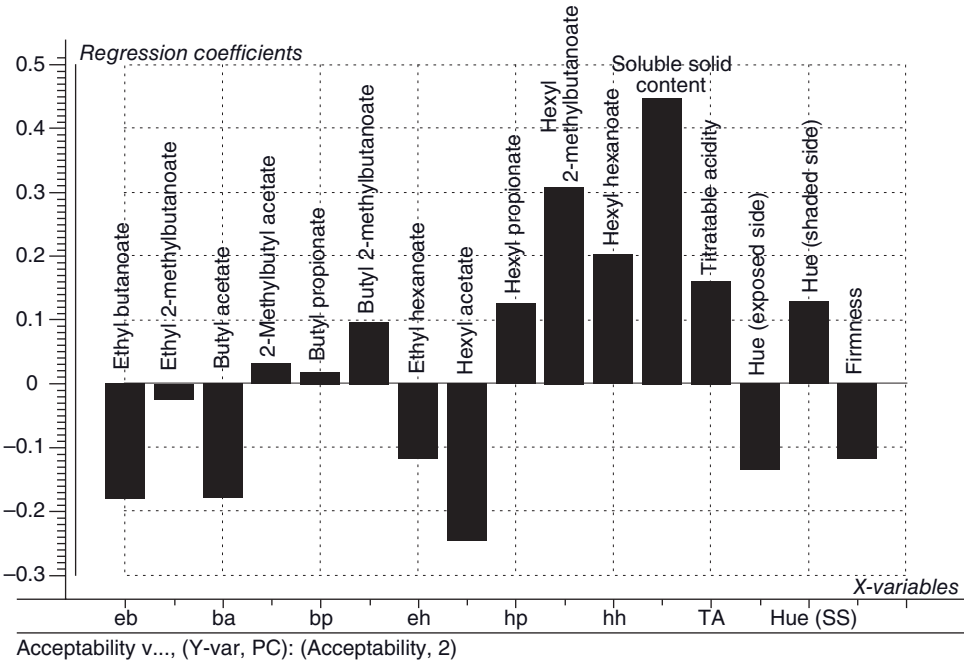

Figure 15.3. Regression coefficient plot of the first principal component (PC1) versus the second principal component (PC2) corresponding to partial least-square regression model for the acceptability of "Pink Lady®" apples cold stored in different atmospheres.

has also been reported by the same authors for "Fuji" (Echeverría et al. 2004d) and also for "McIntosh" apples (Liu and King 1978).

FINAL REMARKS

Using sensory analysis of fruit flavor based on consumer perception combined with instrumental analysis should further define the contribution of individual volatile compounds to total flavor quality and help to refine our understanding of fruit ripening and consumer preferences. This, in turn, should help to optimize the production and postharvest handling of fruit. This process includes identifying the role of volatile compounds in influencing the perception of flavor by consumers and also in identifying their role in defining the "ripeness," "eating quality," and "freshness" of apple fruits from the perspective of flavor.

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REFERENCES

Aaby K, Haffner K, Skrede G. 2002. Aroma quality of Gravenstein apples influenced by regular and controlled atmosphere storage. *Lebensm Wiss Technol* 35:254–9.

Ackermann J, Fischer M, Amado R. 1992. Changes in sugars, acids and amino acids during ripening and storage of apple (Cv. Glockenapfel). *J Agric Food Chem* 40:1131–4.

Ampun W, Hewett EW. 1997. Aroma volatile enhancement in stored apples. In Saltveit MK (ed.), *Proceedings 7th International Controlled Atmosphere Research Conference*, July 13–18, Davis, CA, pp. 241–5.

Bourne MC. 1979. Rupture tests vs small-strain tests in predicting consumer response to texture. *Food Technol* 10:67–70.

Brackmann A, Streif J, Bangerth F. 1993. Relationship between a reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. *J Am Soc Hortic Sci* 118:243–7.

Brockhoff P, Skovgaard I, Poll L, Hansen KA 1993. Comparison of methods for linear prediction of apple flavour from gas chromatographic measurements. *Food Qual Prefer* 4:215–22.

Buettner A, Schieberle P. 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia Late and Navel Oranges by quantitation of key odorants and flavour reconstitution experiments. *J Agric Food Chem* 49:2387–94.

Burdock GA. 2002. *Fenaroli's Handbook of Flavor Ingredients*, 4th Ed. New York: CRC Press.

Buttery RG. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree TE, Teranishi R (eds.), *Flavor Science*. Washington, DC: ACS Professional American Chemical Society, pp. 259–86.

Cunningham DG, Acree TE, Barnard J, Butts RM, Braell PA. 1986. Charm analysis of apple volatiles. *Food Chem* 19:137–47.

Diirr P, Schobinger U. 1981. The concentration of some volatiles to the sensory quality of apple and orange juice odour. In Schreier P (ed.), *Flavour '81 3rd Weurman Symposium Proceedings of the International Conference*, Munich, April 28–30, Berlin, New York, Walter de Gruyter, pp. 179–93.

Dimick PS, Hoskin JC. 1983. Review of apple flavor: State of the art. *Crit Rev Food Sci Nutr* 18:387–409.

Dirinck PJ, Schamp N. 1989. Instrumental aroma analysis for objective evaluation of parameters influencing aroma formation in apples and for prediction of the optimum picking date. *Acta Hort* 258:421–8.

Dixon J, Hewett EW. 2000. Factors affecting apple aroma/flavour volatile concentration: A review. *N Z J Crop Hortic Sci* 28:155–73.

Dixon J, Hewett EW. 2001. Exposure to hypoxia conditions alters volatile concentrations of apple cultivars. *J Sci Food Agric* 81:22–9.

Durán L, Costell E. 1999. Revisión: Percepción del gusto. Aspectos fisicoquímicos y psicofísicos. *Food Sci Technol Int* 5:299–309.

Echeverría G. 2003. Evaluación instrumental y sensorial de la calidad en manzanas "Fuji" durante su maduración en campo y posterior almacenamiento en atmósfera controlada. PhD thesis, University of Lleida, Lleida, Spain.

Echeverría G, Fuentes T, Graell J, Lara I, López ML. 2004a. Aroma volatile compounds of "Fuji" apples in relation to harvest date and cold storage technology. A comparison of two seasons. *Postharvest Biol Technol* 32:29–44.

Echeverría G, Fuentes MT, Graell J, López ML. 2004b. Relationships between volatile production, fruit quality and sensory evaluation of "Fuji" apples stored in different atmospheres by means of multivariate analysis. *J Sci Food Agric* 84:5–20.

Echeverría G, Correa E, Ruiz-Altisent M, Graell J, Puy J, López ML. 2004c. Characterization of Fuji apples from different harvest dates and storage conditions from measurements of volatiles by gas chromatography and electronic nose. *J Agric Food Chem* 52(10): 3069–76.

Echeverría G, Lara I, Fuentes T, López ML, Graell J, Puy J. 2004d. Assessment of relationships between sensory and instrumental quality of controlled-atmosphere-stored "Fuji" apples by multivariate analysis. *J Food Sci* 69(9):368–75.

Echeverría G, Graell J, Lara I, López ML. 2008. Physicochemical measurements in "Mondial Gala®" apples stored at different atmospheres: Influence on consumer acceptability. *Postharvest Biol Technol.* doi: 10.1016/j.postharvbio.2008.05.002.

Echeverría G, Graell J, López ML. 2002. Effect of harvest date and storage conditions on quality and aroma production of "Fuji" apples. *Food Sci Technol Int* 8(6):351–60.

Flath RA, Black DR, Guadagni DG, McFadden WH, Schultz TH. 1967. Identification and organoleptic evaluation of compounds in *Delicious* apple essence. *J Agric Food Chem* 15:29–35.

Fellman JK, Mattinson DS, Bostick BC, Mattheis JP, Patterson ME. 1993. Ester biosynthesis in "Rome" apples subjected to low-oxygen atmospheres. *Postharvest Biol Technol* 3:201–14.

Fellman JK, Miller TW, Mattinson DS, Mattheis JP. 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. *HortScience* 35(6):1026–33.

Fellman JK, Rudell DR, Mattinson DS, Mattheis JP. 2003. Relationship of harvest maturity to flavor regeneration after CA storage of "Delicious" apples. *Postharvest Biol Technol* 27:39–51.

Hansen K, Poll L, Olsen CE, Lewis MJ. 1992. The influence of oxygen concentration in storage atmospheres on the post-storage volatile ester production of "Jonagold" apples. *Lebensm Wiss Technol* 25:451–6.

Harada M, Ueda Y, Iwata T. 1985 Purification and some properties of alcohol acetyltransferase from banana fruit. *Plant Cell Physiol* 26:1067–74.

Harb J, Bisharat R, Streif J. 2008. Changes in volatile constituents of blackcurrants (*Ribes nigrum* L. cv. "Titania") following controlled atmosphere storage. *Postharvest Biol Technol* 47:271–9.

Harb J, Streif J, Bangerth F. 2000. Response of controlled atmosphere (CA) stored "Golden Delicious" apples to the treatments with alcohols and aldehydes as aroma precursors. *Eur J Hortic Sci* 65(4):154–61.

Heath HB, Reineccius G. 1986. Biogenesis of flavour fruits and vegetables. In *Flavour Chemistry and Technology*. Westport, CT: AVI, pp. 43–70.

Holland DH, Harkov O, Bar-Ya'Akov I, Bar E, Zax A, Brandeis E, Ravid U, Lewinsohn E. 2005. Development and varietal differences in volatile ester formation and acetyl-CoA: Alcohol acetyl transferase activities in apple (*Malus domestica* Borkh.) fruit. *J Agric Food Chem* 53:7198–203.

Hornung DE, Enns MP. 1994. The synergistic action of the taste and smell components of flavor. In Birch G, Campbell-Platt G (eds.), *Synergy*. Andover, UK: Intercept, pp. 145–54.

Kader AA. 2004. A perspective on postharvest horticulture (1978–2003). *HortScience* 38:1004–8.

Kakiuchi N, Moriguchi S, Fukuda H, Ichimura N, Kato Y, Banba Y. 1986. Composition of volatile compounds of apple fruits in relation to cultivars. *J Jpn Soc Hortic Sci* 55(3):280–9.

Karlsen AM, Aaby K, Sivertsen H, Baardseth P, Ellekjær MR. 1999. Instrumental and sensory analysis of fresh Norwegian and imported apples. *Food Qual Prefer* 10:305–14.

Kays SJ, Wang CY. 2000. Thermally induced flavor compounds. *HortScience* 35(6):1002–12.

Knee M. 1993. Pome fruits. In Seymour GB, Taylor JE, Tucker GA (eds.), *Biochemistry of Fruit Ripening*. London: Chapman and Hall, pp. 325–46.

Knee M, Hatfield SGS. 1976. A comparison of methods for measuring the volatile components of apple fruits. *J Food Technol* 11:485–93.

Kollmannsberger H, Berger RG. 1992. Precursor atmosphere storage induced flavour changes in apples cv. Red Delicious. *Chem Mikrobiol Technol Lebensm* 14:81–6.

Komthong P, Hayakawa S, Katoh T, Igura N, Shimoda M. 2006. Determination of potent odorants in apple by headspace gas dilution analysis. *Lebensm Wiss Technol* 39:472–8.

Lavilla MT. 1998. Effect of different CA conditions on aroma production in Golden Delicious, Starking Delicious, and Granny Smith apples. PhD thesis, University of Lleida, Lleida, Spain.

Lavilla MT, Puy J, López ML, Recasens I, Vendrell M. 1999. Relationships between volatile production, fruit quality and sensory evaluation in "Granny Smith" apples stored in different controlled-atmospheres treatments by means of multivariate analysis. *J Agric Food Chem* 47:3791–803.

Liu FW, King MM. 1978. Consumer evaluations of "McIntosh" apple firmness. *HortScience* 13:162–3.

López ML, Lavilla MT, Graell J, Recasens I, Vendrell M. 1999. Effect of different CA conditions on aroma and quality of Golden Delicious apples. *J Food Qual* 22:583–97.

López ML, Lavilla MT, Recasens I, Graell J, Vendrell M. 2000. Changes in aroma quality of "Golden Delicious" apples after storage at different oxygen and carbon dioxide concentrations. *J Sci Food Agric* 80:311–24.

López ML, Lavilla T, Riba M, Vendrell M. 1998a. Comparison of volatile compounds in two seasons in apples: *Golden Delicious* and *Granny Smith*. *J Food Qual* 21:155–66.

López ML, Lavilla T, Recasens I, Riba M, Vendrell M. 1998b. Influence of different oxygen and carbon dioxide concentrations during storage on production of volatile compounds by Starking Delicious apples. *J Agric Food Chem* 46:634–43.

López ML, Villatoro C, Fuentes T, Graell J, Lara I, Echeverría G. 2007. Volatile compounds, quality parameters and consumer acceptance of "Pink Lady®" apples stored in different conditions. *Postharvest Biol Technol* 43:55–66.

Lo Scalzo R., Lupi D, Giudetti G, Testoni A. 2003. Evolution of volatile composition of whole apple fruit cv "Gala" after storage. *Acta Hort* 600:555–62.

Mannheim CH, Passy N. 1975. Aroma recovery and retention in fruit juices. *Int Flav Food Addit* 6:323–8.

Martens H, Naes T. 1989. Partial least squares regression. In *Multivariate Calibration*. Chichester, UK: John Wiley & Sons, pp. 116–65.

Mattheis JP, Buchanan DA, Fellman JK. 1995. Volatile compound production by Bisbee Delicious apples after sequential atmosphere storage. *J Agric Food Chem* 43:194–9.

Mattheis JP, Buchanan DA, Fellman JK. 1998. Volatile compounds emitted by "Gala" apples following dynamic atmosphere storage. *J Am Soc Hortic Sci* 123(3):426–32.

Mattheis JP, Fellman JK, Chen PM, Patterson ME. 1991. Changes in headspace volatiles during physiological development of "Bisbee Delicious" apple fruit. *J Agric Food Chem* 39:1902–6.

Mehinagic E, Royer G, Symoneaux R, Jourjon F, Prost C. 2006. Characterization of odor-active volatiles in apples: Influence of cultivar and maturity stage. *J Agric Food Chem* 54:2678–87.

Negoias S, Visschers R, Boelrijk A, Hummel T. 2008. New ways to understand aroma perception. Review. *Food Chem* 108:1247–54.

Noble AC, Matysiak NL, Bonnans S. 1991. Factors affecting the time-intensity parameters of sweetness. *Food Technol* 45:121–6.

Paillard NMM. 1979. Biosynthese des produits volatils de la pomme: Formation de alcools et des esters a partir des acides gras. *Phytochemistry* 18:1165–71.

Paillard NMM. 1990. The flavour of apple, pears and quinces. In Morton ID, MacLeod AJ (eds.), *Food Flavours, Part C. The Flavour of Fruits*. Amsterdam: Elsevier Science, pp. 1–41.

Pelayo C, Ebeler SE, Kader AA. 2003. Postharvest life and flavor quality of three strawberry cultivars kept at 5 degrees C in air or air + 20 kPa CO_2. *Postharvest Biol Technol* 27:171–83.

Plotto A. 1998. Instrumental and sensory analysis of "Gala" apple (*Malus domestica, Borkh*) aroma. PhD thesis, Oregon State University, Corvallis, OR, p. 193.

Plotto A, McDaniel M, Mattheis JP. 1999. Characterizations of "Gala" apple Aroma and Flavor: Differences between controlled atmosphere and air storage. *J Am Soc Hortic Sci* 124:416–23.

Plotto A, McDaniel MR, Mattheis JP. 2000. Characterization of changes in "Gala" apple aroma during storage using Osme analysis, a gas chromatography-olfactometry technique. *J Am Soc Hortic Sci* 125(6):714–22.

Rizzolo A, Gras M, Zerbini PE. 2006. Influence of harvest date on ripening and volatile compounds in the scab-resistant apple cultivar "Golden Orange." *J Hortic Sci Biotechnol* 81(4):681–90.

Rizzolo A, Polesello A, Teleky-Vàmossy GY. 1989. CGC/Sensory analysis of volatile compounds development from ripening apple fruit. *J High Resolut Chromatogr* 12:824–7.

Rizzolo A, Visai C, Vanoli M, 1997. Changes in some odour-active compounds in paclobutrazol-treated "Starkspur Golden" apples at harvest and after cold storage. *Postharvest Biol Technol* 11:39–46.

Rychlik M, Schieberle P, Grosch W. 1998. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*. Garching, Germany: Deutsche Forschungsanstalt fuer Lebensmittelchemie.

Sanz C, Olias JM, Perez A. 1997. Aroma biochemistry of fruits and vegetables. In Tomás-Barberán FA, Robbins RJ (eds.), *Phytochemistry of Fruits and Vegetables*. New York: Oxford University Press, pp. 125–55.

Schnabel KO, Belitz HD, Von Ranson C. 1988. Investigations on the structure-activity relationships of odorous substances. Part 1. Detection thresholds and odour qualities of aliphatic and alicyclic compounds containing oxygen functions. *Z Lebensm Unters Forsch* 187:215–23.

Schotsmans W, Prange RK. 2006. Controlled atmosphere storage and aroma volatile production. *Stewart Posthar Rev* 5(1):1–8.

Seong-Jin CH, Jee Eun K. 1999. The change in aroma production of "Fuji" apple fruits as influenced by CA storage. *J Korean Soc Hortic Sci* 40(1):61–4.

Seymour GB, Taylor JE, Tucker GA. 1993. *Biochemistry of Fruit Ripening*. London: Chapman and Hall, pp. 405–442.

Song J, Bangerth F. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. *Acta Hort* 368:150–7.

Song J, Bangerth F. 1996. The effect of harvest date on aroma compound production from "Golden Delicious" apple fruit and relationship to respiration and ethylene production. *Postharvest Biol Technol* 8(4):259–69.

Stow J. 1995. Quality measurements of apples. *Postharvest News Inform* 6(3):32–3.

Takeoka GR, Buttery RG, Flash RA. 1992. Volatile constituents of Asian pear (*Pyrus serotina*). *J Agric Food Chem* 40:1925–9.

Takeoka GR, Buttery RG, Ling L. 1996. Odour thresholds of various branched and straight chain acetates. *Lebensm Wiss Technol* 29:677–80.

Takeoka GR, Flath RA, Mon TR, Teranishi R, Guentert M. 1990. Volatile constituents of apricot (*Prunus armeniaca*). *J Agric Food Chem* 38:471–7.

USDA. 2004. *The US and World Situation: Apples*. USDA Foreign Agricultural Service. http://www.fas.usda.gov/htp/Hort_Circular/2005.

Van Gemert LJ, Nettenbreijer AH. 1977. *Compilation of Odour Threshold Values in Air and Water*. Voorburg, The Netherlands: National Institute for Water Supply.

Vanoli M, Visai C, Rizzolo A. 1995. The influence of harvest date on the volatile composition of "Starkspur Golden" apples. *Postharvest Biol Technol* 6:225–34.

Van Zeebroeck M, Van linden V, Ramon H, De Baerdemaeker J, Nicolaï B, Tijskens E. 2007. Impact damage of apples during transport and handling (review) *Postharvest Biol Technol* 45:157–67.

Visai C, Rizzolo A, Vanoli M. 1993. Sviluppo delle sostanze volatili nelli meli durante la conservazione frigorifera. *Riv Frutticolt* 9:57–60.

Wang Y, Finn CH, Qian MC. 2005. Impact of growing environment on Chickasaw Blackberry (*Rubus* L.) aroma evaluated by gas chromatography olfactometry dilution analysis. *J Agric Food Chem* 53:3563–71.

Yahia EM, Liu FW, Acree TE. 1990. Changes of some odor-active volatiles in controlled atmosphere-stored apples. *J Food Qual* 13:185–202.

Yahia EM, Liu FW, Acree TE. 1991. Changes of some odor-active volatiles in low-ethylene controlled atmosphere stored apples. *Lebensm Wiss Technol* 24:145–51.

Young H, Gilbert JM, Murria SH, Ball RD. 1996. Causal effects of aroma compounds on Royal Gala apple flavours. *J Sci Food Agric* 71:329–36.

CHAPTER 16

Citrus Fruits and Oranges

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INTRODUCTION

The concept of fragrance and aroma includes the presence of high odorant organic compounds of pleasant odor characteristics. They are used in perfumes and perfumed products—fragrances—or in aromatization of foods and beverages—aroma. Flavor, a decisive factor in the choice and acceptance of a food or beverage, is the result of integrated sensations of taste, odor, and buccal perception. The taste is attributed to the nonvolatile compounds that determine the four basic tastes—sour, salty, sweet, and bitter—have a distinct anatomical basis (Bufe and Meyerhof 2006).

Aroma consists of a mixture of hundreds of different organic compounds in low concentrations, parts per million or parts per billion. The literature reports thousands of different identified compounds, among them esters, alcohols, aldehydes, ketones, carboxylic acids, hydrocarbons, amines, mercaptans, terpenes, ethers, and lactones.

According to Plutowska and Wardencki (2007), the characteristics of the resultant food aroma depend on a number of factors: disponibility and structure of the reagents, presence of fat, amino acids and sugar, and reaction conditions (temperature, time, water activity, pH, oxygen level).

The typical aroma of the tropical fruits results from the mixture of many volatile representative substances from different chemical classes, with distinct physical and chemical properties (Thomazini and Franco 2000). Aroma perception depends on the individual impact of each of these compounds, but it is the result of the interdependence of concentration among them (Moshonas et al. 1993; Moshonas and Shaw 1995; Pillonel et al. 2002).

ORANGE AND CITRUS FRUITS

Citrus is botanically a large family whose dominant members are the sweet orange (*Citrus sinensis*), mandarin or tangerine orange (*Citrus reticulata*), grapefruit (*Citrus*

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paradisi), lemon (*Citrus limon*), and lime (*Citrus aurantifolia*). The citrus gene has about 16 species. Food and beverage industries aiming at increase sales are continuously improving their products; for example, the addition of natural citrus flavors is a way of introducing the juice products in the emerging health market (Minh Tu et al. 2002; Porto et al. 2003; Redd and Hendrix 1993).

Citrus fruits are abundant in limonoids, phytochemicals that scientists are currently investigating for their antiviral, antifungal, antibacterial, and antioxidant properties. Limonoids have also shown effectiveness as insecticides both in traditional farming cultures and modern biochemistry labs.

Orange juice is the main product of the citrus industry, but some commercially valuable by-products are obtained during the juice processing: essential oils, D-limonene, terpenes, aromatic liquids, and citric pulp bran.

There are different applications in the internal and external Brazilian markets, which include the manufacture of chemical and solvent products, and the production of aromas and fragrances, substances to be used in the industries of inks, cosmetics, and animal feed, among others (ABECITRUS 2008).

Due to economical and food stability reasons, the mechanical extraction of orange juice is concentrated in the producing countries of this fruit. Three main fractions are obtained: the concentrated juice, the aqueous essence (orange aroma), and the essential oil (Hinterholzer and Schieberle 1998).

CITRUS FLAVOR

Orange juice is a multiphase system constituted by an aqueous phase containing soluble compounds, the serum, and a water-insoluble phase comprising both pulp and cloud. Fresh orange juice aroma is associated with its volatile fractions that are constituted by water-soluble and -insoluble substances. The distribution of aroma compounds between pulp and serum in different fruit juices has shown that hydrocarbons (mono- and sesquiterpene hydrocarbons) are associated with pulp and that oxygenated compounds (ethyl butyrate and octanal) are mainly contained in the serum (Brat et al. 2003).

The volatile constituents present in fresh orange juice originate from three sources. The juice contained in the juice sacs, which is liberated during extraction, is the source for the volatile water-soluble compounds. Two types of oil, juice oil and peel oil, contribute the oil-soluble compounds to the flavor of fresh orange juice. Juice oil is present in globular bodies within the juice sacs, and it becomes dispersed in the juice during extraction (Nagy and Shaw 1990).

Research has been conducted to determine the compounds responsible for orange flavor (Roberts et al. 2000; Wilkes et al. 2000). The conclusion is that orange flavor is not the result of one or a few compounds but rather an interaction of many compounds, although some compounds in certain combinations significantly enhance the flavor of processed orange juice (Niedz et al. 1997).

Aroma or aqueous phase essence is also referred to as natural orange aroma. Some of the most important flavor components of orange aroma include acetaldehyde, ethyl acetate, acetal, and ethyl butyrate. In addition, ethanol is also an important constituent, around 13%.

Poore (1932) was the first to intensively analyze the volatile compounds of the essential oil from the California orange. In 1946, Curl compared different systems

for the determination of volatile components in citric juices. After 1960 research was intensified and much progress was made in the qualitative and quantitative determination of orange oil constituents. Among the researchers, Attaway and coworkers, Kesterson and Hendrikson, and Wolford and collaborators (Figueredo and Uzelac 1976) are prominent. Attaway and coworkers isolated and identified some carbonyl components, alcohols, and volatile organic acids present in natural essence of orange, and Wolford analyzed recovery essences from orange juice processing.

Regarding the orange constituent, the essential oil has been studied most extensively. More than 220 compounds have been identified in its composition. The oil is predominantly composed of 96% terpene hydrocarbons (limonene in higher proportion); however, it also contains 1.6% of aldehydes (mainly, octanal and decanal), 0.8% of alcohols (especially, linalool), 0.3% of esters (neryl acetate and octyl acetate), and 1% of nonvolatile compounds such as carotenoids, tocopherols, flavonoids, hydrocarbons, fatty acids, and sterols (Gaffney et al. 1996; Kimball 1991; Matthews and Braddock 1987; Shaw 1977).

The three major citrus volatiles are methanol, ethanol, and acetaldehyde. Methanol concentrations range from 10 to 80 ppm, ethanol from 90 to 900 ppm, and acetaldehyde from 50 to 190 ppm (w/v). Lund and others (1981) found that orange single-strength juices (fresh and processed) presented a higher acetaldehyde concentration than grapefruit juices, but ethanol and methanol concentrations were lower in grapefruit than in orange juice. Reconstituted commercial concentrates contain less methanol and ethanol and more acetaldehyde than single-strength juices. Similarity between the profiles of volatiles for some concentrates and the profile for single-strength juice suggests that these concentrates contain additional essences. Any correlation among the volatile compounds, physical characteristics, and storage conditions has not been verified (Nagy and Dinsmore 1974; Naim et al. 1993).

Maccarone and others (1998) identified 19 aroma compounds with gas chromatography–mass spectrometry (GC-MS) and quantified by gas chromatography with flame ionization detector (GC-FID) of 72 orange juices from the varieties "Naveline," "Washington Navel," "Valencia Barks," "Ovale Calabrese," "Tarocco," "Sanguinello," and "Glanders" produced in Italy. The juices obtained from the varieties "Naveline" and "Washington Navel" were characterized by a high content of 2 *trans*-2-hexenol; "Valencia Barks" for myrcene; "Ovale Calabrese" for myrcene and linalool; and "Sanguinello and Glanders" for valencene; meantime, no aroma compound differentiated the variety "Tarocco."

Eight constituents were found at higher levels in orange juices: the monoterpene hydrocarbons sabinene, α-phellandrene, 6-3-carene/3-ocimene, and γ-terpinene, and the oxygenated compounds octanol, linalool oxide, and cavone. Although these trace constituents in orange juice have not been directly associated with orange juice flavor, some have been used in synthetic citrus flavors, including α-phellandrene, Δ-3-carene, and β-ocimene. γ-Terpinene in dilute solutions has a pleasant, citrus-like taste and is known to be important in mandarin oil aroma (Wilson and Shaw 1981).

Except for limonene, the levels in juice believed to be optimum for these compounds have not been established. Thus, higher levels of these substances in juice are not necessarily better than lower levels for good fresh orange flavor.

The most important oil-soluble constituents present in the orange juices included α-pinene, myrcene, limonene, octanal, nonanal, decanal, neral, geranial, and linalool. These nine constituents are generally considered to make important contributions

to orange juice flavor. Most of the compounds quantified in this study are water-soluble constituents present prior to juice extraction in the aqueous portion of the juice sacs. The seven water-soluble constituents quantified that are considered important to orange juice flavor include ethyl acetate, ethyl propionate, methyl butanoate, ethyl butanoate, ethyl 3-hydroxyhexanoate, ethanol, and (*Z*)-3-hexen-1-ol (Pollien et al. 1997; Shaw 1991).

Hinterholzer and Schieberle (1998) concluded that the mixture of acetaldehyde, citral, ethyl butanoate, limonene, and α-pinene, when added to the orange juice, presents high punctuation on aroma.

Berlinet and others (2007) determined the partition coefficients of aroma compounds in fresh and pasteurized juices regarding the pulp content. They verified that in fresh juices, the aroma compounds had higher retention in 12% pulp juice than in 6% pulp juice. After pasteurization, the difference between the coefficients was lower. The authors concluded that potential interactions between cloud proteins and aroma compounds may be enhanced by thermal treatment resulting in better retention of several aroma compounds into the matrix. This trend was consolidated by the sensory experiments and headspace solid-phase microextraction (HS-SPME) with gas chromatography (GC) olfactometry that showed fewer sensory differences among the pasteurized juices than among the fresh juices.

Five studies on different orange processing and characterization techniques are summarized in Table 16.1. It is interesting that few compounds of the orange aroma were identified in all five references.

Orange essential oil is a natural flavoring material obtained during orange juice concentration. It is usually condensed on the first stage of a cryo-evaporator and separated from the aqueous phase by centrifugation. The composition and flavor quality of this product vary considerably depending on the orange cultivar, maturity and processing conditions used to extract and concentrate the juice (Haypek et al. 2000; Hognadottir and Rouseff 2003). These oils have larger application in the food and pharmaceutical industries. They can be directly used as flavoring in beverages, ice creams, and other foods, and in medications and cosmetics, as soaps and perfumes. They are still used by the manufacturing industries of cleaning products.

According to Gaffney and others (1996), the essential oils of two orange varieties, Valency and Pear, from Florida (United States) and Brazil presented the following odor profile in order of importance: octanal (description: citrus), linalool (cereal, taste of fruits), an unidentified compound (licorice), β-sinensal (fish), and octanal, then an unidentified compound and linalool, respectively.

In 2003, Quintero and coworkers evaluated the *Citrus aurantium amara* aroma, which has been used to add aroma to beverages and liquors and as an ingredient to give fragrance to soaps, detergents, cosmetics, and perfumes. The fruits were collected in several sites of Venezuela, and the oil was extracted from the cortex by cold pressing. Its components were analyzed by GC with flame ionization detector and GC-MS. The main constituents were monoterpenes (limonene, 77.90%; β-pinene, 3.40%; myrcene, 1.81%; and *trans*-ocimene, 1.16%), sesquiterpenes (valencene, 0.52%), aldehydes (decanal, 3.51%; dodecanal, 0.36%; and geranial, 0.29%), alcohols (β-nerolidol, 0.85% and linalool, 0.89%), and nootkatone as the only ketone. The extraction procedure can be considered as adequate since the oil obtained does not contain *p*-cymene, which is an indicator of oxidation of monoterpenes in citrus essential oils.

TABLE 16.1. Identified Aroma Compounds of Orange and the Methods of Extraction and Characterization (Identification, Separation, and Quantification)

Reference	Processing	Techniques	Compounds	
1	Essential oil	Solvent extraction (fractionation of sample)	Methyl anthranilate	*cis*-Sabinene hydrate
		4 fractions/GC-FID/GC-MS/column SE 52	Caryophyllene	*trans*-Sabinene hydrate
			Citro-α-3-caryophyllene (α-humulene)nel acetate	Neroli
			α-Copaene	Neryl acetate
			α-Cubebene	Sesquiterpene
			β-Elemen	γ-Terpinene
			δ-Elemen	α-Thujene
			Methyl geraniate	Undecanal
2	Essential oil	Solvent extraction/GC-FID/GC-MS/ Crompack WCOT CP Wax 52 CB	Acetoin	Hexanol
			Hexanoic acid	*cis*-3-Hexenol
			1-Butanol	*trans*-2-Hexenol
			2-Methyl-1-butanol	1-Penten-3-ol
			2-Methyl-1-propanol	3-Penten-2-ol
			4-Methyl-2-pentanol	
1	Essential oil	Solvent extraction (fractionation of sample—4 fractions)/GC-FID/GC-MS/column SE 52	*p*-Cymene	
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG		
1	Essential oil	Solvent extraction (fractionation of sample—4 fractions)/GC-FID/GC-MS/column SE 52	Octanol	
2	Essential oil	Solvent extraction/GC-FID /GC-MS/Crompack WCOT CP Wax 52 CB		
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX		

TABLE 16.1. ***Continued***

Reference	Processing	Techniques	Compounds		
1	Essential oil	Solvent extraction (fractionation of sample—4 fractions)/GC-FID/GC-MS/column SE 52	Canphene		α-Farnesene
			Citronellal		α-Felandrene
			β-Cubebene		Geranial
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX			
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	Neryl acetate	Decanol	*cis*-Limonene oxide
			α-Terpenil acetate	Tetradecane	Geraniol
			2-Methylbutyl butyrate	Octadecane	Germacrene D
			Cadinene	β-Elemol	Bicyclogermacrene
			δ-3-Carene	β-Farnesene	Aliphatic hydrocarbon
			β-Caryophyllene (β-humulene)	β-Felandrene	Perillaldehyde
			β-Citronelol	Menthone	Peryl alcohol
					α-Sinensal
1	Essential oil	Solvent extraction (fractionation of sample 4 fractions)/GC-FID/GC-MS/column SE 52	Nonanal		
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701e SE-54			
2	Essential oil	Solvent extraction/GC-FID/GC-MS/Crompack WCOT CP Wax 52 CB	Hexanal		
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701and SE-54			
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG	Butyl acetate	Monoterpene hydrocarbon	
			Nonanol	Ocimene	
			3-Heptanone	Isopropanol	
			Methanol		

5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701e SE-54	Phenyl acetaldehyde	3-Ethyl hydrohexanoate
			Ethyl acetate	Hex-3-enal
			Acetic acid	Non-2-enal
			Butanoic acid	Nona-2,6-dienal
			2-Methyl butanoic acid	Nona-2,4-dienal
			3-Methyl butanoic acid	Methynal
			Ethyl butanoate	Met-1-eno-8-thiol
			Ethyl-2-methyl butanoate	Octa-1,5-dien-3-one
			2-Methyl-butanal	1-Penten-3-one
			3-Methyl-butanol	2-Isopropyl-3-methoxypyrazine
			Butan-2,3-dione	Ethyl 2-propionate
			4,5-Epoxy-2-decenal	Ethyl methylpropionate
			4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Vanillin
			Ethyl hexanoate	
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG	B-ionone	
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701and SE-54		
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	Octanal	
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701e SE-54		
1	Essential oil	Solvent extraction (fractionation of sample—4 fractions)/GC-FID/GC-MS/column SE 52		
2	Essential oil	Solvent extraction/GC-FID/GC-MS/Crompack WCOT CP Wax 52 CB	α-Terpinene	
			α-Terpineol	
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG		

TABLE 16.1. ***Continued***

Reference	Processing	Techniques	Compounds
1	Essential oil	Solvent extraction (fractionation of sample—4fractions)/GC-FID/GC-MS/column SE 52	β-Pinene
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG	
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	
1	Essential oil	Solvent extraction (fractionation of sample—4fractions)/GC-FID/GC-MS/column SE 52	Decanal
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701e SE-54	
1	Essential oil	Solvent extraction (fractionation of sample—4fractions)/GC-FID/GC-MS/column SE 52	
2	Essential oil	Solvent extraction/GC-FID/GC-MS/Crompack WCOT CP Wax 52 CB	4-Terpineol Valencene
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG	
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	
1	Essential oil	Solvent extraction (fractionation of sample–4 fractions)/GC-FID/GC-MS/column SE 52	
2	Essential oil	Solvent extraction/GC-FID/GC-MS/Crompack WCOT CP Wax 52 CB	
3	Juice	Solvent extraction/GC-FID/GC-MS/column PEG	Limonene (D-limonene) Linalool Mircene α-Pinene
4	Essential oil	Solvent extraction/GC-FID/GC-MS/column DB-WAX	
5	Juice	Solvent/AEDA/GC-MS/GC-FID/columns FFAP, OV-1701and SE-54	

1, Dugo and others (1988); 2, Maccarone and others (1998); 3, Moufida and Marzouk (2003); 4, Min Tu and others (2002); 5, Hinterholzer and Schieberle (1998).
PEG, polyethyleneglycols; AEDA, aroma extract dilution analysis; FFAP, polar column.

Tangerine essential oil, like most of the citrus family, has particularly refreshing and rejuvenating characteristics. Its aroma clears the mind and can help to eliminate emotional confusion. Aroma therapists also consider it to be very comforting, soothing, and warming. Users may also see tangerine used in perfumes and soaps, and as an antispasmodic, carminative, digestive, diuretic, sedative, stimulant (digestive and lymphatic), and tonic agent. Tangerine essential oil has the typical citrus scent—fresh, radiant, and tangy sweet. With only subtle differences, it smells a lot like the mandarin, with some even considering them identical. In comparison to sweet orange, tangerine can be seen as lighter with more candy-like tones (Johnson and Vora 1983).

In an extract of the peel from clementines, prepared by solvent extraction, 42 odor-active compounds were detected by application of an aroma extract dilution analysis and subsequently identified by using the respective reference odorants. Among them, by far the highest flavor dilution factors were determined for the flowery smelling linalool, the fatty smelling (*E*,*E*)-deca-2,4-dienal, and the wine lactone eliciting a sweet odor quality. These were followed by α-pinene, myrcene, and octanal with pine tree-like, geranium leaf-like, and citrus-like aromas. Among the 30 odor-active compounds identified, 11 aroma compounds are reported here for the first time as important contributors to clementine peel aroma, for example, wine lactone, (*E*,*E*)-nona-2,4-dienal, carvone, (*Z*)-hex-3-enal, or *trans*-4,5-epoxy-(*E*)-dec-2-enal (Buettner et al. 2003).

Sawamura and others (2004) studied the cold pressure oil from *C. reticulata* (ponkan) and its oxygenated fraction by GC and GC-MS and sensory analyses. The monoterpene group was predominant, accounting for more than 89.6% (w/w), of which limonene was the most abundant (80.3%). Among the oxygenated compounds, octanal and decanal were the major ones among 12 aldehydes, accounting for >1.5%. Six alcohols were identified with a total concentration of >0.7%, while oxides, ketones, and esters did not quantitatively or qualitatively contribute to the oil. Sniffing the ponkan cold-pressed oil and its oxygenated fraction demonstrated that octanal and decanal were the characteristic odor components of ponkan.

Chida and others (2006) evaluated 23 odor substances from three citrus essential oils (lemon, Valencia orange, and *Citrus sudachi*), which were selected as the potent character-impact compounds on the basis of their limited odor unit values, and then every chemical was cross-matched by sensory test to the three oils to attribute each aroma character to one of the three citrus oils. It was found that the aroma character of lemon oil was mainly represented by citral, with a high matching frequency of 0.89 (59 counts out of 66 trials). The orange character was consisted by linalool and nonanal. α, β-Pinene, α-sinensal, and myrcene were related to the aroma of the *C. sudachi* oil.

CITRUS JUICE PROCESSING AND AROMA LOSSES

Frozen concentrated orange juice (FCOJ) is obtained by an extraction process. In a single operation, the juice is extracted and separated from the pulp, the essential oil, and the peel. The extracted juice goes through finishers for the removal of excess pulp. Then, it is sent to centrifuges where it will go through an even more refined separation of insoluble solids. After the centrifuges, the orange juice flows to evaporators where the natural juice (around 12°Brix) is concentrated up to 66°Brix

(ASSOCITRUS 2008; Neves et al. 2001). Concentration is traditionally carried out through the use of high temperatures, promoting significant sensory and nutritional changes of the fruit juice quality since these characteristics are conferred by volatile components and vitamins, which are thermosensitive compounds. So, at this point, essences and oils (recovered during the vacuum concentration process) are added back to restore the flavor.

The orange juice industries use the essential oil and the originated aqueous and oil essences to reincorporate the volatile compounds to the concentrated juice to recuperate the fresh fruit note so much appreciated by the fruit juice consumers (Bazemore 1995; Bettini et al. 1998; Garcia 2000; Kaanane et al. 1988; Maccarone et al. 1996; Moshonas and Shaw 1990).

Pasteurized Valencia and Temple orange juices concentrated to 45°Brix by freeze concentration retained their fresh juice flavor. Direct steam infusion heating to inactivate enzymes allowed more rapid heating than indirect heating and successfully lowered juice peel oil during vacuum cooling. Except for considerable pulp reduction of feed stream juices, there were few differences from normal citrus juice recovery procedures for freeze concentration. Since the product retained most of the aroma constituents of fresh juice, careful handling and high-quality feed juice prior to freeze concentration was much more important than for evaporation. Fresh juice freeze concentrated to 45°Brix and then pasteurized at temperatures of 80, 97, and 111°C had reduced sucrose (up to 25%) as the temperature increased to 111°C (Lee and Chen 1998; Lee and Nagy 1988; Manheim and Havkin 1981).

Freshly squeezed Valencia orange juice, distilled at low temperature and reduced pressure, yielded an aqueous distillate with the necessary balance of volatile flavor and aroma constituents to retain the fresh juice characteristics. Flavor comparisons by an experienced sensory panel determined that orange juice reconstituted by combining the aqueous distillate with the residual juice solids had significantly more fresh juice character than reconstituted commercial FCOJ; commercial not pasteurized single-strength orange juice; pasteurized, not from concentrate; canned, from concentrate; or aseptically packed, from concentrate. The panel also found no significant difference in fresh juice characteristics between freshly squeezed orange juice and distillate and residual solids recombined from the same juice sample. Fifty-four volatile constituents were identified from the aqueous distillate of freshly squeezed orange juice (Braddock and Marcy 1987).

Almeida (2006) evaluated the stability and sensory quality of reconstituted orange juice aromatized with four different natural aroma: I—distilled essential oil; II—fractions of oil essence (oil phase); III—formulated aroma containing distilled fractions of essential oil and oil essence; and IV—formulated aroma containing distilled fractions of essential oil, distilled fractions of oil essence, and aqueous essence (water phase). Chromatographic analyses of all the formulations indicated the presence of 55 odor compounds. The identified volatile substances of higher odorant importance were β-myrcene, ethyl butanoate, α-pinene, hexanal, citral, and decanol, and the compounds of low odorant power were limonene, linalool, α-terpineol, and decanal.

Orange, grapefruit, and lemon juices were concentrated over twofold in a pilot scale reverse osmosis (RO) process using a commercially available membrane system. Major sugars, acids, vitamin C, aroma volatiles, and over 20 minerals were examined in feed, concentrate, and permeate streams. Typically, 15–20 aroma

compounds were identified in feed juices and concentrates. Compared with less volatile compounds (e.g., ethyl butyrate, limonene), poorer retention during processing was noted for more volatile molecules (methanol, acetaldehyde, ethanol). °Brix of membrane concentrates were orange (25.3°Brix), grapefruit (25.1°Brix), and lemon (22.5°Brix) (Braddock et al. 1988).

One method for improving the quality is to strip the aromas from the ongoing raw juice stream before processing and to feed them back into the final product. Vacuum distillation is a commercially preferable process for recovery due to its high yield and easiness in controlling the system. The disadvantage is that there is additional heat treatment, which is not desirable for heat-sensitive products such as orange. Pervaporation is a new technique that can be operated at low feed pressure, ambient temperature without any additional chemical. It is developed to replace distillation for heat-, stress-, and chemical-sensitive products (Braddock 1999).

Loss of aroma compounds during food processing is a well-known problem in the food industry that causes considerable decrease in the quality of beverages especially in fruit juices (Holley 2006). Pervaporation is a membrane separation process that has been widely evaluated as a potential technology to aroma recovery and concentration due to its beneficial advantages over conventional aroma recovery processes such as high selectivity, low energy consumption, physical separation mechanism, moderate operating temperatures, and no additive requirement (Assis et al. 2007; Peng and Liu 2003; Pereira et al. 2005; Rajagopalan and Cheryan 1995).

Addition of volatile flavor fractions recovered during processing, such as aqueous or essential oil, restores some of its characteristic aroma, but the natural juice aroma has not yet been reproduced in processed citrus products. More information on the volatile flavor components present in fresh citrus juices and their proportional concentration can enhance the understanding of the basis of these unique flavor attributes.

CONCLUSIONS

Citrus is by far the most technologically developed juice industry. Its operation can range in size and technology from the immense and global to small and village-oriented.

The three major citrus volatiles are methanol, ethanol, and acetaldehyde.

For citrus oils, the terpenes represent the main composite class, constituted mainly of D-limonene. The oxygenated terpenoids, which are the lesser constituents, contribute a higher intensity to the oil aroma.

REFERENCES

Ahmed EM, Dennison RA, Dougherty RH, Shaw PE. 1978b. Effect of nonvolatile orange juice components, acid, sugar and pectin on the flavour threshold of D-limonene in water. *J Agric Food Chem* 26:192–4.

Ahmed EM, Dennison RA, Shaw PE. 1978c. Effect of selected oil and essence volatile components on flavour quality of pumpout orange juice. *J Agric Food Chem* 26:368–72.

Almeida SB. 2006. Efeito de diferentes aromas cítricos sobre a qualidade e estabilidade sensoriais de suco de laranja pronto para beber. PhD thesis (Food Technology), Faculty of Food Engineering, Universidade Estadual de Campinas, Campinas, São Paulo.

Assis AR, Bizzo HR, Matta VM, Cabral LMC. 2007 Recuperação e concentração de componentes do aroma de caju (*Anacardium occidentale* L.) por pervaporação. *Ciênc Tecnol Aliment* 27:349–54.

ASSOCITRUS. 2008. Saude Com Laranja. http://www.associtrus.com.br (accessed January 2010).

Bazemore RA. 1995. Osme and sensory analysis of aqueous orange essence. Thesis (Master Science), Oregon State University, Corvallis, OR.

Berlinet C, Guichard E, Fournier N, Ducruet V. 2007. Effect of pulp reduction and pasteurization on the release of aroma compounds in industrial orange juice. *J Food Sci* 72(8):s535–43.

Bettini MFM, Shaw PE, Lanças FM. 1998. Sensory and analytical evaluations of Brazilian orange juices and aromas. *Fruit Process* 7:283–7.

Braddock RJ. 1999. Juice processing operations. In *Handbook of Citrus By-Products and Processing Technology*. New York: Wiley, pp. 35–51.

Braddock RJ, Marcy JE. 1987. Quality of freeze concentrated orange juice. *J Food Sci* 52(1):159–62.

Braddock RJ, Nikdel S, Nagy S. 1988. Composition of some organic and inorganic compounds in reverse osmosis-concentrated citrus juices. *J Food Sci* 53(2):508–12.

Brat P, Rega B, Alter P, Reynes M, Brillouet J-M. 2003. Distribution of volatile compounds in the pulp, cloud and serum of freshly squeezed orange juice. *J Agric Food Chem* 51:3442–7.

Buettner A, Mestres M, Fischer A, Guasch J, Schieberle P. 2003. Evaluation of the most odour-active compounds in the peel oil of clementines (*Citrus reticulata* Blanco cv. clementine). *Eur Food Res Technol* 216(10):11–14.

Bufe B, Meyerhof W. 2006. The human perception of taste compounds. In Voilley A, Etievant P (eds.), *Flavour in Food*. Boca Raton, FL; Boston; New York; Washington, DC: CRC Press, p. 471.

Chida M, Yamashita K, Izumiya Y, Watanabe K, Tamura H. 2006. Aroma impact compounds in three *Citrus* oils: Cross-matching test and correspondence analysis approach. *J Food Sci* 71(1):S54–8.

Curl AL. 1946. Off-flavor development in processed tangerine juice. *Fruit Prod J* 25(12):326–57.

Dugo G, Cotroneo A, Trozzi A. 1988. On the genuineness of citrus essential oils. Part XXV. The essential oil from "Wenzhou honey" oranges. *Flav Frag J* 3:161–6.

Figueredo IB, Uzelac M. 1976. *Aromas em Alimentos [Food Aroma]*. Campinas, São Paulo, Brasil: ITAL.

Gaffney B, Havekotte M, Jacobs B, Costa L. 1996. Charm analysis of two *Citrus sinensis* peel oil volatiles. *Perfumer and Flavorist* 21:1–2, 4–5.

Garcia LV. 2000. Efeito do óleo essencial de laranja no aroma e sabor do suco concentrado congelado: a colaboração do consumidor. 2000. PhD thesis (Food Technology), Faculty of Food Engineering, Universidade Estadual de Campinas, São Paulo.

Haypek E, Silva LHM, Batista E. 2000. Recovery of aroma compounds from orange essential oil. *Braz J Chem Eng* 17(4–7):705–12.

Hinterholzer A, Schieberle P. 1998. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia Late oranges by odour dilution techniques. *Flav Frag J* 13:49–55.

Hognadottir A, Rouseff RL. 2003. Identification of aroma active compounds in orange essence oil using gas chromatography–olfactometry and gas chromatography–mass spectrometry. *J Chromatogr A* 998:201–11.

Holley A. 2006. Processing information about flavour. In Voilley A, Etievant P (eds.), *Flavour in Food*. Boca Raton, FL; Boston; New York; Washington, DC: CRC Press, p. 471.

Johnson JD, Vora JD. 1983. Natural citrus essences. *Food Technol* 37:92–3.

Kaanane A, Kane D, Labuza TP. 1988. Time and temperature effect on stability on Moroccan processed orange juice during storage. *J Food Sci* 53(5):1470–3.

Kimball DA. 1991. *Citrus Processing: Quality Control and Technology*. New York: Van Nostrand Reinhold.

Lee HS, Chen CS. 1998. Rated of vitamin C loss and discoloration in clear orange juice concentrate during storage at temperatures of 4–24°C. *J Agric Food Chem* 46:4723–7.

Lee HS, Nagy S. 1988. Relationship of sugar degradation to detrimental changes in citrus juice quality. *Food Technol* 42:91–4.

Lund ED, Kirkland CL, Shaw PE. 1981. Methanol, ethanol, and acetaldehyde contents of citrus products. *J Agric Food Chem* 29:361–6.

Maccarone E, Campisi S, Cataldi Lupo MC, Fallico B, Nicolosi Asmundo C. 1996. Effetti dei trattamenti tecnologici sui costituenti del succo di arancia rossa di Sicília. *Ind Bevande* 25:335–41.

Maccarone E, Campisi S, Fallio B, Rapisarda P, Sgarla R. 1998. Flavor components of Italian orange juices. *J Food Chem* 46:2293–8.

Manheim CH, Havkin M. 1981. Shelf-life of aseptically bottled orange juice. *J Food Process Preserv* 5:1–6.

Matthews RF, Braddock RJ. 1987. Recovery and applications of essential oils from oranges. *Food Technol* 41(1):57–61.

Minh Tu NT, Thanh LX, Une A, Ukeda H, Sawamura M. 2002. Volatile constituents of Vietnamese pummelo, orange, tangerine and peels oils. *Flav Frag J* 17:169–74.

Moshonas MG, Shaw PE. 1990. Flavor and compositional comparison of orange essences and essence oils produced in the United States and in Brazil. *J Agric Food Chem* 38(3):799–801.

Moshonas MG, Shaw PE. 1994. Quantitative determination of 46 volatile components in aseptically package orange juice during storage. *J Agric Food Chem* 42:1525–8.

Moshonas MG, Shaw PE. 1995. Fresh orange juice flavour: A quantitative and qualitative determination of the volatile constituents. In Charalambous G (ed.), *Food Flavors: Generation, Analysis and Process Influence*. Amsterdam: Elsevier Science, pp. 1479–92.

Moshonas MG, Shaw PE, Buslig BS. 1993. Retention of fresh orange juice flavor and aroma in an aqueous distillate from Valencia orange juice. *J Food Qual* 16(2):101–8.

Moufida S, Marzouk B. 2003. Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. *Phytochemistry* 62:1283–9.

Nagy S, Dinsmore HL. 1974. Relationship of furfural to temperature abuse and flavour change in commercially canned single-strength orange juice. *J Food Sci* 39:1116–9.

Nagy S, Shaw PE. 1990. Factors affecting the flavour of citrus fruit. In Morton ID, MacLeod AJ (eds.), *Food Flavour Part C. The Flavour of Fruit*. New York: Elsevier, pp. 93–124.

Naim M, Wainish S, Zehavi U, Peleg H, Rouseff RL. 1993. Inhibition by thiol compounds of off-flavour formation in stored orange juice. Effect of L-cysteine and N-acetyl-L-cysteine on 2,5-dimethyl-4-hydroxy-3(2H)-furanone formation. *J Agric Food Chem* 41:1355–8.

Neves MF, Do Val AM, Marino MK. 2001. The orange network in Brazil. *Fruit Process* 12:486–90.

Niedz RP, Moshonas MG, Peterson B, Shapiro JP, Shaw PE. 1997. Analysis of sweet orange (*Citrus sinensis* (L.) Osbeck) callus cultures for volatile compounds by gas chromatography with mass selective detector. *Plant Cell Tissue Org Cult* 51:181–5.

Peng M, Liu SX. 2003. Recovery of aroma compounds from dilute model blueberry solution by pervaporation. *J Food Sci* 68:2706–10.

Pereira CC, Rufino JRM, Habert AC, Nobrega R, Cabral LMC, Borges CP. 2005. Aroma compounds recovery of tropical fruit juice by pervaporation: Membrane material selection and process evaluation. *J Food Eng* 66:77–87.

Pillonel L, Bosset JO, Tabacchi R. 2002. Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review. *Lebensm Wiss Technol* 35:1–4.

Pino JA, Ranivoelisoa V, Torricella R. 1987. Influência de la temperatura de almacenamiento sobre la calidad del zumo de naranja envasado em frascos de vidrio. *Revista de Agroquímica y Tecnologia de Alimentos* 27(2):261–9.

Plutowska B, Wardencki W. 2007. Aromagrams—Aromatic profiles in the appreciation of food quality. Analytical, nutritional and clinical methods. *Food Chem* 101(2):845–72.

Pollien P, Ott A, Montigon F, Baumgartner M, Muñoz-Box R, Chaintreau A. 1997. Hyphenated headspace-gas chromatography-sniffing technique: Screening of impact odorants and quantitative aromagram comparisons. *J Agric Food Chem* 45:2630–7.

Poore HD. 1932. Analysis and composition of California lemon and orange oils. *US Dept Agr Tech Bull* 241.

Porto C, Pizzale L, Bravin M, Conte LS. 2003. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MS. *Flav Frag J* 18:66–72.

Quintero A, Gonzalez CN, Sanchez F, Usubillaga A, Rojas L. 2003. Constituents and biological activity of *Citrus aurantium amara* L. essential oil. *Acta Hortic* 597:115–17.

Rajagopalan N, Cheryan M. 1995. Pervaporation of grape juice aroma. *J Memb Sci* 104(3):243–50.

Redd JB, Hendrix CL Jr. 1993. Processing of natural citrus oils and flavour. In Nagy S, Chen CS, Shaw PE (eds.), *Fruit Juice Processing Technology*. Auburndale, FL: Agscience, pp. 83–109.

Roberts DD, Pollien P, Milo C. 2000. Solid-phase microextraction method development for headspace analysis of volatile flavour compounds. *J Agric Food Chem* 48:2430–7.

Sawamura M, Thi Minh Tu N, Onishi Y, Ogawa E, Choi HS. 2004. Characteristic odor components of *Citrus reticulata* Blanco (ponkan) cold-pressed oil. *Biosci Biotechnol Biochem* 68(8):1690–7.

Shaw PE. 1977. Essential oils. In Nagy S, Shaw PE, Veldhuis MK (eds.), *Citrus Science and Technology*. Westport, CT: Publishing Company, pp. 427–62.

Shaw PE. 1991. Fruits II. In Maarse H (ed.), *Volatile Compounds in Foods and Beverages*. New York: Dekker, pp. 305–28.

Thomazini M, Franco MRB. 2000. Metodologia para análise dos constituintes voláteis do sabor. *Ciênc Tecnol Aliment* 34:52–9.

Wilkes JG, Conte ED, Kim Y, Holcomb M, Sutherland JB, Miller DW. 2000. Sample preparation for the analysis of flavors and off-flavors in foods. *J Chromatogr A* 880:3–33.

Wilson CW III, Shaw PE. 1981. Importance of thymol, methyl N-methylanthranilate, and monoterpene hydrocarbons to the aroma and flavor of mandarin cold-pressed oils. *J Agric Food Chem* 29:494–6.

CHAPTER 17

Flavor in Grapes: Its Characterization and Commercial Applications

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INTRODUCTION

Grape (*Vitis vinifera* L.), one of the earliest fruit crops known to man, is widely cultivated all over the world. It is believed that grape cultivation began in Asia Minor, the region between and to the south of the Black and Caspian seas (Salunkhe and Desai 1984). This region, now known as Armenia, is the home of *V. vinifera*; from there, grape culture spread both west and east (Patil et al. 1995; Winkler et al. 1974). Grape is grown most successfully in the temperate regions of the world but is being cultivated in several tropical and subtropical countries also.

Flavor and aroma contribute significantly to fruit quality. The growing dissatisfaction among consumers about the flavor quality of fruits and vegetables has affected repeat buys. First-time purchases are often based on external quality parameters like color, appearance, and firmness; however, repeat buys are influenced by internal quality of fruits, such as mouthfeel and flavor. Fruit breeders often select lines based on external horticultural traits like color, size, disease resistance, and yield, thereby inadvertently leaving out flavor and aroma, which has affected repeat sales of fruits resulting in industry's concern (Baldwin 2002).

BOTANY, CULTIVARS, AND FRUIT CLASSIFICATION IN RELATION TO FLAVOR

Botany

The grapevine belongs to the genus *Vitis* of the family Ampelidaceae (Vitaceae), including several species and their cultivars. The genus *Vitis* (chromosome number 2n = 38) is native to the temperate region of the Northern Hemisphere with a few outlying species extending southward to the tropical region (Olmo 1993; Patil et al. 1995). The genus *Vitis* includes two subgenera, viz., *Euvitis* or true grapes and

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Muscadinia. Out of the several species of *Vitis* and *Muscadinia* found in different parts of the world, *V. vinifera* produces over 90% of the world's grapes, which are either pure *vinifera* or its hybridized versions (Patil et al. 1995). Based on morphological characteristics and geographic distribution, Negrul (1968) divided the cultivated *vinifera* into three groups: the *occidentalia* group containing winter-hardy wine grapes of Western Europe with small, compact clusters and berries; the *orientalia* or Eastern group with large papery leaves, often showing tendencies toward seedlessness and large-clustered table grape varieties with thin skin and firm flesh; and the *pontica* group representing intermediate types of both Asia Minor and Eastern Europe. The *Muscadinia* grapes (chromosome number 2n = 40) are generally found in southeastern humid parts of the United States.

Peynaud and Ribereau-Gayon (1971), however, classified grapes according to their origin into following four groups: (1) *V. vinifera* or European grape, subdivided into several cultivars bearing black, white, red, or green grapes; (2) American vines, *Vitis riparia*, *Vitis rupestris*, *Vitis labrusca*, generally bearing black grapes; (3) French hybrids and *Vitis rotundifolia* or *Muscadine* grapes; and (4) Asian vines, *Vitis amurensia.*

Cultivars

The most popular European cultivars are Thompson Seedless and Muscat of Alexandria. While all grapes can be fermented into wine when crushed and most of them can be dried as raisins or eaten fresh, only a limited number of cultivars can make wines of standard quality. Also, the raisins of commercial interest are produced from three cultivars, viz., Thomson Seedless, Black Corinth, and Muscat of Alexandria. Similarly, most of the sweet grape juice produced in the United States comes from Concord and only one or two seedless cultivars, namely, Thomson Seedless and Canner (Salunkhe and Desai 1984).

Table grapes are consumed as fresh fruit after harvest. They have large clusters and berries of attractive appearance and fine flesh with lower acidity and very few or no seeds, reflecting their derivation from selection in a desert environment of middle Asia. They require high temperature and isolation to ensure yield and fruit maturity (Olmo 1993; Patil et al. 1995).

Raisin grapes are a special class derived from mideastern Asian table grapes, mainly in Iran and Afghanistan. The berries have thin skin and firm flesh, with high sugar content and moderate to low acidity, with berries loosely arranged in the cluster. Occasionally, when left on the vine in the dry desert environment, the berries shrivel due to water loss, producing dried raisins *in situ* (Patil et al. 1995).

Wine grapes are processed by yeast and microbial fermentation, occasionally followed by distillation into a plethora of beverages. Most wine grapes have small clusters with roundish berries set compactly and with soft, juicy flesh of high acidity.

Fruit Classification in Relation to Flavors

The grape belongs to the large group of fleshy fruits and is classified as a berry, which develops after fertilization of the ovary (Coombe 1976; Kanellis and Roubelakis-Angelakis 1993; Pratt 1971). Grape is a nonclimacteric fruit with a relatively low rate of physiological activity, based on the very small amounts of ethylene produced during its development and ripening on the vine (Seymour et al. 1993).

Pigments in grapes are normally found in the skin, where they are confined to the outer three layers of cells. Based on color, grapes are usually classified as white, black, red, and intermediate color types (Patil et al. 1995). In some red or black cultivars, such as Salvador, the inner cells of the skin rupture and exude color when ripe or overripe, so that the pulp near the skin becomes colored. In Alicante Bouschet, juice is colored both in the skin and the pulp. The grape pigments are anthocyanins (red, purple, blue, and black), modified by their attachment with glucose molecule. Five anthocyanins, viz., cyanidin, peonidin, delphinidin, petunidin, and malvidin, together make up the basic part of grape pigment (Patil et al. 1995). Black *vinifera* cultivars usually contain monoglucosides of malvidin in highest percentage. Yanidin is the predominant pigment of red grape cultivars. The *Muscadine* varieties contain diglucoside. Yellow pigments constituting grape flavor and red pigments (anthocyanins) in the skin appear at *veraison*, reaching their complete expression at full maturity (Olmo 1993). Other commonly occurring pigments in green, immature fruits are carotenoids, xanthophylls, and chlorophyll, which disappear as the fruits ripen on the vine and reach their harvest maturity. The development of the pigment (color) is mainly influenced by light and temperature. The disorder known as "pink berry syndrome" has been observed in Maharashtra, India, which is manifested by an erratic appearance of dull pink color on berries during their development. The pink berry syndrome is believed to be caused by the development of anthocyanins and influenced by the application of growth regulators (Patil et al. 1995).

WORLD PRODUCTION, IMPORTS, AND EXPORTS

Europe has the maximum area under grapes and accounts for nearly 40% of the 69 million tons of all grape types produced in the world (Anon. 2008). China accounts for 10% of the world production and ranks the second, whereas the United States is the fifth largest producer of grapes. Italy, France, and Spain are leading grape producers in Europe. Other important grapes producers are Turkey, Argentina, and South Africa. According to Olmo (1993), around 70% of the total world production of 60 million tons of grapes is used for wine making, producing around 30 million tons of wine, with the remaining produce being utilized as table grapes (20%), raisins (11%), and fresh juice (1%). The principal grape exporting countries are Chile, the United States, South Africa, Italy, Greece, Afghanistan, Turkey, and Australia. The major importers in 2006 were the United States, Germany, Russia, the United Kingdom, The Netherlands, Canada, France, Poland, and Belgium (Anon. 2008). The consumption of grapes is the highest in China, followed by Iran, Turkey, Egypt, India, the United States, and Italy.

FLAVOR QUALITY IN GRAPES

History and Background

Flavor in fruits is a complex, elusive trait and is determined by several factors, such as fruit genetics (Baldwin et al. 1992), environment (Baldwin et al. 1995; Romani et al. 1983), cultural practices (Wright and Harris 1985), harvest maturity (Baldwin

et al. 1999a; Fellman et al. 1993; Maul et al. 1998), and postharvest handling (Baldwin et al. 1999a,b; Fellman et al. 1993; Mattheis et al. 1991, 1995; Maul et al. 1998, 1999). According to Baldwin (2002), flavor is composed of sweetness, sourness, bitterness, saltiness, and aroma. There is a need to conduct an elaborate sensory panel testing to measure these traits and quantify the corresponding chemical components. There is a further need to understand fruit flavor in terms of flavor components, their biosynthetic pathways, and their perception by human beings. Baldwin (2004) concluded that flavor in fruits is complex, both in terms of chemical and sensory measurements and in terms of interfacing the two approaches. Flavor quality of fresh fruits is at maximum at harvest (except for climacteric fruits that continue to ripen after harvest) and can only be maintained, at best, during storage, shipping, and marketing, mainly because the bottom line for fruit flavor is its genetic character. Obviously, breeders need more information and analytic tools in order to select lines for flavor quality, such as molecular markers relating to flavor, which could help identify vital enzymes in flavor biosynthesis. Flavor life in fruits is normally shorter than their shelf life based on appearance. Also, the effects of harvest maturity on flavor quality need to be determined for each commodity. With the global market getting more competitive, flavor quality in fruits and vegetables is achieving increasing importance for consumers and consequently for the horticultural industry. Maintenance of flavor quality in fresh and perishable horticultural products after harvest is a special challenge, while marketing distances are increasing as a result of newer storage, handling, and transport technologies. Thus, postharvest physiologists, sensory scientists, flavor chemists, breeders, and molecular biologists, armed with newer biotechnologies, need to take an integrated approach to confront the flavor quality (Baldwin 2002).

Grape Flavor and Aroma

During ripening, grapes develop characteristic flavor or aroma by synthesizing certain volatile compounds. According to Patil and others (1995), the principal flavor constituents in grapes include sugars, acids, anthranilate, volatile ester acids, alcohols, and aldehydes. Linalool and genaniol have been shown to contribute to the aroma of Concord grapes, closely resembling the aroma of methyl anthranilate (Bolin and Salunkhe 1971; Olmo 1993). Most but not all of the aromatic compounds are concentrated in the hypodermal tissue. The grape cultivars have natural aroma typical of most varieties of *V. vinifera*. Some aromatic hydrocarbons, including xylene, toluene, and alkyllenzene, have also been reported (Olmo 1993). However, these being insoluble in water, their presence are not expected in the processed products. Muscat varieties often contain the acetates of some monoterpene alcohols. The Concord and Niagara have strong foxy flavors, which is attributed to the presence of methyl anthranilate. The most widely occurring terpene alcohols in Muscat grape cultivars are linalool and geraniol, nerol, citronellol, terpinool, and hortrienol. The waxes covering the berry surface are aliphatic *n*-alkenes and *n*-alkanes (Olmo 1993; Patil et al. 1995).

A study of terpenic derivatives occurring in the aroma of Muscat grapes by gas and thin-layer chromatography (TLC) and by infrared and mass spectrophotometry (Ribereau-Gayon et al. 1975) identified the following eight compounds: linalool, geraniol, nerol, α-terpineol, two furanic, and two piranic oxides of linalool. The

terpenes of the Muscat juice were titrated individually by gas chromatography (GC), after concentration by salting out of the aqueous solution into an extractive solvent. The total terpene content varied between 1 and 3 mg/L. The influence of each substance on the whole aroma was precisely checked by determining thresholds (100–6000 μg/L). This study indicated that some transformations of terpenes may explain the losses of aroma observed during processing and storage of grape juices and wines.

The accumulation of aroma in the grape berry appears to differ significantly from the accumulation processes normally associated with berry ripening (Coombe and McCarthy 1997). A distinctive feature is increase in concentration of free and glycosylated aroma compounds in the advanced stages of ripening, when sugar increase per berry has slowed. A similar pattern is shown for the levels of non-anthocyanin glycosides in Shiraj berries. Coombe and McCarthy (1997) named the process of accumulation of aroma compounds as "engusting," meaning that the berry is acquiring attractive aromas and flavors (to engust). Park and others (1991) reported accumulation of major free and glycosidically bound monoterpenes of Muscat grapes during ripening. Around 90% of the monoterpenes were glycosidically bound, while only 10% were in the free odor-producing form in whole berries. The distribution of free and bound monoterpenes in the skins and mesocarp changed constantly during berry ripening. At harvest, 4.6% and 5.9% of the three major monoterpenes (linalool, geraniol, and nerol) occurred as free terpenes located in the skin and mesocarp, respectively, whereas 31% and 59% of total terpenes were found as glycosides in the skin and mesocarp.

Grape wine aroma is known to consist of 600–800 aroma compounds from which especially those, typical for the variety, are already present in the grapes (Rapp 1995). Significant varietal differences among the "aromagrams" (fingerprint pattern) have indicated the amount of key flavor compounds to be typically dependent on the variety. Monoterpenes especially play an important role in the differentiation of wine varieties.

Dokoozlian and Kliewer (1996) reported the effects of light on berry growth and variation in composition during fruit development of *V. vinifera* var. "Cabernet Sauvignon" and "Pinot Noir." Fruit softening and berry coloration were delayed when berries were grown without light during the early two stages. Clusters exposed to light through fruit development had greater skin anthocyanins and phenolics than fruit grown without light. Free and bound aroma compounds of eight Spanish grape cultivars used in wine making have been reported by Lopez-Tamames and others (1997). A single-extraction procedure was used to provide information on the potential flavor of these varieties. Bound aroma was determined directly by trifluoroacetic acid (TFA) glycoside analysis and indirectly by enzyme hydrolysis release of aglycon. Grape characteristics, such as sugars, acidity, polyphenols, and juice yield, have been correlated with the aroma composition as influenced by climatic and cultivar factors. Non-terpenyl compounds, such as 2-phenylethanol and benzyl alcohol, were the predominant glycosidically bound compounds of Spanish varieties. This study indicated that direct determination of glycosides is better than aglycon hydrolysis as an approach to the potential aroma of grapes.

Kennedy and others (2000) determined the quantity and characterization of extracted polyphenols (flavan-3-ol monomers and procyanidins) in seeds during fruit ripening. The per berry extractive yield of all polyphenols decreased with

maturity and followed a second-order kinetics. During fruit ripening, the mean degree of polymerization of extracted procyanidins remained unchanged when analyzed by high-performance liquid chromatography (HPLC) but decreased by thiolytic degradation. Changes in vine water status affected polyphenol amounts, indicating that cultural practices can be used to influence composition.

Volatile compounds (aroma) in musts and skins of grapes of Aren, Macabeo, and Chardonnay white varieties determined during ripening by Garcia and others (2003) indicated that skins of these varieties were both qualitatively and quantitatively richer in volatiles (c-3-hexanol, t-3-hexanol, 2,4-hexadienal) than the musts. Changes in the concentration of the volatile compounds during ripening were not uniform, making it difficult to determine the optimum level of ripening for each variety on the basis of the volatile compound content.

The *V. vinifera* Albarino is a considerably acid white Galician grape cultivar, with an important aromatic potential. Dieguez and others (2003) identified and quantified aromatic compounds extracted from the grape sample using C18 cartridges by gas chromatography–mass spectrometry (GS-MS). The grapes had a high content of bound aromatic compounds. The varietal aroma was contributed by predominating linalool, geraniol, benzyl alcohol, and 2-phenylethanol.

Flavor Components and Profiles

Flavor in fruits is contributed by several factors such as sweetness (sugars), sourness (organic acids), bitterness (terpenoids, flavonoids), saltiness (natural salts), and astringency (flavonoids, alkaloids, tannins). Fruit sweetness is primarily related to soluble sugars, including sucrose, glucose and/or fructose, sorbitol, and several others. Fructose is sweeter than sucrose, and sucrose, in turn, is sweeter than glucose. The total soluble solids (TSSs) in fruits are often regarded to be synonymous with soluble sugars and can be easily measured. Breeders often select lines for higher TSS in an attempt to increase sweetness and postharvest shelf life of fruits (Salunkhe and Desai 1984). The general chemical composition of grapes, which varies according to environmental factors, temperature, soil fertility, moisture status, and light, is shown in Table 17.1.

Sourness (acidity) in fruits is related to organic acids, such as tartaric acid (grapes), citric acid (citrus fruits), malic acid (apples), quinic acid (pears) (Kanellis and Roubelakis-Angelakis 1993), and oxalic acid (banana), which provides some astringency to complement the citric and malic acids (Seymour 1993).

Bitterness in fruits depends mainly on terpenoid lactones such as limonin in oranges, or flavonoid glucosides, such as naringin in grapefruit. The saltiness is contributed by various natural salts, and astringency is due to flavonoids, alkaloids, tannins, and other factors. These compounds often interact with each other chemically or in terms of perception to influence intensity of flavor components and descriptors (Baldwin 2002). Malundo and others (1995) demonstrated that sourness or acid levels, and aroma compounds affect perception of sweetness in tomato. Aroma is gaining increasing attention in fresh fruits for their flavor quality. Baldwin and others (1998) showed that in addition to contributing to fruit odor, aroma compounds can affect perception of sweetness and sourness in tomato.

Rocha and others (2007) recently established the varietal volatile profile of musts from white *V. vinifera* L. varieties. The free volatile components were extracted using

TABLE 17.1. General Composition of Grapes

Constituents	Freshly Expressed Juice by Volume
Water (%)	70–80
Carbohydrate (%)	15–25
Dextrose (glucose) (%)	8–13
Levulose (fructose) (%)	7–12
Pentose (mg/L)	100–500
Pectin (mg/L)	100–1000
Inositol (mg/L)	200–800
Total organic acids (g/L) (tartaric, malic, citric, and tannins)	0.3–1.5
Nitrogenous compounds (g/L)	0.1–1.7
Proteins (g/L)	0.01–1.0
Amino acids (g/L)	0.17–1.1
Minerals (%)	0.3–0.6

Adapted from Amerine and others (1967).

a liquid–liquid continuous method and analyzed by GC-MS. The potential volatile compounds (PVCs) were determined after heat treatment, followed by an enzymatic treatment. The PVC fraction contained the compounds released by the enzymes from the glycosidically linked components plus those produced by the heat treatment at the must pH 3.2 as well as compounds arising from the thermal degradation of sugars. Based on three varietal chemical groups, viz., terpenoids, C13 norisoprenoids, and aromatic alcohols, the volatile profile of each of the four varieties (Fernao-Pires [FP], Bical, Cerceal, and Arinto) was established, which allowed their aroma potential to be defined. The results suggested that it is possible to establish markers (volatiles) for the characterization of must varieties independently of the harvest effect (Rocha et al. 2007). Coelho and others (2007) analyzed the variety- and prefermentation-related volatile compounds of white grape berries to define their evolution profile.

Volatile Compounds in Grapes (Grape Aroma)

Volatile compounds in fruits contribute to their aroma. Those volatiles that are present in concentrations and can be perceived by the human nose are assumed to contribute to fruit flavor or aroma. The "Ascending Method of Limits," prescribed by the American Society of Testing and Materials (ASTM 1991), is employed to determine odor thresholds by placing a compound in a background similar to a food medium and testing to determine the level at which it can be detected by smell. Log odor units are then calculated from the ratio of the concentration of a component in a food to its odor threshold. Compounds with positive odor units are assumed to contribute to the flavor of a food. The aroma perception of volatile compounds is, however, influenced by the medium of evaluation (Baldwin 2002).

Aroma is a significant and complex character of grape quality (Kanellis and Roubelakis-Angelakis 1993). Gunata and others (1985a,b) and Strauss and others (1986) showed that characteristic aroma of grapes and wines is a natural blend of

several hundred chemically different compounds, synthesized during fruit ripening. These aromatics are primarily located in the skin of the berries, although their precursors are synthesized in the leaves. The synthesis and evolution of aroma takes place in the berries (Winkler et al. 1974).

Grape aroma is a distinctive varietal character. Whereas *labrusca* and *rotundifolia* cultivars have a distinct and pronounced odor, those of *vinifera* (barring the Muscat group) have a more delicate and subdued aroma (Kanellis and Roubelakis-Angelakis 1993). Winkler and others (1974) reported that the foxy aroma of *V. labrusca* is attributed to methyl anthranilate. Muscat varieties have an aroma almost as distinguished as that of the *labrusca* cultivars and their hybrids. Thomson Seedless, on the other hand, has a milder aroma.

The major aromatic fraction of Muscat odor is composed of monoterpenes, such as linalool and geraniol. Certain alcohols like benzyl and 2-phenylethyl alcohols, as well as ethers, aldehydes, hydrocarbons, and polyfunctional derivatives, are believed to be mainly contributing to grape aroma (Gunata et al. 1985b). Whereas genaniol and nerol are localized in the berry skin, linalool is equally distributed between the juice and berry solids. The terpenes are found either in the free or glycosidically bound forms, the latter occurring as disaccharide glucosides, that is, β-rutinosides and 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides (Williams et al. 1982a,b).

The free and bound monoterpene levels increase during development and maturation of the berry (Kanellis and Roubelakis-Angelakis 1993). The bound forms predominate in the green berry stage, exhibiting higher levels than the free forms throughout maturation (Gunata et al. 1985a,b; Wilson et al. 1984). The bound fraction continues to increase even after overripeness, whereas accumulation of free forms may stop or decline (Gunata et al. 1985a,b). This release of the glycosidic forms is thought to contribute for the enhancement of grape juice aroma, which may take place via either acid or enzymatic hydrolysis (Gunata et al. 1988, 1989, 1990; Williams et al. 1982b). Gunata and others (1988) further showed that enzymatic hydrolysis of grape monoterpenyl disaccharide glycosides in berries is a sequential reaction requiring three glycosidases: α-L-arabinofuranosidase or α-L-rhamnopyranosidase, and β-D-glucopyranosidase. Both Muscat and non-Muscat cultivars have these enzymes in their berries, and they increase considerably during ripening (Aryan et al. 1987; Gunata et al. 1989). These authors also demonstrated that while β-glycosidase and β-galactosidase activities were mostly present in the pulp/juice rather than in the skins, bound and free glycosidases were mostly localized in the berry skin.

According to Van Wyks and others (1967), lipids may contribute adversely to the aroma of grapes, via the actions of lipoxygenase (Caryel et al. 1983) and subsequently, by the actions of cleaving enzyme and alcohol dehydrogenase (Kanellis and Roubelakis-Angelakis 1993), resulting in the production of six-carbon aldehydes and alcohols (hexanal, 2-hexanal, etc.). This necessitates the crushing of grape berries so that previously isolated aroma precursors come in to contact with the corresponding enzymes. Caryel and others (1983) reported the occurrence of lipoxygenase and alcohol dehydrogenase activities in grapes. Kanellis and Roubelakis-Angelakis (1993), however, emphasized that despite the considerable work on identification of aromatic components in grapes and wines, their enzymatic synthesis and regulation need to be elucidated by further research.

Sweetness (Sugars) in Grapes

Grape sweetness can be accounted for by the presence of over 99% of glucose and fructose in grape juice, which also constitute from 12% to 27% or more of the fresh weight of mature grape berry as the TSSs (Winkler et al. 1974). During the early stages of berry development (green berries), glucose accounts for 85% of the total sugar content, glucose predominating over fructose during veraison. In ripe berries, the ratio of glucose to fructose content approaches unity, whereas in the overripe grapes, fructose content often exceeds glucose (Coombe 1987; Peynaud and Ribereau-Gayon 1971; Winkler et al. 1974). Fructose being sweeter than glucose, cultivars containing higher amounts of fructose at maturity may be picked earlier than those containing more glucose (Kanellis and Roubelakis-Angelakis 1993). According to Lavee and Nir (1986), warmer climates tend to result in a lower glucose-to-fructose ratio in grapes.

Several other sugars, in addition to glucose and fructose, are present in small amounts, including sucrose (less than 0.1% in ripe berries), raffinose, stachyose, melibiose, maltose, and galactose (Winkler et al. 1974). *V. vinifera* cultivars appear to contain less sucrose (0.019–0.6%) than *V. rotundifolia* or *V. labrusca* varieties (0.2–5%). Also, grape flesh has lower sucrose content than in the skin and brush (Coombe 1987). Winkler and others (1974) reported that pentoses, mainly arabinose and traces of xylose, are present in small amounts (0.3–1.09/L of juice) in ripe grapes.

Sugars begin to accumulate rapidly at the onset of ripening (Hrazdina et al. 1984; Possner and Kliewer 1985) coinciding with the beginning of berry softening (Coombe and Phillips 1982). Peynaud and Ribereau-Gayon (1971) attributed this dramatic increase in sugar content during phase III to an enhanced photosynthetic activity. Berries appear to exert stronger sink capacity as compared with other developing organs of the vine after veraison (Alleweldt et al. 1975; Lavee and Nir 1986).

Most sugars are first synthesized photosynthetically in the leaves and are then transported through the phloem to the berries, primarily in the form of sucrose (Lavee and Nir 1986; Winkler et al. 1974), which is hydrolyzed to glucose and fructose in the berries (Saito and Kasai 1978). Hawker (1969) demonstrated that sugar translocation to, and accumulation in, the grape berries takes place through following sequential reactions: phosphorylation of glucose and fructose, synthesis of sucrose phosphate, hydrolysis of sucrose phosphate to sucrose in the leaves, and transport followed by hydrolysis of sucrose to glucose and fructose in the berries.

Sourness (Acids) in Grapes

Organic acids are known to affect the nature and content of other organic compounds in grapes significantly, as well as wine flavor, color, and stability (Lamikanra et al. 1995). Winkler and others (1974) stated that sourness (acid fraction) in grapes consists mainly of tartaric and malic acids, accounting over 90% of the total grape acidity. Other organic acids found in variable but low concentrations are citric (5–10%), succinic acid, fumaric acid, acetic acid, glycolic acid, lactic acid, aconitic acid, quinic acid, shikimic acid, and mandelic acid (Kanellis and Roubelakis-Angelakis 1993). With all these organic acids being intermediates of glycolysis, tricarboxylic acid (TCA) cycle, glyoxylate cycle, and shikimic acid pathway, it is inferred that all these metabolic pathways are operative in grapes (Ruffner 1982; Winkler et al. 1974).

Sourness in grapes (high acids in some, whereas extremely low in others) is considered a vital characteristic of grape quality (Coombe and Phillips 1982; Lavee and Nir 1986; Winkler et al. 1974), since high acidity not only affects the palatability of table purpose grapes, but also influences suitability of wine grapes for vinification (Ruffner 1982). Kanellis and Roubelakis-Angelakis (1993) stated that excessive tartness of the fruit normally correlates with low sugar concentrations, resulting in poor wine quality. The low acid levels at harvest may be accompanied either by low or high sugar content, depending on the preceding climatic conditions, resulting in "flat" wines made from such grapes.

Total acidity (TA) in grapes is influenced by several factors, primarily by temperature (Klenert et al. 1978; Ruffner 1982; Winkler et al. 1974). Ruffner (1982) reported that continuous warmer conditions resulted in enhanced degradation of malate during ripening, resulting in lower acid content at maturity.

Grape cultivars vary greatly in their ratio between tartarate and malate contents, the varieties with higher tartarate-to-malate ratio are considered more suitable for wine making, especially in warmer climates (Buttrose et al. 1971; Kanellis and Roubelakis-Angelakis 1993; Lavee and Nir 1986). Ruffner (1982) proposed that malic and tartaric acids are synthesized mainly in the fruit berry, most probably from carbohydrate precursors. Although these two acids are closely similar in their chemistry, they appear to exhibit distinctly different accumulative patterns during grape berry ontogeny.

Based on inverse correlation between acid decrease and massive sugar accumulation in the grape berries at veraison, it has been hypothesized that a probable metabolic interrelationship exists between these two important physiological processes. The mechanism controlling the rate of malate remetabolism during ripening is also thought to play a role in controlling the malate concentration during development and senescence of grape berry (Kanellis and Roubelakis-Angelakis 1993).

Lamikanra and others (1995) determined changes by HPLC in organic acid contents of red muscadine grapes with fruit maturity and their distribution within mature berries. The three major acids identified were succinate, tartarate, and malate. Succinate was the most abundant immediately after fruit set, but its concentration dropped sharply as fruits matured. Tartarate was the prominent acid from veraison until fruits were fully mature. Malate content increased gradually until veraison, after which it decreased with fruit ripening. Within the berries, these acids were unevenly distributed. While the grape skin had the highest acid content, the amount of acids in grape seeds was the lowest.

Soyer and others (2003) suggested the following ranges of acid concentrations in grapes: citrate, 30–164 mg/L; tartarate, 4.98–7.48 g/L; and malate, 1.43–3.40 g/L; in grape juice, the corresponding ranges were the following: citrate, 31–181 mg/L, tartarate, 4.07–4.92 g/L; and malate, 1.36–3.47 g/L. The tartaric acid was the major acid in every grape variety analyzed. Grape juices had lower tartaric acid content than berries due to detartration process (Soyer et al. 2003).

Bitterness, Astringency, and Saltiness (Phenolics and Flavonoids) in Grapes

Bitterness, astringency and saltiness in grapes depend on their contents of terpenoids, flavonoid glycosides and alkaloids, tannins, and organic salts. The flavonoids

and phenolics in grapes are of special significance because these compounds contribute significantly to the color, taste, and flavor of fresh fruit as well as the color, taste, and body of wines and other products made from grapes (Kanellis and Roubelakis-Angelakis 1993). The flavonoids have a C15 skeleton with a chromane ring bearing a second aromatic ring B in second, third, or fourth positions. Major phenolics present in grapes include anthocyanins, benzoic acids, cinnamic acids, flavonoids, and tannins (Peynaud and Ribereau-Gayon 1971).

Anthocyanins are major pigments producing various skin colors in berries, and grape cultivars are often classified as white, red, and black varieties. Anthocyanins in grapes are mainly the anthocyanidins, as modified by the attachment of a glucose molecule at 3-OH position or by attachment of glucose both at the 3 and the 5 positions (diglucosides). The latter is characteristic of the American *Vitis* species, viz., *rupestris*, *riparia*, *labrusca*, and so on; *V. vinifera* has only monoglucosides (Kanellis and Roubelakis-Angelakis 1993). The glucose hydroxyls can be esterified with organic acids to produce complex compounds. Some of the organic acids acylating anthocyanins in grapes are *p*-coumaric acid, caffeic acid, and acetic acid (Webb 1970).

Anthocyanins begin to accumulate in berries at or shortly after an enhanced accumulation of sugars. Anthocyanins are at equilibrium between the red-colored flavylium salt, the purple anhydrous base, and the colorless carbinol base forms (Hrazdina and Moskowitz 1980). According to Kanellis and Roubelakis-Angelakis (1993), cyanidin and delphinidin contents increase sharply during the first few weeks of ripening but decrease thereafter, thus indicating that they are transformed into further stable pigments.

Pirie and Mullins (1976, 1977) proposed that endogenous sugars probably cause the synthesis of anthocyanins and other phenolics. However, other reports have shown that treatments that increase the synthesis of anthocyanins (light and ethylene) do not affect the levels of sugars in grape skins (Wicks and Kliewer 1983). Several external factors, including light, temperature, crop size, leaf area, and plant growth regulators influence the synthesis and accumulation of anthocyanins in grape berries (Kataoka et al. 1982; Ribereau-Gayon 1971, 1972; Roubelakis-Angelakis and Kliewer 1986).

Depending on their total content, as well as the structure of the elementary units and on their degree of polymerization, tannins cause astringency in grapes. In white grapes, major acidic phenols include *trans*-caffeoyltartarate, *cis*-coumaroyl tartarate, and *trans*-coumaroyl tartarate, and the major neutral phenols are catechin, epicatechin, and two unidentified compounds (Kanellis and Roubelakis-Angelakis 1993).

FLAVOR ANALYSIS

Flavor components can be measured by employing various analytic techniques, such as refractometry, paper chromatography, TLC, GC, and HPLC. Soluble solids are generally measured by refractometry, while individual sugars require either gas or paper chromatography or HPLC. Individual organic acids are measured by HPLC and TA by volumetric titration or by pH meter. Often the measurement of total solids, the ratio of solids to TA or pH relate better to sourness than TA itself (Baldwin 2002).

Flavonols are localized in the solid parts of the grape cluster. In red grape cultivars, flavonols are present in much smaller quantities than anthocyanins. The copigmentation of anthocyanins with flavonols does influence plant colors, including grapes (Kanellis and Roubelakis-Angelakis 1993). Paper chromatographic analyses have identified the following flavonols in red grape varieties: the 3-glucosides of kaempferol, quercetin, and myricetin and quercetin-3 glucuronide. The myricetin derivatives are absent in the white cultivars (Cheynier and Rigaud 1986). Roggero and others (1986) employing the HPLC technique verified the above flavonols in grape skins and reported the presence of the following flavonols: kaempferol-3-galactoside, isorhamnetin-3-glucoside, kaempferol and myricetin-3-glucuronides, and three diglycosides.

The grape seeds are rich in phenolics, such as gallic acid, monomer flavonols (catechin and epicatechin), polymeric flavans, such as procyanidins B_1, B_2, B_3, B_4, and epicatechin gallate and epigallocatechin (Oszmianski et al. 1986). These compounds contribute significantly to the oxidative browning of grapes (Kanellis and Roubelakis-Angelakis 1993).

During ripening of grape berries, the content of total phenols rises sharply early in the development process, followed by a steady decline thereafter (Kataoka et al. 1983). Singleton (1966), however, demonstrated that total phenols per berry increase until rather late in maturation, indicating that there is synthesis, which is insufficient to match berry enlargement. Despite appreciable work done on grape flavonoids, their biosynthetic pathway, transformations, and the molecular regulation of the enzymes mediating these reactions remain to be elucidated (Kanellis and Roubelakis-Angelakis 1993).

Macheix and others (1990) described HPLC techniques used for the accurate determination of anthocyanosides in grape and wine extracts, making it possible to compare cultivars. A systematic effort is being made today to determine the criteria to be retained to enable characterization of a cultivar, based on analyses of the anthocyanins in different grape varieties, and the experimental data being processed by computer using factorial analysis. Macheix and others (1990) concluded that the following parameters could be selected from the chemotaxonomic point of view: malvidin monoglucoside contents, anthocyanin acetic ester contents, anthocyanin cinnamic ester contents, and the ratio of malvidin and peonidin glucosides contents.

Initially, flavor isolation procedures of steam distillation and solvent extraction were commonly used to extract and even quantify aroma compounds (Teranishi and Kint 1993). Schamp and Dirinck (1982) later showed that these techniques can modify the flavor profile of a sample both qualitatively and quantitatively. In addition, they are time-consuming and thus, difficult to apply to larger sample sets. According to Baldwin (2002), internal standards that cover the boiling points of volatiles of interest must be incorporated to determine the recovery. The resulting concentration of material allows the identification of compounds by GC-MS.

Currently, purge and trap headspace sampling methods are becoming increasingly popular (Baldwin 2002). These methods employ trapping and concentrating volatile components on a solid support. The volatiles are later released from trap using heat and analyzed by GC or GC-MS (Schamp and Dirinck 1982; Teranishi and Kint 1993). Static headspace methods reflect the true flavor profile; however, the compounds present at very low levels may not be detected and quantified easily.

According to Teranishi and Kint (1993), cryo-focusing (using a cold trap) of static headspace volatiles can help overcome this problem, since samples can be concentrated and recovered without heating losses; while avoiding the possible adulteration, Moshonas and Shaw (1997) successfully employed this method to quantify orange juice volatiles.

Solid phase microextraction (SPME) is a rapid sampling technique where volatiles made to interact with a fiber-coated probe inserted into the sample headspace (Baldwin 2002). The probe is later transferred to a GC injection port where the volatiles are desorbed. This technique has been successfully applied in apples, tomatoes, and strawberries (Baldwin 2002).

The electronic noses, where volatile components are interacted with various sensors allowing for discrimination between samples, have been described by Baldwin (2002). The pattern recognition helps to identify a particular sample or flavor component mixture. This equipment, however, cannot be used to identify or quantify individual components of flavor. Baldwin (2002) further mentioned four basic technologies that have been commercialized, employing two classes of sensors. These four types of sensors are metal oxide semiconductors (MOS), metal oxide semiconductors field effect transistors (MOSFET), conducting organic polymers (CP), and piezoelectric crystals (bulk acoustic wave [BAW]) or quartz crystal microbalance. They can be divided into two types, since they either operated "hot" (MOS, MOSFET) or "cold" (CP, BAW). The "hot" types have low sensitivity to moisture and also have less carryover from one to the next measurement. Modified mass spectrometer can combine the sensor output with the mass data. The new generation "electronic noses" may employ fiber-optic, electrochemical, and bimetal sensors that are currently being developed (Baldwin 2002; Schaller et al. 1998).

The determination of glycosyl glucose (G-G) of black grape berries was found to be optimal, if measured on an aliquot taken from extract made for 1 h with 50% ethanol of homogenates prepared from either fresh or stored (frozen –20°C) berries (Iland et al. 1996). The homogenization of berries resulted in compounds, presumably phenolic, being extracted from the seeds, which interfered with the glucose enzyme assay. This could be eliminated by passing the control and hydrolysate solutions through a C18 RP solid phase extraction (SPE) cartridge prior to glucose analysis.

Palma and Barroso (2002) optimized an ultrasound-assisted extraction (UAE) method to determine tartaric and malic acids from grapes and wine-making products. A fractional factorial experimental design allowed for the determination of the effects of seven extraction variables. The best extraction conditions could be obtained by applying graphic analysis. The most important variables were the extracting liquid and the extraction temperature. Later, a central composite design was applied for optimizing the temperature and the composition of the extracting liquid. The recovery of tartaric and malic acids was established by studying repeatability (precision) of the method. Organic acids were quantified by liquid chromatography (LC) using a post-column buffer and a conductivity detector.

The odorants released by mild acid hydrolysis from an Amberlite XAD-2 odorless fraction of flavor precursors isolated from Tempranillo and Grenache grapes were analyzed in an aroma extract concentration analysis (AECA) experiment (Lopez et al. 2004). AECA detected 98 odor-active regions; the most important odorants released were unsaturated fatty acid derivatives, such as hexanal, octanal,

1-octen-3-one, *E*-2-heptanal, *E*,*E*-2,4-decadienal, fatty acids, and several aliphatic lactones: γ-nona, -deca-, and undecalactones; δ-deca-lactone; and ε-dodecalactone. Some shikimic acid derivatives, such as guaiacol, 2-phenylethanol, ethyl dihydrocinnamate, ethyl cinnamate, 2,6-dimethoxyphenol, 4-vinylphenol, isoeugenol, phenyl acetic acid, and vanillin, were noticed.

Volatile compounds of grapes are responsible for varietal aroma. The current methods used for analysis of volatile compounds are solvent based, time-consuming, and generally requiring large amounts of sample. Sanchez-Palomo and Diaz-Coello (2005) developed the headspace–solid phase microextraction (HS-SPME) method in order to obtain an appropriate technique to study grape volatiles. The optimal sampling conditions were 70°C for 20 min with a 65-μm PDMS/DVB fiber. Employing this technique, 16 volatile compounds were quantified in the pulp and skins of Muscat grapes. Terpenes, mainly linalool, geraniol, and nerol predominated and contributed to a larger extent, to the aroma of Muscat grapes and wines. Jesus Ibarz and others (2006) developed a procedure for GC-MS analysis of the aromas released in the fast acid hydrolysis of precursor fractions from grape musts and skins, comparing different sorbents for the extraction of the precursors. The best results were obtained with Lichrolut EN polymeric resins, which displayed two- and sixfold extraction capacity over Amberlite XAD-2 resins and C18 sorbents, respectively. C18 sorbents are more suitable for the selective extraction of less polar precursors. The initial imprecise method could be improved by greater crushing of the solid parts and SPE instead of liquid–liquid extraction (LLE), which improved reproducibility. In the method finally proposed by these authors, around 100 aromatic components belonging to four larger groups (lipid derivatives, shikimic acid derivatives, norisoprenoids, and terpenes) could be determined with high precision and good reproducibility.

The composition of proanthocyanidins in the grape (*V. vinifera* L. cv. Cabernet Franc) skin was determined by HPLC reversed phase, after fractionation and thiolysis (Cadot et al. 2006). Further information about the proanthocyanidins was obtained by complementing HPLC analytic approach with histochemistry. The 2-μm-thick sections were stained according to the 4-dimethylaminocinnamaldehyde method.

Coelho and others (2007) analyzed the variety- and prefermentation-related volatile compounds of FP white grape berries during ripening by HS-SPME coupled to gas chromatography–quadrupole mass spectrometry (GC-qMS). Grapes were first crushed and macerated before HS-SPME analysis. The sampling started at veraison (beginning of berry ripening) and continued up to 5 weeks in two vineyards. Sixteen terpenoids, two C13 norisoprenoids, two aromatic alcohols, two C6-aldehydes, and three C6-alcohols were identified. The amount of all volatiles increased since veraison toward day 20, followed by a sharp decrease after this day. The maximum amount of varietal volatile compounds coincided with the harvesting day for white table wine production as defined by the ratio of sugar/acid content. The varietal volatile evolution observed in FP grapes indicated that the maximum amount of volatiles remained only for a very short period. Thus, the establishment of the optimum time for harvesting of FP white variety, based on its volatile content, deserves higher accuracy than other varieties. The analysis of the evolution of the terpenoids with higher GC peak area represents the evolution of all varietal compounds. For FP grapes, the screening of linalool, α-terpeneol, and geraniol during

ripening may be used to define the evolution profile of the varietal volatile compounds (Coelho et al. 2007).

SENSORY EVALUATION OF FLAVOR

Sensory science deals with measurement of human perception of flavor in fruits, vegetables, and other fresh products. Consumer preference and acceptance testing, investigating the likes and dislikes of consumers, typically require a large number of panelists, and the results of such sensory evaluation tests vary considerably, depending on the socioeconomic, ethnic, and geographic background (Baldwin 2002). This requires segmenting of subpopulations for a particular study (O'Mahony 1995), and generally, a traditional 9-point hedonic scale is employed for sensory examination of flavor and aroma. Baldwin and others (1995) showed that a very simple 3-point test (outstanding, acceptable, and unacceptable) was effective to evaluate tomatoes.

Difference testing is often used to measure slight differences between food quality, usually arising from one particular aspect of flavor. Descriptive analyses measure intensities of a set of sensory attributes (O'Mahony 1995). For the latter, panelists are required to be trained to detect a range of flavor attributes and score their intensities, often on a 150-mm unstructured line scale. Sensory evaluation in fruits has been used to identify optimal harvest maturity, to estimate flavor quality in breeding lines, to determine optimal storage and handling conditions, to assess the effects of disinfestations or preconditioning techniques on flavor quality, and to measure flavor quality over the postharvest life of the product (Baldwin 2002).

Flavor can be evaluated more precisely by relating chemical data with sensory studies in order to determine important flavor compounds as well as how to measure best those corresponding to the sensory experience. Similarly, sensory data alone cannot provide information on how flavor components and their combinations and relative proportions result in desirable or undesirable flavor quality. Sniff ports (olfactometry detectors) are used with GCs, which allow a person to determine if odors are detectable, as well as their relative intensity and characteristics, as they are separated by the GC column. Young and others (1996) employed this technique successfully for apples. In flavor bioassays, descriptive terms were assigned to the respective peaks on the GC chromatogram that had odor activity (Acree 1993). However, the interactive effects of volatile components with each other and with sugars and acids, both chemically and in terms of human perception, are eliminated in this method.

Statistics have been employed most successfully to relate sensory attributes, preferences, and flavor intensity to chemical components in foods (Bett 1993; Martens et al. 1994). Correlation of physical measurements with sensory analysis can provide meaning to instrumental data, as demonstrated in fruits like apple and tomato (Baldwin et al. 1998; Guadagni et al. 1966; Sinesio et al. 2000). Correlation, and linear and multiple regression (stepwise forward or backwards) techniques have been used in sensory evaluation (Baldwin et al. 1998). Peters and Van Amerongen (1998) used the linear regression technique to establish a relationship between levels of sesquiterpene lactones and bitterness in chicory. According to Baldwin (2002), multivariate methods require large data sets. These regression methods, such

as principle component or discriminant analysis, produce useful results to discriminate between fruit or fruit juice samples based on data for flavor volatiles or for other flavor compounds, as has been shown for tomato (Maul et al. 1998), citrus (Moshonas and Shaw 1997; Shaw et al. 1998), and strawberry (Shamaila et al. 1992).

FLAVOR APPLICATION IN FOOD PROCESSING

The phenolic composition of the grape at maturity is major information for the winemaker. These compounds, anthocyanins, flavonols, and proanthocyanidins, are important for the quality of the processed products, especially for red wines, because they are responsible for their bitterness, astringency, and color properties (Cadot et al. 2006; Cheynier 1999; Haslam 1998). Among these are the proanthocyanidins or condensed tannins, commonly called "tannins" by the winemakers. The proanthocyanidins are oligomeric and polymeric flavan-3-ols, linked together by C (4)-C (6) or C (4)-C (8) interflavonoid bonds. The content of flavan-3-ols is an important factor in determining the quality of grape juices and wines. Several environmental and cultural practices can affect the accumulation of the flavan-3-ols. Also, the composition of these compounds in grapes of different varieties could be affected by water stress, nitrogen, shading, and temperature. These changes during berry development under different conditions are well-known, particularly after veraison (Cadot et al. 2006).

Rioja is the most emblematic Spanish area producing high-quality red wines. *V. vinifera* cv. Tempranillo and Grenache are the most important Spanish red grape cultivars. These two cultivars with minor quantities of Graciano grapes form the base of Rioja wines. As neutral grape cultivars, the aroma and flavor of Tempranillo and Grenache grapes are stable. There is an important but unknown part of wine aroma that comes from the odorless flavor precursor present in grape (Lopez et al. 2004).

Terpenols released from grape precursors have been known to play an actual or potential role in the aroma of Muscat wines (Williams et al. 1980, 1982b) or in the formation of some characteristic off-flavors in Riesling wines (Winterhalter 1991). In nonfloral grape cultivars, several studies have established a connection between the aroma attributes of hydrolyzed flavor precursors from the grapes and aroma attributes of their wines (Abbot et al. 1991; Francis et al. 1992). Francis and others (1999) demonstrated that important sensory attributes were correlated with the concentrations of specific components of the hydrolysates. Lopez and others (2004) identified and classified the main odor-active chemicals released by mild acid hydrolysis from a fraction of odorless flavor precursors extracted from grapes, according to their potential aroma importance and possible biochemical origin.

The main grape varieties used in Madeira (Portugal) to produce quality wine include Boal, Malvasia, Sercial, and Verdelho. Camara and others (2004) estimated the free fraction of varietal aroma compounds of these varieties, using 39 samples of musts analyzed to determine their contents of monoterpenes and C13 norisoprenoids (terpenoids). The musts had differential contents of terpenoids. In contrast to Verdelho musts, Mavasia musts showed the highest free terpenoids content. The relationship between the compounds and the varieties under study was established by applying principal component analysis and linear discriminant analysis to the

data, revealing a good separation and classification power between the four groups as a function of varietal origin.

Masa and Vilanova (2008) reported flavonoids and the volatile composition of wines of Albarin Blanco, a white *V. vinifera*, cultivar native of Asturias (North of Spain). Sixteen flavonoids (five dihydroxyflavonols, seven quercetin derivatives, and four kaempferol derivatives) and 34 aroma compounds were identified and quantified by HPLC and GC, respectively. The dihydroflavonols were the most abundant flavonoids, and the higher alcohols and esters were quantitatively the largest group of volatile compounds in wines made from this cultivar, making up over 80% of the free volatiles. Linalool, β-ionone, isoamyl alcohols, ethyl acetate, isoamyl acetate, ethyl hexanoate, and ethyl octanoate were found to be the most important contributors to the aroma of Albarin Blanco wines.

TRANSGENIC AND GENETIC IMPROVEMENTS IN RELATION TO FLAVORS

Genetically modified (GM) or transgenic crops have been the focus of considerable attention of the farmers, agricultural/horticultural scientists, molecular biologists, the general public, and the media (Desai 2004). One of the most promising approaches currently available to improve the quality, diversity, and yield of crops is the evolution of transgenic crops through genetic engineering. The GM or transgenic crops and their varieties can be produced by manipulating plant cells or organs at the molecular level leading to the introduction, integration, and expression of specific and useful characteristics of foreign genetic material in a host plant. Such genetic manipulations have enabled us to produce new crops/varieties with useful agronomic traits such as higher yields, better quality, and insect, viral, or herbicidal resistance as well as to generate custom-made male-sterile plants useful in hybridization. Also, GM crops can be produced with improved postharvest qualities of perishable foods (shelf-life enhancement) and crops that can synthesize a variety of pharmaceutical chemicals, vaccines, and a number of other useful industrial products (Potrykus et al. 1998; Pueyo and Hiatt 1998; Topfer and Martini 1998).

Tomato plants expressing a bacterial ACC deaminase gene, which reduces ethylene synthesis, produced mature fruits that remained firm for at least 6 weeks longer than nontransgenic controls (Klee et al. 1991). Transgenic lettuce and tomato have been obtained that express monellin, which is a sweet protein isolated from African berries that increases flavor and sweetness around 10^4 times that of sucrose on a molar basis (Penarrubia et al. 1992).

Sparse research data are available on transgenity and genetic improvements in relation to flavors in fruits, including grapes. Lucker and others (2004), however, published a report on *V. vinifera* terpenoid cyclases: functional identification of two sesquiterpene synthase cDNAs, encoding (+)-valencene synthase and (–)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. Grapevine flowers emit a volatile sesquiterpene valencene. A full-length cDNA from the cv. Gewurztraminer was functionally expressed in *Escherichia coli* and found to encode valencene synthase. Sesquiterpene synthase and monoterpene synthase transcripts were not detected in the mesocarp and exocarp during the early stages of fruit development, but transcripts

hybridizing with valencene synthase appeared during late ripening of berries. Sesquiterpene synthase transcripts were also detected in young seeds.

CONCLUSIONS

Grapes have significantly contributed to the world fruit production and processing industry in the form of wines, raisins, and grape juice, as well as table grapes. Owing to its "nonclimacteric" nature, grape has not been an attractive candidate for research in the areas of metabolic processes during development and ripening and their regulation at the biochemical and molecular level, which have still been poorly understood. In addition, the high levels of carbohydrates, organic acids, and flavonoids in grapes constitute a barrier to biochemical and molecular studies (Kanellis and Roubelakis-Angelakis 1993).

Flavor in fruits, including grapes, is an elusive, complex parameter of fruit quality, both in terms of chemical and sensory evaluation and in terms of interfacing the two approaches. Flavor quality of grapes is gaining an increasing attention from both the consumers and industry. The bottom line for fruit flavor being genetic (Baldwin 2002) is that grape breeders need more information and analytic tools to select new lines for flavor quality, such as molecular markers that relate to flavor and aroma of fruits in general and grapes in particular. These markers could help to identify vital enzymes in flavor biosynthetic pathways. An integrated approach taken by breeders, molecular biologists, postharvest physiologists, sensory scientists, and flavor chemists, armed with newer biotechnologies, can effectively face the challenge of enhancing as well as maintaining flavor quality after harvest in genetically engineered (transgenic) fruits.

REFERENCES

Abbot NA, Coombe BG, Williams PJ. 1991. The contribution of hydrolyzed flavor precursors to quality differences in Shiraz juice and wines: An investigation by sensory descriptive analysis. *Am J Enol Vitic* 42:167–74.

Acree TE. 1993. Bioassays for flavor. In Acree TE, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: ACS Books, pp. 1–20.

Alleweldt G, During H, Waitz G. 1975. Utersuchungen zum mechanismus der zuckereinlagerung in die qachsenden weinbeeren. *Angew Bot* 49:65–73.

American Society for Testing and Materials (ASTM). 1991. *Standard Practice E679. Determination of Odor and Taste Thresholds by a Force Choice Ascending Concentration Series Methods of Limits, 1-5*. Philadelphia, PA: American Society for Testing and Materials.

Amerine MA, Berg HW, Crues WV. 1967. *The Technology of Wine Making*, 2nd Ed. Westport, CT: AVI Publishing.

Anon. 2008. *World Markets and Trade—Fresh Table Grapes*. Washington, DC: US Department of Agriculture, Foreign Agricultural Service.

Aryan AP, Wilson B, Strauss CB, Williams PJ. 1987. The proportion of glycosides of *Vitis vinifera* and a comparison of their β-glycosidase activity with that of exogenous enzymes, as assessment of possible applications in enology. *Am J Enol Vitic* 38:182–8.

Baldwin EA. 2002. Fruit flavor, volatile metabolism and consumer perceptions. In Knee M (ed.), *Fruit Quality and Its Biological Basis*. Boca Raton, FL: CRC Press, pp. 89–106.

Baldwin EA. 2004. Flavor. In: Gross KC, Wang CY, Saltveit M (eds.). *The Commercial Storage of Fruits, Vegetables and Florist and Nursery Stocks*. Agricultural handbook number 66. Beltsville, MD: Agricultural Research Service.

Baldwin EA, Malundo TMM, Bender R, Brecht JK. 1999a. Interactive effects of harvest maturity, controlled atmosphere and surface coatings on mango (*Mangifera indica* L.) flavor quality. *HortScience* 34:514.

Baldwin EA, Burns JK, Kazokas W, Brecht JK, Hagenmaier RD, Bender RJ, Pesis E. 1999b. Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica* L.) ripening during storage. *Postharvest Biol Technol* 17:215–26.

Baldwin EA, Nisperos-Carriedo MO, Scott JW. 1992. Levels of flavor volatiles in a normal cultivar, ripening inhibitor and their hybrid. *Proc Fla State Hortic Soc* 140:86–9.

Baldwin EA, Scott JW, Einstein MA, Malundo TMM, Carr BT, Shewfelt RL, Tandon KS. 1998. Relationship between sensory and instrumental analysis for tomato flavor. *J Am Soc Hortic Sci* 12:906–15.

Baldwin EA, Scott JW, Shewfelt RL. 1995. Quality of ripened mutant and transgenic tomato cultigens. *Proc Tomato Qual Workshop* 503:47–57.

Bett K. 1993. Measuring sensory properties in meat in the laboratory. *Food Technol* 47:121–5.

Bolin HR, Salunkhe DK. 1971. Physico-chemical and volatile flavor changes occurring in fruit juices during concentration and foam-mat drying. *J Food Sci* 36:665–7.

Buttrose MS, Hale CR, Kliewer WM. 1971. Effect of temperature on the composition of "Cabernet Sauvignon" berries. *Am J Enol Vitic* 22:71–5.

Cadot Y, Minana Castello MT, Chevalier M. 2006. Flavan-3-ol compositional changes in grape berries (*Vitis vinifera* L. cv. Cabernet Franc) before veraison, using two complementary analytical approaches, HPLC reversed phase and histochemistry. *Anal Chim Acta* 563:65–75.

Camara JS, Harbert P, Marques JS, Alves MA. 2004. Varietal flavor compounds of four grape varieties producing Madeira wines. *Anal Chim Acta* 513:203–7.

Caryel A, Crouzet J, Chan HW. 1983. Evidence for the occurrence of lipoxygenase activity in grapes (variety Carignane). *Am J Enol Vitic* 34:77–82.

Cheynier V. 1999. Tannins in livestock and human nutrition. In Broker JD (ed.), Proceedings of an International Workshop, May 31–June 2, 1990, Australian Centre for International Agricultural Research, Canberra, Australia.

Cheynier V, Rigaud J. 1986. HPLC separation and characterization of phenols in the skins of *Vitis vinifera* var. Cinsault. *Am J Enol Vitic* 37:248–51.

Coelho E, Rocha SM, Barros AS, Delgadillo I, Coimbra MA. 2007. Screening of variety- and pre-fermentation related volatile compounds during ripening of white grapes to define their evolution profile. *Anal Chim Acta* 597:257–64.

Coombe BG. 1976. The development of fleshy fruits. *Annu Rev Plant Physiol* 27:207–28.

Coombe BG. 1987. Distribution of solutes within the developing grape berry in relation to its morphology. *Am J Enol Vitic* 38:127–9.

Coombe BG, McCarthy MG. 1997. Identification and naming of the inception of aroma development in ripening grape berries. *Aust J Grape Wine Res* 3:18–20.

Coombe BG, Phillips PE. 1982. Development of the grape berry. II. Compositional changes during veraison measured by sequential hypodermic sampling. Proceedings of the International Symposium on Grapes and Wines, pp. 132–6, June 1980, Davis, CA.

Desai BB. 2004. *Seeds Handbook: Biology, Production, Processing and Storage*, 2nd Ed., Revised and Expanded. New York: Marcel Dekker, pp. 705–34.

Dieguez SC, Lois LC, Gomez EF, De La Pena MLG. 2003. Aromatic composition of the *Vitis vinifera* grape Albarino. *Food Sci Technol* 36:585–90.

Dokoozlian NK, Kliewer WM. 1996. Influence of light on grape berry growth and composition varies during fruit development. *J Am Soc Hortic Sci* 121:860–74.

Fellman JK, Mattheis JP, Patterson ME, Mattinson DS, Bostic BC. 1993. Study of ester biosynthesis in relation to harvest maturity and controlled-atmosphere storage of apples (*Malus domestica* Borkh). Proceedings of the 6th International Controlled Atmosphere Research Conference, June 1993, Cornell University, Ithaca, NY.

Francis IL, Kassara S, Noble AC, Williams PJ. 1999. The contribution of glycoside precursors to Cabernet Sauvignon and Merlot Aroma. In Waterhouse A, Ebeler E (eds.), *Chemistry of Wine Flavor*. Washington, DC: ACS Books, pp. 13–30.

Francis IL, Sefton MA, Williams PJ. 1992. Sensory descriptive analysis of the aroma of hydrolyzed precursor fractions from Semillon, Chardonnay and Sauvignon Blanc grape juices. *J Sci Food Agric* 59:511–20.

Garcia E, Chacon JL, Martinez J, Izquierdo PM. 2003. Changes in volatile compounds during ripening in grapes of Airen, Macabeo and Chardonnay white varieties grown in La Mancha region (Spain). *Food Sci Technol* 9:33–41.

Guadagni DG, Okano S, Buttery RG, Burr HK. 1966. Correlation in sensory and gas-liquid chromatographic measurements of apple volatiles. *Food Technol* 20:166–9.

Gunata YZ, Bayonove CL, Baumes RL, Cordonnier RE. 1985a. The aroma of grapes. 1. Extraction and determination of free and glycosidically bound fractions of some grape aroma compounds. *J Chromatogr* 331:83–90.

Gunata YZ, Bayonove CL, Baumes RL, Cordonnier RE. 1985b. The aroma of grapes. Localization and evolution of free and bound fractions of some grape aroma compounds (cv. Muscat) during first development and maturation. *J Sci Food Agric* 36:857–62.

Gunata YZ, Bayonove CL, Cordonnier RE, Arnaud A, Galzy P. 1990. Hydrolysis of grape monoterpenyl glycosides by *Candida molisschiana* and *Candida wickerhamii* β-glycosides. *J Sci Food Agric* 50:499–506.

Gunata YZ, Biron C, Sapis JC, Bayonove CL. 1989. Glycosidase activities in sound and rotten grapes in relation to hydrolysis of grape monoterpenyl glycosides. *Vitis* 28:191–7.

Gunata YZ, Bitteur S, Brillouet JM, Bayonove CL, Cordonnier RE. 1988. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydr Res* 184:139–49.

Haslam E. 1998. *Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action*. Cambridge: Cambridge University Press.

Hawker JS. 1969. Changes in the activities of malic enzyme, malate dehydrogenase, phosphopyruvate carboxylase during the development of a non-climacteric fruit (the grape). *Phytochemistry* 8:19–23.

Hrazdina G, Moskowitz H. 1980. Subcellular status of anthocyanins in grape skins. Proceedings of the International Symposium on Grape skins. Proceedings of the International Symposium on Grape and Wine, pp. 245–3, Davis, CA.

Hrazdina G, Parsons GF, Mattick LR. 1984. Physiological and biochemical events during development and maturation of grape berries. *Am J Enol Vitic* 35:220–7.

Iland PG, Cynkar W, Francis IL, Williams PJ, Coombe BC. 1996. Optimization of methods for the determination of total and red-free glycosyl glucose in black grape berries of *Vitis vinifera*. *Aust J Grape Wine Res* 2:171–8.

Jesus Ibarz M, Ferreira V, Hernandez-Orte P, Loscos N, Cacho J. 2006. Optimization and evaluation of procedure for the gas chromatographic-mass spectrometric analysis of the

aromas generated by fast acid hydrolysis of flavor precursors extracted from grapes. *J Chromatogr* 116:217–29.

Kanellis AK, Roubelakis-Angelakis KA. 1993. Grape. In Seymour GB, Taylor JE, Tucker GA (eds.), *Biochemistry of Fruit Ripening*. London: Chapman & Hall, pp. 189–234.

Kataoka I, Suglura A, Utsunomiya N, Tomana I. 1982. Effect of abscisic acid and defoliation on anthocyanin accumulation in Kyoto grapes (*Vitis vinifera* L × *V. labruscana* Bailey). *Vitis* 21:325–32.

Kataoka I, Yasutaka K, Sugiura A, Tomana T. 1983. Changes in L-phenylalanine ammonia lyase activity and anthocyanin synthesis during berry ripening of three grape cultivars. *J Jpn Soc Hortic Sci* 29:273–9.

Kennedy JA, Matthews MA, Waterhouse AL. 2000. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* 55:77–85.

Klee HJ, Hayford MB, Kretzmer KA, Barry GP, Kishore GM. 1991. Control of enzyme synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Plant Cell* 3:1187.

Klenert M, Rapp A, Alleweldt G. 1978. Einfluss der traubentemperatur aut Beerenwachstum und Beeren reipe de Rebsorte Silvaner. *Vitis* 17:350–60.

Lamikanra O, Inyand ID, Leong S. 1995. Distribution and effect of grape maturity on organic acid content of red muscadine grapes. *J Agric Food Chem* 43:3026–8.

Lavee S, Nir G. 1986. Grape. In Monselise SP (ed.), *CRC Handbook of Fruit Set and Development*. Boca Raton, FL: CRC Press, pp. 167–91.

Lopez R, Ezpeleta E, Snchez I, Cacho J, Ferriera V. 2004. Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysis of odorless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. *Food Chem* 88:95–103.

Lopez-Tamames E, Carro-Marino N, Gunata YZ, Sapis C, Baumes R, Bayonove C. 1997. Potential aroma in several varieties of Spanish grapes. *J Agric Food Chem* 45:1729–35.

Lucker J, Bowen P, Bohllmann J. 2004. *Vitis vinifera* terpenoid cyclases: Functional identification of two sesquiterpene synthase cDNAs encoding (+)-valencene synthase and (–)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grape vine flowers and berries. *Phytochemistry* 65:2649–59.

Macheix JJ, Fleuriet A, Billot J. 1990. *Fruit Phenolics*. Boca Raton, FL: CRC Press, pp. 39–85.

Malundo TMM, Shewfelt RL, Scott JW. 1995. Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill) as affected by sugar and acid levels. *Postharvest Biol Technol* 6:103–10.

Martens M, Risvik E, Martens H. 1994. Matching sensory and instrumental analysis. In Piggt JR, Paterson A (eds.), *Understanding Natural Flavors*. New York: Blackie Academic Professional/Chapman & Hall, pp. 60–76.

Masa A, Vilanova M. 2008. Flavonoid and aromatic characterization of cv. Albarin blanco (*Vitis vinifera*). *Food Chem* 107:273–81.

Mattheis JP, Buchanan DA, Fellman JK. 1991. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. *J Agric Food Chem* 39:1602–5.

Mattheis JP, Buchanan DA, Fellman JK. 1995. Volatile compound production by Bisbee Delicious apples after sequential atmosphere storage. *J Agric Food Chem* 43:194–9.

Maul F, Sargent SA, Balaban MO, Baldwin EA, Huber DJ, Sims CA. 1998. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: An application for electronic nose technology. *J Am Soc Hortic Sci* 123:1094–101.

Maul F, Sargent SA, Huber DJ, Balaban MO, Sims CA, Baldwin EA. 1999. Harvest maturity and storage temperature affect volatile profiles of ripe tomato fruits: Electronic nose and gas chromatographic analysis. In Hurs WJ (ed.), *Electronic Noses and Sensor Array Based*

Systems: Design and Applications. Proceedings of the 5th International Symposium on Olfaction and the Electronic Nose. Lancaster, PA: Technomic Publishing, pp. 1–13.

Moshonas MG, Shaw PE. 1997. Dynamic headspace gas chromatography combined with multivariate analysis to classify fresh and processed orange juices. *J Essent Oil Res* 9:133–9.

Negrul AN. 1968. Question of the origin and breeding of the grapevine on a genetical basis. *Genetica* 4:84.

Olmo HP. 1993. Grapes. In MaCrae R, Robinson RK, Sadler MJ (eds.), *Encyclopedia of Food Science Food Technology and Nutrition*. London: Academic Press, p. 2252.

O'Mahony M. 1995. Sensory measurement in food science: Fitting methods to goals. *Food Technol* 49:72–82.

Oszmianski J, Romeyer FM, Sapis JC, Macheix JJ. 1986. Grape seed phenolics extraction as affected by some conditions occurring during wine processing. *Am J Enol Vitic* 25:107–17.

Palma M, Barroso CG. 2002. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. *Anal Chim Acta* 458:119–30.

Park SK, Morrison JC, Adams DO, Noble AC. 1991. Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *J Agric Food Chem* 39:514–8.

Patil VK, Chakrawar VR, Narwadkar PR, Shinde GS. 1995. Grape. In Salunkhe DK, Kadam SS (eds.), *Handbook of Fruit Science and Technology: Production, Composition, Storage and Processing*. New York: Marcel Dekker, pp. 1–38.

Penarrubia L, Kim R, Giovannoni J, Kim SH, Fisher RI. 1992. Production of the sweet protein, monellin in transgenic plants. *Biotechnology* 10:561.

Peters AM, Van Amerongen A. 1998. Relationship between levels of sesquiterpene lactones in chicory and sensory evaluation. *J Am Soc Hortic Sci* 123:326–9.

Peynaud E, Ribereau-Gayon GP. 1971. The grape. In Hulme AC (ed.), *The Biochemistry of Fruits and Their Products*, Vol. 2. London: Academic Press, pp. 179–205.

Pirie A, Mullins MG. 1976. Changes in anthocyanin and phenolic component of grape vine leaf and fruit tissues treated with sucrose, nitrate and abscisic acid. *Plant Physiol* 58:468–72.

Pirie A, Mullins MG. 1977. Interrelationships of sugars, anthocyanins, total phenols, and dry weight in the skin of grape berries during ripening. *Am J Enol Vitic* 26:43–6.

Possner D, Kliewer WM. 1985. The location of acids, sugars, potassium and calcium in developing grape berries. *Vitis* 24:229–40.

Potrykus I, Bilang R, Futterer J, Sautter C, Schrott M. 1998. Genetic engineering of crop plants. In Altman A (ed.), *Agricultural Biotechnology*. New York: Marcel Dekker, pp. 119–60.

Pratt C. 1971. Reproductive anatomy in cultivated grapes—A review. *Am J Enol Vitic* 22:92–106.

Pueyo JJ, Hiatt A. 1998. Production of foreign compounds in transgenic plants. In Altman A (ed.), *Agricultural Biotechnology*. New York: Marcel Dekker, pp. 251–61.

Rapp A. 1995. Possibilities of characterizing wine varieties by means of volatile flavor compounds. *Dev Food Sci* 37:1703–22.

Ribereau-Gayon GP. 1971. Evolution des composes phenoliques au cours de la maturation du raisin. I. Experimentation 1969. *Connaissance de la Vigne et du Vin* 2:247–61.

Ribereau-Gayon GP. 1972. Evolution des composes phenoliques au cours de la maturation du raisin. II. Discussion des resultants obtenus en 1969, 1970 et 1971. *Connaissance de la Vigne et du Vin* 2:161–75.

Ribereau-Gayon GP, Boidron JN, Terrier A. 1975. Aroma of Muscat grape varieties. *J Agric Food Chem* 23:1042–7.

Rocha SM, Coutinho P, Barros A, Delgadillo I, Coimbra MA. 2007. Establishment of the varietal volatile profile of musts from white *Vitis vinifera* L. varieties. *J Sci Food Agric* 87:1667–76.

Roggero JP, Coen S, Ragonnet B. 1986. High performance liquid chromatography. Survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. *Am J Enol Vitic* 37:77–83.

Romani R, Labavitch J, Yamashita T, Hess B, Rae H. 1983. Preharvest AVG treatment of Bartlett pear fruits: Effects on ripening, color change and volatiles. *J Am Soc Hortic Sci* 108:1046–9.

Roubelakis-Angelakis KA, Kliewer WM. 1986. Effects of exogenous factors on phenylalanine ammonia lyase activity and accumulation of anthocyanins and total phenolics in grape berries. *Am J Enol Vitic* 37:275–80.

Ruffner HP. 1982. Metabolism of tartaric and malic acids in *Vitis*: A review—Part A. *Vitis* 21:247–59.

Saito K, Kasai Z. 1978. Conversion of labeled substrates to sugars, cell wall polysaccharides, and tartaric acid in grape berries. *Plant Physiol* 62:215–19.

Salunkhe DK, Desai BB. 1984. Grape. In Salunkhe DK, Desai BB (eds.), *Postharvest Biotechnology of Fruits*, Vol. 1. Baca Raton, FL: CRC Press, p. 95.

Sanchez-Palomo E, Diaz-Coello MS. 2005. Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC-MS. *Talanta* 66:1152–7.

Schaller E, Bosset JO, Escher F. 1998. "Electronic nose" and their application to food. *Lebensm Wiss Technol* 31:305–16.

Schamp N, Dirinck P. 1982. The use of head space concentration on Tenax of objective evaluation of fresh fruits. In Chralambous G (ed.), *Chemistry of Foods and Beverages*. New York: Academic Press, pp. 25–47.

Seymour GB. 1993. Banana. In Seymour GB, Taylor JR, Tucker GA (eds.), *Biochemistry of Fruits Ripening*. New York: Chapman & Hall, pp. 83–106.

Seymour GB, Taylor JE, Tucker GA. 1993. *Biochemistry of Fruit Ripening*. London: Chapman and Hall.

Shamaila M, Powrie WD, Skura BJ. 1992. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). *J Food Sci* 57:1173–6.

Shaw PE, Buslig BS, Goodner KL. 1998. Monitoring changes in citrus juice quality during processing. *Proc Fla State Hortic Soc* 111:280–2.

Sinesio F, Natale C, Quaglia GB, Bucarelli FM, Moneta E, Macagnano A, Paolesse R, D'Amico A. 2000. Use of electronic nose and trained sensory panel in the evaluation of tomato quality. *J Sci Food Agric* 80:63–71.

Singleton VL. 1966. The total phenolic content of grape berries during the maturation of several varieties. *Am J Enol Vitic* 25:107–17.

Soyer Y, Koca N, Karadeniz F. 2003. Organic acid profile of Turkish white grapes and grape juices. *J Food Compos Anal* 16:629–36.

Strauss CR, Wilson B, Gooley PR, Williams PJ. 1986. The role of monoterpenes in grape and wine flavor—A review. In Parliment TH, Crotean RB (eds.), *Biogeneration of Aromas. ACS Symposium Series 317*. Washington, DC: American Chemical Society, pp. 222–42.

Teranishi R, Kint S. 1993. Sample preparation. In Acree T, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: ACS Books, pp. 137–67.

Topfer R, Martini N. 1998. Engineering of crop plants for industrial traits. In Altman A (ed.), *Agricultural Biotechnology*. New York: Marcel Dekker, pp. 161–81.

Van Wyks CJ, Webb AD, Kepner RE. 1967. Some volatile compounds of *Vitis vinifera*, variety White Reisling. 1. Grape juice. *J Food Sci* 32:660–4.

Webb AD. 1970. Anthocyanin pigments in grapes and wines. *Suomen Kemistilehti* A43:64–74.

Wicks AS, Kliewer WM. 1983. Further investigations into relationships between anthocyanins, phenolics and soluble carbohydrates in grape berry skins. *Am J Enol Vitic* 34:114–6.

Williams PJ, Strauss CR, Wilson B. 1980. New linalool derivatives in Muscat of Alexandria grapes and wines. *Phytochemistry* 19:1137–9.

Williams PJ, Strauss CR, Wilson B, Massy-Westropp RA. 1982a. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* 21:2013–20.

Williams PJ, Strauss CR, Wilson B, Massy-Westropp RA. 1982b. Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides rationalizing the monoterpene composition of grapes. *J Agric Food Chem* 30:1219–23.

Wilson B, Strauss CR, Williams PJ. 1984. Changes in free and glycosidically bound monoterpenes in developing Muscat grapes. *J Agric Food Chem* 32:919–24.

Winkler AJ, Cook JA, Kliwer WM, Lider LA. 1974. *General Viticulture*. Berkeley, CA: University of California Press.

Winterhalter P. 1991. 1,1,6-Trimethyl-1, 2-dihydronaphthalene (TDN) formation in wine. I. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro [4,5] dec-6-ene-2, 8-diol rationalizing the origin of TDN and related c13 norisoprenoids in Riesling wine. *J Agric Food Chem* 39:1825–9.

Wright DH, Harris ND. 1985. Effect of nitrogen and potassium fertilization on tomato flavor. *J Agric Food Chem* 33:355–8.

Young H, Gilbert JM, Murray SH, Ball RD. 1996. Causal effects of aroma compounds on Royal Gala apple flavors. *J Sci Food Agric* 71:326–9.

CHAPTER 18

The Aroma of Wine

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Wine is a foodstuff product that comes exclusively from the fermentation of either the grape grains or the must of fresh grapes. It has been a well-known product since the Neolithic period (McGovern et al. 1996), and it has accompanied humankind ever since. In the Mediterranean area, wine has been an important ingredient in the daily diet together with olive oil and white bread. For years, wine has had two main tasks: to supply the calories quickly and to quench the thirst in a pleasing way. Nowadays, the first is no longer required; the second one is the most important feature for the consumers. In fact, wine has turned into a luxury product that has led enologists to pursue more and more perfect wines and researchers to identify the molecules responsible for their good and bad properties.

The five senses take part in the sensations linked to wine tasting, but without any doubt, the odor, taste, and chemostatic sensations are the most important ones. For this reason, the aroma of wine is perhaps the most valuable quality, and, as any other attribute, it has to be well balanced, in perfect harmony with wine's characteristic molecules. This is why knowing the composition of the aroma of wine is of great interest.

It is a well-known fact that most of the varieties of *Vitis vinifera* provide grapes with poor aromatic characteristics. However, the wines produced from them can be very aromatic and even have hints specific of each variety, which can be developed and enhanced with time and age. The enologists classify these aromas as primary of prefermentative, secondary or from the fermentation, and tertiary or from aging. Nowadays, knowledge about the chemical composition of the grape grains and about the real contribution of the different volatile molecules of the wine to its aroma makes this classification incomplete or at least susceptible to improvement.

Now we know that on one hand, there are volatile molecules in grapes that contribute to the aroma of wine, and on the other hand, there are nonvolatile and non-odorous molecules that can be converted into volatile and odorous molecules through two different mechanisms: either a simple break of a chemical bond or a more complex chemical reaction. Such compounds are named aroma precursors.

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The simple breakdown of the chemical bond usually takes place via enzymatic methods, mainly during the fermentation or alternatively by hydrolysis during either fermentation or aging.

Prefermentative Aromas

The odorous molecules of the grapes are usually characteristic of the variety (Bayonove 1998). That is why this primary aroma is called free varietal aroma. The first compounds of this class identified belong to the monoterpene family, mainly monoterpenols, such as linalool and nerol, and although they are very common in several grape varieties, their concentration is very low. Only the most floral varieties such as Muscat and Alsacian varieties have quite high concentrations of these compounds, and that is why their odor matches that of their wines.

Other important molecules belong to the sesqui-methoxypyrazines, among which the isobutylmethoxypyrazine plays the main role (Allen et al. 1991). The characteristic odor of the compounds is herbal and vegetal, and, in most of the wines, it cannot be considered as a positive scent. The content of pyrazines in grapes diminishes as the grapes ripen. That is why a clear perception of this scent in the wine indicates almost always an early vintage and can be considered as a failure.

These molecules are present in many grape varieties, such as the Cabernet family, including Cabernet Sauvignon, Cabernet Franc, and Merlot, which have the highest concentration of them (Baumes 2006).

The rest of the odorant molecules of the *V. vinifera* varieties do not contribute much to the varietal aroma of wine, and, consequently, they are not indicators of grape variety. For this reason, the quality of wine is primarily dependent on, besides the characteristics of the soil, climate, and culture, the compounds generated by the aroma precursors along with the fermentation and the aging aromas.

Herbal scents are often found in the musts, and in some wines, it could be thought that they are prefermentative, varietal aromas. This is not true. On one hand, the compounds responsible for these scents are aldehydes formed from unsaturated fatty acids present in both the grape skin and the pulp. The break of the grains in contact with the air triggers the enzymatic processes that produces hexanal and 2-hexenal. Although the content of fatty acids and the enzymatic activity, which also depends on the ripening stage, are different in each variety of grapes, the final products are always the same. For this reason, these products cannot be recognized as varietal aromas (Drawert 1974).

On the other hand, during fermentation, the yeasts reduce the aldehydes to alcohols, with hexanol as the major compound. This compound is slightly odorous, then its contribution to the aroma of wine may be minimum or even null. In any case, it would be a failure.

Secondary Aromas

The fermentative aromas are those that give the essential aroma to wine, and they are also the most abundant. These compounds come of course from the yeast's

substrates such as the sugars (polysaccharides), nitrogenated compounds, lipids, sulfur compounds, and, in general, from many other molecules that directly or indirectly intervene in the fermentation. The metabolism of these families supplies the alcohols, the fatty acids and their ethyl esters, the branched fatty acids and their ethyl esters, with acetate, fusel alcohols, acetoin, and diacetyl among the best known. The aromas originating from them are always the same. This means that they are not dependent on grape variety and do not produce specific aromas. Consequently, the yeast's substrates are not classified or named as aroma precursors, although they are. Only the phenolic acids and the amino acids are the exception to this rule.

The cinnamic phenolic acids, mainly the cutaric and fetaric acids, are the precursors of vinyl and ethyl phenols and vinyl and ethyl guayacols, respectively. These acids are found in the skin of the grapes more than in the juice, and for this reason, their content in the white musts is lower than in rose wines and much lower than in red wines. Despite these facts, the content of the vinylic aromas is higher in white wines because of their enzymatic activity. In fact, the decarboxylation of the free cinnamic phenolic acids is carried out by the enzyme cinnamate decarboxylase, which is present in the *Saccharomyces cerevisae* yeasts. However, this enzyme is inhibited by the catechins. As in the musts macerated with the skin, the content of these tannins is higher than in the white musts; the enzymatic action is lower (Chatonnet et al. 1993).

The amino acids play an important role in the metabolism of both yeasts and lactic bacteria as a nitrogen source for the biosynthesis of proteins, as energy source, and as a system to regulate the pH in an acidic medium, as well as to regenerate the co-substrate. The first step in the catabolism of amino acids could be either a decarboxylation, a desamination, or an elimination carried out by the enzymes of the following groups: decarboxylases, transaminases, deaminases, lyases, and dehydratases. The final compounds produced by them are alpha-keto acids, aldehydes, alcohols, carboxylic acids, and others (Ardo 2006). The amino acidic profile of different varieties of *V. vinifera* is different, and it has been demonstrated that their contribution to the global aroma of the obtained wines is different too and very characteristic of each variety. Consequently, the aromatic profile generated by the amino acids could be considered as varietal (Hernández Orte et al. 2002).

During the fermentation and as mentioned above, some aromas coming from other non-odorous molecules appear. Among them, glycosylate, carotenoid, cysteine, and glutation derivatives are the most important. The glycosylate derivatives are molecules that combine one molecule of glucose with one volatile compound named aglycone. In addition, glucose can be linked to another sugar, giving a diglycoside such as alpha-arabinofuranose, alpha-ramnopyranose, or beta-apioeritrofuranose.

The molecule of aglycone can belong to several families, but it needs an acidic, alcohol, or phenol function to be linked to glucose. The presence of many molecules having such a function in the grapes makes the number of glycosides known as aroma precursors surpass 100.

The release of aglycone via the break of the glycosidic bond is mainly a chemical phenomenon, hydrolysis, and as such is affected by temperature and pH. The temperature is never very high during the wine-making process, and the pH of wine must is not low; thus, the kinetic of this process is slow. For this reason, it is better

for the generation of these aromas to occur during aging (Marais 1983; Voirin 1990; Winterhalter 1993).

The glycosidic bond can also be broken by the enzymatic action of glycosidase. These enzymes are present in grapes; it could then be expected that they were the major enzymes responsible for these aromas. However, this is not true, since the glucose inhibits the action of glycosidase, which, together with the low pH of the must, limits the natural enzymatic activity in the fermentation process (Günata et al. 1990, 1998).

The bio-enologic industry supplies the enologists with the enzymatic preparations of filamentous fungi, which have a good performance at the end of the fermentation process, when most of sugars have already disappeared. In these conditions, the release of aromas is noticeable, and, on top of that, they can act over the yeasts to transform them in other odorant compounds, as in the case of the formation of citronellol from geraniol.

The first group of glycosylate compounds found in grapes was the terpene. This finding about its aromatic role has produced many papers and has even been a guide to classify grapes (Mateo and Jiménez 2000).

The study of monoterpenes in both wine and grapes started in the 19th century when Ordenneau found the first compound, the terebene, in 1885. However, the glycosylate terpenes were not found until Cordonier (1956) detected the presence of linalool, geraniol, and alpha-terpineol, suggesting that they were both free compounds and bonded to other molecules giving hydrolyzable aroma precursors. Further on, he pointed out their sensory importance (Cordonier and Bayonove et al. 1974; Ribéreau-Gayon et al. 1975).

The detection of terpenes and their glycosylate precursors in grapes implied a change in the research of the aroma of wine. Many researchers worked on identifying and quantifying these compounds in any wine, independently of the grape variety or the wine-making process, and they wrongly attributed the aroma of many wines to the presence of terpenes. It was only in the 1990s when gas chromatography–olfactometry (GC-O) was applied to the sensory analysis of aromas when it was demonstrated that the content of terpene glycosides was significant only in some grapes and that their free aromas contribute to the final aroma of wine. One of these examples is the Melon B grape, in which 14 terpenes have been found (Schneider et al. 2001).

The presence of these compounds in the grape grain starts in the veraison and increases during ripening (Marais 1983; Wilson et al. 1984). However, at the end of the ripening process, there is no linear relationship between the classical parameters of evolution and the content of glycosides. This is why there is no index of precursors similar to that of phenols.

Other very interesting aroma precursors are the carotenoid derivatives, which are mainly in the skin of the grapes. They are the biogenetic precursors of the glycosides of C13-norisoprenoids (Winterhalter 1993) from which beta-damascenone and vitispyrane are coming. Surprisingly, an important odorant, such as the beta-ionone, does not originate in the degradation of any glycosidic precursor, but in an *in vitro* degradation of beta-carotene. This pathway is quite common, and that is why the presence of several norisoprenoids in wine could occur because of the simultaneous degradation of corresponding glycosides, norisoprenoids, and carotenoids. This is the case of beta-damascenone that comes from different precursors.

Obviously, if these substances are present in the skin of the grapes, their role in the aroma will be the most important one in red wines with a high alcoholic degree and with a long fermentation period (Baumes 2006).

The last families of non-odorous compounds that generate aromas are the S-conjugated L-cysteine and glutation. This fact has been shown only very recently, although the thiol function of their aromas is very odiferous.

Contrary to the terpenic and carotenoid derivatives, the number of S-conjugated compounds is quite small. Only the *S*-(1-hydroxyhex-3-yl)-L-cysteine (P3MH), the *S*-(4-methyl-2-oxopent-4-yl)-L-cysteine (P4MMP), the *S*-(4-methyl-2-hydroxypent-4-yl)-L-cysteine (P4MMPOH), and the *S*-(1-hydroxyhex-3-yl)-glutation are present. Their content in grapes is quite low, and they are distributed either in both the pulp and the skin such as P4MMP and 4MMPOH or just in the skin such as P3MH. Consequently, film maceration is very important. During fermentation, the thiols 3-sulfanylhexanol (3-mercaptohexanol, 3MH), 3-sulfanylhexyl acetate (3-mercaptohexyl acetate, ac3MH), 4-methyl-4-sulfanylpentan-2-one (4-methyl-4-mercapto pentanone, 4MMP), and 4-methyl-4-sulfanylpentanol (4-methyl-4-mercapto pentanol, 4MHPH) are released. The 3MH is the most abundant thiol, and it is found in all varieties of grapes (Darriet et al. 1991, 1993, 1995; Tominaga et al. 1996, 2000).

The thiol function is extremely labile, which together with a very low concentration level make the analysis of these compounds difficult. That is why very scarce data are available in the literature.

Tertiary Aromas or Aging Aromas

Along with time, wines in barrels and in bottles suffer from a substantial change in their aroma. In the case of barrels, the main cause is the migration of odorous components from the wood and the action of oxygen combined with microbiological processes (Jarauta 2004). The description of all of these effects is over the scope of this chapter.

The changes in the aroma of bottled wine or in an inert container without contact with air are of a chemical nature and are mainly due to the disappearance of important odorant components as a consequence on one hand of reaching the chemical equilibrium and on the other hand of the appearance of other components coming from the reactions of hydrolysis, combination, or structural changes.

In fermentation, the yeasts provide ethyl esters of fatty acids and acetates of high alcohols at concentrations much higher than those corresponding to the chemical equilibrium acid–alcohol. Consequently, during aging, the ester function is slowly lowering to reach equilibrium, and the fresh and fruity aromas characteristic of young wines disappear. On the other hand, as mentioned above, at the typical pH of wine, the precursor molecules are not very stable and slowly decompose to give birth from glycosides to non-terpenic alcohols, C6-compounds, volatile phenols, monoterpenols, and C13-norisoprenoids. Once released, these substances suffer from chemical reactions that drive them to their final decrease. The most important compounds generated in this step are linalool, rose-*cis* oxide, 1,8-cineole, rotundone, w-lactone, eugenol, guayacol, zingerone, methyl salicylate, 1,1,6-trimethyl-1,2-dihydroxynaphthalene (TDN), (*E*)-1-(2,3,6 trimethyphenyllbuta,1,3dien(TPB)-Riesling acetal), vitispyrane, 1-beta-ionone, 2,2,6-trimethylcyclohexanone, and

beta-cyclocitral among the carotenoid derivatives (Francis et al. 1992, 1996; Schneider et al. 2001).

Concerning the thiols, their content generally diminishes, although their evolution is closely linked to the presence or absence of agents that prevent the modification of the redox potential of the wine. An important compound with a content that increases with aging is dimethyl sulfide (DMS). This compound is an important component of truffle aroma and appears in top quality wines and in late vintage wines. It contributes to enhance the perception of reduction bouquet. Its role is then contradictory as its presence in young white wines is considered as negative (Segurel et al. 2005).

Depending on the storage conditions, intense aromas from amino acids can appear as well in wines. Among the most important ones is sotolon.

THE ROLE OF THE ODORANTS IN THE AROMA WINE

Knowledge about the volatile composition of wine has been a subject of constant research for many years. By classical procedures, the major components from fermentation such as alcohols and esters were identified and the minor components by instrumental analysis such as gas chromatography with capillary columns and mass spectrometry detectors. In 1989, about 800 molecules present in the wine were known (Maarse and Vischer 1989), and this number is still growing. Thus, it could be assumed that the problem of knowing the molecules responsible for the aroma in wine and their odorant power were solved. Unfortunately, this is not true, since knowing the analytic concentration of one compound does not mean knowing the aroma properties such as intensity and specific contribution to the sensory perception identified as the aroma. For this reason, it has been necessary to combine the instrumental analytic techniques with the sensorial techniques, starting with the perception thresholds of the odorants.

From the study of both the volatile composition via GC-O and the odor values (Guadagni et al. 1966) of the compounds present in wines elaborated from different varieties of grapes, several interesting conclusions have been obtained (Campo et al. 2005; Culleré et al. 2004; Escudero et al. 2004; Ferreira et al. 1998, 2002; Guth 1997a; Kotseridis and Baumes 2000; López et al. 1999, 2003; Schneider et al. 2003). Also, the tests carried out in the laboratory by adding and subtracting odorants in wines and in model solutions, and the tests of reconstitution of the wine aromas, have allowed scientists to discover the role that several odorants play in the perception of aroma (Campo et al. 2005; Culleré et al. 2007; Escudero et al. 2004, 2007; Ferreira et al. 2002; Guth 1997b; Segurel et al. 2004).

The relationship between the analytic concentration of one compound and its detection threshold value indicates the odorous power, or its number of odor aroma units (OAUs). Obviously, to perceive the aroma of a pure compound, its OAU should be equal or higher than 1.

Surprisingly, among the hundreds of volatile odorous compounds present in wine, only about 60 contribute to its aroma, because most of them have odor values much lower than 1. To explain the different behaviors found, it is better to group the odorants into families and to classify them as basic aromas, subtle aromas, and impact aromas.

Basic Aromas Twenty compounds are present in all wines, from the simplest to the most complex scents in terms of odor, and their aroma values are quite high. Often, 13 among them surpass the 5 AOU and even some of them surpass 20 AOU. These 20 compounds are in fact the base of the wine aroma. Apparently, only one compound, the beta-damascenone, comes directly from the grapes, since the others are produced by the metabolism of the yeasts. These compounds are high alcohols such as butyric, isoamylic, hexylic, and phenylethylic; acids such as acetic, butyric, hexanoic, octanoic, and isovalerianic; ethylic esters from fatty acids; and acetates and compounds such as diacetyl. There is little variation in the concentration of these compounds within the wines. Of course, there is ethanol in all of them. The global odor is named "wine odor," and it is very difficult to describe, as all the scents are perfectly harmonized: fruity scents, banana, pineapple, apple, alcohol, pollen, acids, or red fruits from the forest. On top of that, there is ethanol that has a particular effect on the perception (Cacho 2006).

The behavior of the set of odorants of the base aroma is particular. Their effect is to eliminate or minimize the variation of the aroma by addition or subtraction of some of the odorants, even when they are at a concentration level much higher than their perception threshold. This behavior is similar to a buffer for the pH. In a similar way, to break the aromatic buffer, it is necessary to add one molecule or a group of them with chemical or odor characteristics much more intense than and different from their own buffer compounds. These groups or families of molecules should have similar chemical and odorant properties, and these will be grouped in the paragraphs below (Ferreira et al. 2007).

The breakage of the buffer generates a new aroma perception. This can either smell as the substance originally added or modify a generic perception such as sweet flower, increasing or diminishing it.

The content of ethanol has a great influence in the perception of the fruity scent supplied by the esters. It has been demonstrated that by adding increasing amounts of ethanol to a solution of nine esters at the same concentration level as that found in wine, initially having an intense apple odor, the fruity scent quickly goes down. When the alcohol degree reaches 14.5, the perceived scent is just a hydroethanolic solution, which means that the aroma has been completely overlapped (Escudero et al. 2007).

Subtle Aromas About 16 compounds different from those mentioned above are nearly in all wines as well, although their AOU are low, usually lower than 1. They come from either the fermentative process or the grapes, and they are the responsible for the subtle secondary generic aromas in wines (fruity, sweet, etc.). This means that acting either individually or as a group, they have been capable of breaking the buffer of basic aromas but incapable of providing their specific aroma. Their odor action is additive or synergic, in a way that their characteristics scents can be perceived, although the individual odors of each compound were lower than 1 (Jarauta et al. 2006).

They belong to the following nine chemical families in which the above-mentioned compounds are included too (Escudero et al. 2007): volatile phenols such as guayacol, eugenol, isoeugenol, 2,6-dimethoxyphenol, and allyl-2,6-dimethoxyphenol; ethyl esters of fatty acids—their scents are fruity and apple like, and the components of this series are in close relationship (Ferreira et al. 1995); acetates of high

alcohols, ethyl esters of cyclic, or branched fatty acids recently identified (Campo et al. 2006, 2007); aliphatic aldehydes with 8, 9, or 10 carbon atoms (Ferreira et al. 2006); branched aldehydes such as 2-methylpropanol, 2-methylbutanol, and 3-methylbutanol (Culleré 2005); ketones (Culleré et al. 2006); aliphatic gamma-lactones responsible for the peachy aroma of some red wines (Jarauta et al. 2006); vanillin and its derivatives (methyl vanillate, ethyl vanillate, and acetovanillone); and compounds with caramel scent such as furaneol, homofuraneol, and maltol (Jarauta 2004). Certain carotene and terpene derivatives should be added as well to these families.

Impact Aromas Finally, some of the 14 compounds named as "impact aromas" can be found in some particular wines. These compounds give a characteristic scent to the wine, even at very low concentration. This criterion eliminates from this category most of the esters obtained from the fermentation, since although they have low detection threshold, the odor they provide at the low concentration level at which they are does not permit their identification. For instance, ethyl 2-methyl-butyrate has a threshold of 0.1 ppb, and at this concentration, it only provides a fruity pleasant scent that is very different from its characteristic strawberry scent when it is at a higher concentration level.

The study of the compounds' impact in the wine that is not included in the off-flavors indicates that most of them are varietals and belong to a few families. One of them is pyrazine. The most important is the 2-isopropyl-3-methoxypyrazine, with a detection threshold of 2 ng/L. It has been found in Sauvignon and Albariño white wines, giving them a fresh and green aroma, and in red wines (mainly Cabernet Sauvignon), where it is responsible for the pepper odor.

Among the monoterpenes, the most important ones are linalool, rose-*cis* oxide, and rotundone. Linalool plays an important role in the aroma of many white wines, mainly in the Muscat varieties. The Spanish varieties of Albariño y Treixadura, cultivated in Galicia, produce wines with intense varietal characteristics (Campo et al. 2005; Versini et al. 1994). Linalool also contributes to the floral and citric scents of other grape varieties (Arrhenius et al. 1996; Campo et al. 2005; Lee and Noble 2003; Palomo et al. 2006), and its role is quite complex due to its antagonistic action with thiol compounds.

The second terpene of impact is the rose-*cis* oxide, characteristic of the Gewürztraminer wines. Its sweet flower odor is very pleasant and was identified in this grape for the first time by Guth (1997). Later, it was found as well in another variety of Muscat, Devin (Petka et al. 2006), and in hydrolyzed non-terpenic varieties (Ibarz et al. 2006).

Another compound is rotundone, which was found only recently, and for this reason, information about it and its presence in wines is rare. Its odor is similar to green pepper.

The second wide family of aromatic compounds with impact characteristics is the multifunctional mercaptans. Their discovery and the attribution of the sensorial importance was done in 1991. The role that they play in the aroma of many white wines is decisive because of the personality that they impart, although they are also important in red wines. These thiol compounds are very sensitive to oxygen, which destroys them quickly, at least partially. Often, this variation of concentration implies a change in the perceived scents in some young wines, with a positive effect, as some

too intense green scents disappear. Of course, this is an additional difficulty in their quantitative analysis.

Nowadays, six compounds are described, four of them varietals. The best known is 4-methyl-4-mercaptopentanone that gives the Sauvignon Blanc and Scheurebe wines an odor similar to that of the box tree (Darriet et al. 1991, 1993, 1995; Guth 1997). Its detection threshold is 4.2 ng/L. Another mercaptan with a similar scent is the 3-mercaptohexyl acetate, which is also present in Sauvignon Blanc (Tominaga et al. 1996). It is also the responsible for the tropical fruit scent in the Spanish Verdejo wines, which could be attributed to the joint action with linalool (Campo et al. 2005). Their detection threshold is 60 ng/L.

The 2-mercaptohexanol smells like mango, with a detection threshold of 60 ng/L, and appears in white wines (Tominaga et al. 1998) such as the Petit Arvine (Fretz et al. 2005), red wines (Bouchilloux et al. 1998; Tominaga et al. 2000), and rose wines (Ferreira et al. 2002; Murat et al. 2001). In a model rose wine made from Garnacha grapes, the absence of this compound produces the disappearance of citric and fruity odors and to a simultaneous increase in caramel and floral scents. It is also responsible for the increase in red fruit scents in Merlot and Cabernet Sauvignon, although these odors are well integrated in the global aroma. Its detection threshold is only 0.8 ng/L. Concerning the 4-methyl-4-mercaptopentanol, it seldom surpasses its threshold level of 55 ng/L in wines.

Among the thiol families, there are two compounds that do not have varietal origin: 2-furylmethanethiol (or furfuryl-tiol) and benzyl mercaptan. The first one has been detected in Champagne aged wines and in Chardonnay aged on lees (Tominaga et al. 2003a,b). It has a toasted aroma and gives a torrefacted scent in combination with furfuryl-tiol.

The second one comes from the reaction with sulfhydric acid generated during the fermentation with the furfural present in the barrels as a consequence of the toasted process (Blanchard et al. 2001). This compound has a pleasant coffee odor and has been described in several wines (Tominaga et al. 2003b) and in the Champagne wines.

An interesting impact compound is the lactone named soloton. It smells like curry—intense and very persistent. Its sensorial description depends on its concentration. At high level, it smells like soy sauce. Its taste is sweet, and that is why the industry started to use it as a sweetener instead of flavoring. In oxidized white wines, it is responsible for the liquor scent. It origin seems to be in the oxidative degradation of threonine. For this reason, it is not surprising that it appears in special wines such as sherry aged under a yeast bed (Blanchard et al. 2001; Martin et al. 1990, 1992; Moreno et al. 2005), the Oporto wines (Ferreira et al. 2003a,b), Madeira wines (Camara et al. 2004), natural sweet wines (Cutzach et al. 1998, 1999), top quality white and red wines (Ferreira et al. 2003c), as well as the wines made from botrytized grapes. Its detection threshold is 0.9 μg/L, and its concentration in wine can surpass 50 μg/L.

Another impact compound present in grapes at very low concentration levels but at very high concentrations in the toasted wood is the whiskey lactone. It migrates to the wine in the aging process (Masuda et al. 1984) and gives it a coconut scent. If the concentration increases, it changes to an unpleasant scent that is similar to varnish (Polinitz et al. 2000).

Three compounds of fermentative origin can be included as well in this group: isoamyl acetate, diacetyl, and DMS. The first one gives wines the well-known banana aroma. It is the only ester that belongs to this category. In red wines made from

Tempranillo or Pinotage, it is present at a quite high concentration level, and the same happens in some neutral white wines (Ferreira et al. 1995; Van Wyk et al. 1979).

Diacetyl plays a surprising role in wines as some authors describe it as a failure (Clarke and Bakker 2004), while others consider it as an important contribution to the pleasant bakery aromas of Chardonnay wines (Bartowsky and Henschke 2004; Bartowsky et al 2002; Martineau et al. 1995a,b) or to the sweet aromas in Oporto (Rogerson et al. 2001) and Madeira wines. This compound was one of the first described in the aroma of wines, and nowadays, it is under evaluation for health reasons.

The DMS also plays an ambiguous role in the aroma of wines. Its sulfur aroma is unpleasant (Park et al. 1994), but recent publications demonstrate that it increases the floral aromas in some wines such as those made from the Syrah grapes (Culleré et al. 2007; Escudero et al. 2007).

Finally, it has been demonstrated that little amounts of off-flavors and taints ruin the aroma of wine. They do not destroy the odor compounds, but they overlap the perception. Consequently, they are high-impact compounds.

Interactions within the Aromas

Beginning with the work of Bucks and Axel in 1990 on the gene receptors for aromas in the human pituitary gland, research in this area has been very intense. Nowadays, it is known that an aroma receptor can be specific for one chemical group. Consequently, a complex molecule with several chemical groups can simultaneously activate several receptors, generating an image in the brain that is associated to an object. For example, when the vapor of 1,8-cineole, a terpene that smells like eucalyptus, is breathed in, the image of the eucalyptus appears in the brain. According to this theory, different molecules having the same chemical groups could generate, among others, the eucalyptus image and be identified as eucalyptus aroma as well (Otín et al. 2006).

Taking into account on one hand the great number of aroma receptors existing in our olfactory system and on the other hand the large number of different molecules present in wines and their additive, synergic, and antagonistic effects, it is not surprising that the nuances of wine are unlimited. It can be understood as well that the specific molecules responsible for these nuances, as the enologists expected, have not been found yet. Perhaps, in the 21st century, advances in molecular biology and in neurophysiology will let us predict the exact aroma of a wine just by knowing its chemical composition. Now, this is only possible in very simple wines. For this reason, the only thing we can do is to establish the empirical relationship between the aroma and the chemical composition, to follow the recommendations of the experts, and, of course, to enjoy the aroma of complex wines without worrying about the molecules responsible for it.

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REFERENCES

Allen MS, Lacey MJ, Harris RLN, Brown WV. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Am J Enol Vitic* 42:109–12.

Ardo Y. 2006. Flavor formation by amino acid catabolism. *Biotechnol Adv* 24:238–42.

Arrhenius SP, McCloskey LP, Sylvan M. 1996. Chemical markers for aroma of *Vitis vinifera* var. Chardonnay regional wines. *J Agric Food Chem* 44:1085–90.

Bartowsky EJ, Francis IL, Bellon JR, Henschke PA. 2002. Is buttery aroma perception in wines predictable from the diacetyl concentration? *Aust J Grape Wine Res* 8:180–5.

Bartowsky EJ, Henschke PA. 2004. The "buttery" attribute of wine-diacetyl-desirability, spoilage and beyond. *Int J Food Microbiol* 96:235–52.

Baumes R. 2006. *Le potential aromatique des raisins un parameter multiple*. Bordeaux: Mondiaviti 06, pp. 111–18.

Bayonove C. 1998. Aromes. In Flanzy C (ed.), *Œnologie: Fondemants scientifiques et technologiques*. Paris: Lavoisier Tec & Doc, pp. 165–81.

Blanchard L, Tominaga T, Dubourdieu D. 2001. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. *J Agric Food Chem* 49:4833–5.

Bouchilloux P, Darriet P, Henry R, Lavigne-Cruege V, Dubourdieu D. 1998. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J Agric Food Chem* 46:3095–9.

Cacho J. 2006. La percepción de notas aromáticas del vino y el efecto de ciertas moléculas volátiles. Acta XVII Congreso Anual de la A.C.E. Vilanova del Valles, Barcelona.

Camara JS, Marques JC, Alves MA, Ferreira ACS. 2004. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone levels in fortified Madeira wines: Relationship to sugar content. *J Agric Food Chem* 52:6765–9.

Campo E, Cacho J, Ferreira V. 2007. Solid phase extraction, multidimensional gas chromatography mass spectrometry determination of four novel aroma powerful ethyl esters—Assessment of their occurrence and importance in wine and other alcoholic beverages. *J Chromatogr A* 1140:180–8.

Campo E, Ferreira V, Escudero A, Cacho J. 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. *J Agric Food Chem* 53:5682–90.

Chatonnet P, Dubordieu D, Boidron JN, Lavigne V. 1993. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J Sci Food Agric* 62:191–202.

Clarke RJ, Bakker J. 2004. *Wine Flavour Chemistry*. Oxford, UK: Blackwell Publishing.

Cordonier R. 1956. Recherches sur l'aromatisation et le parfum des vins doux naturels et des vins de liqueur. *Ann Technol Agric* 1:75–110.

Cordonier R, Bayonove CL. 1974. Mise en evidence dans la baie de raisin, var. Muscat d'Alexandrie, de monoterpenes lies revelables par une ou plusieurs enzymes du fruit. *C R Acad Sci Paris D* 278:3387–90.

Culleré L. 2005 Contribución al estudio de componentes carbonilicos del vino. Nuevos métodos de análisis y caracterización de su papel sensorial. PhD thesis, Universidad de Zaragoza, Zaragoza.

Culleré L, Cacho J, Ferreira V. 2006. Validation of an analytical method for the solid phase extraction, in cartridge derivatization and subsequent gas chromatographic-ion trap tandem mass spectrometric determination of 1-octen.-one in wines at ng L^{-1} level. *Anal Chim Acta* 563:51–7.

Culleré L, Cacho J, Ferreira V. 2007. An assessment of the role played by some oxidation-related aldehydes in wine aroma. *J Agric Food Chem* 55:876–81.

Culleré L, Escudero A, Cacho J, Ferreira V. 2004. Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *J Agric Food Chem* 52:1653–60.

Cutzach L, Chatonnet P, Dubourdieu D. 1999. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J Agric Food Chem* 47:2837–46.

Cutzach L, Chatonnet P, Henry R, Pons M, Dubourdieu D. 1998. Etude sur l'arome des vins doux naturels non muscatés. 28 partie: Dosages de certains composés volatils intervenant dans l'arôme des vins doux naturels au cours de leur élevage et de leur vieillissement. *J Int Sci Vigne Vin* 32:211–21.

Darriet P, Lavigne V, Boidron J, Dubourdieu D. 1991. Caracterisation de l'arome varietal des vins de Sauvignon par couplage chromatographique en phase gazeuse-odometrie. *J Int Sci Vigne Vin* 25:167–74.

Darriet P, Tominaga T, Demole E, Dubourdieu D. 1993. Mise en évidence dans le raisin de *Vitis vinifera* var. Sauvignon d'un précurseur de la 44-mercapto-4-méthylpentan-2-one. *C R Acad Sci Paris D* 316:1332–5.

Darriet P, Tominaga T, Lavigne V, Boidron JN, Dubourdieu D. 1995. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flav Frag J* 10:385–92.

Drawert F. 1974. Chemistry of winemaking as a biological-technological sequence. *Adv Chem Ser* 137:1–10.

Escudero A, Campo E, Fariña L, Cacho J, Ferreira V. 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J Agric Food Chem* 55:4501–10.

Escudero A, Gogorza B, Melús MA, Ortín N, Cacho J, Ferreira V. 2004. Characterization to the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity value. *J Agric Food Chem* 52:3516–24.

Ferreira ACS, Barbe JC, Bertrand A. 2003a. 3-Hydroxy-4,5-dimethyl-3(5H)-furanone: A key odorant of the typical aroma of oxidative aged Port wine. *J Agric Food Chem* 51:4356–63.

Ferreira ACS, Hogg T, de Pinho PG. 2003b. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J Agric Food Chem* 51:1377–81.

Ferreira V, Jarauta I, López R, Cacho J. 2003c. Quantitative determination of sotolon, maltol and free furaneol in wine by solid-phase extraction and gas chromatography-ion-trap mass spectrometry. *J Chromatogr A* 1010:95–103.

Ferreira V, Culleré L, Loscos N, Cacho J. 2006. Critical aspects of the determination of pet-afluorobencyl derivatives of aldehydes by gas chromatography with electron capture or mass spectrometric detection. Validation of an optimized strategy for the determination of oxygen-related odor-active aldehydes in wine. *J Chromatogr A* 1122:255–65.

Ferreira V, Escudero A, Campo E, Cacho J. 2007. The chemical foundations of wine aromas a role game aiming at wine quality personality and varietal expression. 13th Australian Meeting of Enology, Adelaide.

Ferreira V, Fernández P, Peña C, Escudero A, Cacho J. 1995. Investigation on the role placed by fermentation esters in the aroma of young Spanish wines by multivariate analysis. *J Sci Food Agric* 67:381–92.

Ferreira V, López R, Escudero A, Cacho JF. 1998. The aroma of Grenache red wine: Hierarchy and nature of its main odorants. *J Sci Food Agric* 77:259–67.

Ferreira V, Ortín N, Escudero A, López R, Cacho J. 2002. Chemical characterization of the aroma of Grenache rosé wines. Aroma extract dilution analysis, quantitative determination and sensory reconstitution studies. *J Agric Food Chem* 50:4048–54.

Francis IL, Sefton MA, Williams PJ. 1992. Sensory descriptive analysis of the aroma of hydrolyzed precursor fractions from Semillon, Chardonnay and Sauvignon Blanc grape juices. *J Sci Food Agr* 59(4):511–20.

Francis IL, Tate ME, Williams PJ. 1996. The effect of hydrolysis conditions on the aroms released from Semillon grape glycosides. *Australian J Grape Wine Res* 2:70.

Fretz CB, Luisier JL, Tominaga T, Amado R. 2005. 3-Mercaptohexanol: An aroma impact compound of Petite Arvine wine. *Am J Enol Vitic* 56:407–10.

Guadagni DG, Okano S, Buttery RG, Burr HK. 1966. Correlation of sensory and gas-liquid chromatographic measurements of apple volatiles. *Food Tech* 20(4):518.

Günata YZ, Bayonove CL, Tapiero C, Cordonnier RE. 1990. Hydrolysis of grape monoterpenyl beta-D-glucosides by various beta-glucosidases. *J Agric Food Chem* 38:1232–6.

Günata Z, Bitteur S, Brillonet JM, Bayonove C, Cordonier R. 1998. Sequential enzymatic hydrolysis of potential aromatic glycosides from grape. *Carbohydr Res* 184:139–48.

Guth H. 1997a. Identification of character impact odorants of different white wine varieties. *J Agric Food Chem* 45:3022–6.

Guth H. 1997b. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J Agric Food Chem* 45:3027–32.

Hernández Orte P, Cacho J, Ferreira V. 2002. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solution and chemometric study. *J Agric Food Chem* 50:2891–9.

Ibarz MJ, Ferreira V, Hernandez-Orte P, Loscos N, Cacho J. 2006. Optimization and evaluation of a procedure for the gas chromatographic-mass spectrometric analysis of the aromas generated by fast acid hydrolysis of flavor precursors extracted from grapes. *J Chromatogr A* 1116:217–29.

Jarauta I. 2004. Estudio analítico de fenómenos concurrentes en la generación del aroma durante la crianza en barricas de roble con diferentes grados de uso. Nuevos métodos de análisis de importantes aromas y caracterización de su papel sensorial. PhD thesis, Universidad de Zaragoza, Zaragoza

Jarauta I, Ferreira V, Cacho J. 2006. Synergic, additive and antagonistic effects between odorants with similar odour properties. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Advances and Trends*. Amsterdam: Elsevier, pp. 2005–208.

Kotseridis Y, Baumes R. 2000. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J Agric Food Chem* 48:400–406.

Lee SJ, Noble AC. 2003. Characterization of odor-active compounds in Californian Chardonnay wines using GC-olfactometry and GC-mass spectrometry. *J Agric Food Chem* 51:8036–44.

López R, Ferreira V, Hernández P, Cacho JF. 1999. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: A comparative study. *J Sci Food Agric* 79:1461–7.

López R, Ortin N, Perez-Trujillo JP, Cacho J, Ferreira V. 2003. Impact odorants of different young white wines from the Canary Islands. *J Agric Food Chem* 51:3419–25.

Maarse H, Vischer CA. 1989. *Volatile Compounds in Food. Alcoholic Beverages. Qualitative and Quantitative Data*. AJ Zeist, the Netherlands: TNO-CIVO, Food Analysis Institute.

Marais J. 1983. Terpenes in the aroma of grapes and wine: A review. *S Afr J Enol Vitic* 4(2):49–57.

Martin B, Etievant P, Henry RN. 1990. The chemistry of sotolon: A key parameter for the study of a key component of Flor Sherry wines. In Bessiere Y, Thomas AF (eds.), *Flavour Science and Technology*. Chichester, UK: Wiley, pp. 53–6.

Martin B, Etievant PX, Quere J, Schlich P. 1992. More clues about sensory impact of sotolon in some Flor Sherry wines. *J Agric Food Chem* 40:475–8.

Martineau B, Acree TE, Henickkling T. 1995a. Effect of wine type on the detection threshold for diacetyl. *Food Res Int* 28:139–43.

Martineau B, Henickkling T, Aeree T. 1995b. Reassessment of the influence of malolactic fermentation on the concentration of diacetyl in wines. *Am J Enol Vitic* 46:385–8.

Masuda M, Okawa E, Nishimura K, Yunome H. 1984. Identification of 4,5-dimethyl-3-hydroxy-2(5H)-furanone (sotolon) and ethyl 9-hydroxynonanoate in botrytised wine and evaluation of the roles of compounds characteristic of it. *Agric Biol Chem* 48:2707–10.

Mateo JJ, Jiménez M. 2000. Monoterpenes in grape juice and wines. *J Chromatog A* 881:557–67.

McGovern PE, Fleming SJ, Katz SH. 1996. *The Origins and Ancient History of Wine*. Amsterdam: Gordon and Breach.

Moreno JA, Zea L, Moyano L, Medina M. 2005. Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. *Food Control* 16:333–8.

Murat M, Tominaga T, Dubourdieu D. 2001. Mise en évidence de composés clefs dans l'arôme des vins rosés et clairets de Bordeaux. *J Int Sci Vigne Vin* 35:99–105.

Otín N. 2006. Caracterización química del aroma de vinos de alta calidad. Contribución al análisis y caracterización de importantes aromas tiolicos del vino. Doctoral thesis, Universidad de Zaragoza.

Palomo ES, Perez-Coello MS, Diaz-Maroto MC, Vinas MAG, Cabezudo MD. 2006. Contribution of free and glycosidically-bound volatile compounds to the aroma of Muscat "a petit grains" wines and effect of skin contact. *Food Chem* 95:279–89.

Park SK, Boulton RB, Bartra E, Noble AC. 1994. Incidence of volatile sulfur-compounds in California wines—A preliminary survey. *Am J Enol Vitic* 45:341–4.

Petka J, Ferreira V, Gonzaiez-Vinas MA, Cacho J. 2006. Sensory and chemical characterization of the aroma of a white wine made with Devin grapes. *J Agric Food Chem* 54:909–15.

Polinitz AP, Pardon KH, Sefton MA. 2000. 4-Ethylphenol, 4-ethylguaiaeol and oak lactones in Australian red wines. *Aust Grapegrow Winemak* 438:47–50.

Ribéreau-Gayon P, Boidron JN, Terrier A. 1975. Aroma of Muscat grape varieties. *J Agric Food Chem* 23:1042–7.

Rogerson FSS, Castro H, Fortunato N, Azevedo Z, Macedo A, De Freitas VAP. 2001. Chemicals with sweet aroma descriptors found in Portuguese wines from the Douro region: 2,6,6-Trimethylcyclohex-2-ene-1,4-dione and diacetyl. *J Agric Food Chem* 49:263–9.

Schneider R, Razungles A, Augier C, Baumes R. 2001. Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melong B. as precursors of odorants in Muscadet wines. *J Chromatog A* 936(1–2):145–57.

Schneider R, Kotseridis Y, Ray JL, Augier C, Baumes R. 2003. Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *J Agric Food Chem* 51:3243–8.

Segurel MA, Razungles AJ, Riou C, Salles M, Baumes RL. 2004. Contribution of dimethyl sulfide to the aroma of Syrah and Grenache Noir wines and estimation of its potential in grapes of these varieties. *J Agric Food Chem* 52:7084–93.

Segurel M, Razungles A, Riou C, Trigueiro MGL, Baumes R. 2005. Ability of possible precursors to release DMS during wine aging and in the conditions of heat alkaline treatment. *J Agric Food Chem* 53:2637–45.

Tominaga T, Baltenweck-Guyot R, Peyrot des Gachons C, Dubourdieu D. 2000. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am J Enol Vitic* 51:178–81.

Tominaga T, Darriet P, Dubourdieu D. 1996. Identification del'acétate de 3-mercaptohexanol, composé a forte odeur de buis, intervenant dans l'arome des vins de Sauvignon. *Vitis* 35:207–10.

Tominaga T, Furrer A, Henry R, Dubourdieu D. 1998. Identification of new volatile thiols in the aroma of *Vitis vinifera*. L. var. Sauvignon blanc wines. *Flav Frag J* 13:159–62.

Tominaga T, Guimbertau G, Dubourdieu D. 2003a. Contribution of benzenemethanethiol to smoky aroma of certain *Vitis vinifera* L. wines. *J Agric Food Chem* 51:1373–6.

Tominaga T, Guimbertau G, Dubourdieu D. 2003b. Role of certain volatile thiols in the bouquet of aged Champagne wines. *J Agric Food Chem* 51:1016–20.

Van Wyk CJ, Augustyn OPH, De Wet P, Joubert WA. 1979. Isoamyl acetate, a key fermentation volatile of wines of *Vitis vinifera* cv. Pinotage. *Am J Enol Vitic* 30:167–73.

Versini G, Orriols L, Dallaserra A. 1994. Aroma components of Galician Albarino, Loureira and Godello wines. *Vitis* 33:165–70.

Voirin S. 1990. Connessance de l'arome du raisin: analyse et syntpese de precurseus heterosidiques. These de Doctorat es Sciences, Univesité Montpellier II.

Wilson B, Strauss CR, Williams PE. 1984. Changes in free and glycosidically bound monoterpenes in developing Muscat grapes. *J Agric Food Chem* 32:919–24.

Winterhalter P. 1993. The generation of C-13 norisoprenoid volatils in Riesling wine. In Crouzet J, Flanzy C, Martin C, Sapis JC (eds.), *Connaissance aromatique des cepages et qualite des vins*. Montpellier, France: Revue Française d'Oenologie, pp. 65–73.

CHAPTER 19

Mango Flavor

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INTRODUCTION

Mango (*Mangifera indica*) is grown commercially in 87 countries, and the geographic distribution covers the entire tropical region encompassing Brazil, Mexico, Middle Africa, Pakistan, India, Indonesia, and the Philippines. Countries like Australia, Egypt, United States (Florida, Hawaii), and West Indies Islands are also known for many exotic varieties of mango. The nativity of mango has been researched extensively, and the origin of mango in India dates back to the Mughal era during which many exotic varieties of mango were introduced from the Persian region. Since then, the native varieties of mango and the imported varieties constituted the parentage for the ultimate development of many domestic varieties by selection, grafting, and other breeding practices. The ancient texts of India describe the existence of mango there in the medieval periods, and over the years, the mango crop in India grew by leaps and bounds, and now grows in states like Uttar Pradesh, Maharashtra, Uttarakhand, Andhra Pradesh, Bengal, Tamil Nadu, Karnataka, Punjab, and Gujrat. India contributes about 60% of the world's production of mango (FAO 1990). The major varieties of mango include Alphonso, Banganapalli, Totapuri, Dashehari, Langra, Chausa, Amrapali, Neelam, and Mulgoa. The modern trends of marketing in the international trade warranted certain quality requirements including sensory attributes to meet the preferences of a wider section of society, and the breeders responded by obtaining exotic cultivars suitable for international trade. Varieties such as Alphonso, Badami, Banganapalli, Dashehari, and Chausa are widely acceptable in the international trade, and the remaining varieties are largely subjected to domestic marketing.

BOTANY

Mango belongs to the family Anacardaceae and to the genus *Mangifera* and was known to have originated in Southeast Asia. The genus was reported to contain 41

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species, but only *Mangifera indica* was cultivated and includes almost all the edible cultivars (Kalra et al. 1995).

Most of the mango cultivars grown in India are of monoembryonic nature, and the polyembryonic cultivars are usually attributed with inferior fruit quality and size. The polyembryonic cultivars include Bellari, Kasargud, Majhgaon, Nileshwar Dwarf, and Salem and are mostly confined to the southern states of India along the western coast. The polyembryonic cultivars reported from other countries include Savre, Pico, Saigon, and Higgins (Majumdar and Sharma 1990). In the United States, Florida region is well-known for mango cultivation, and the major variety grown is Tommy Atkins. In South Africa, the cultivars include Zill, Kent, and Haden. In the case of Australia, the major variety is Kensington, planted extensively in Queensland. Carabao and Pico are widely cultivated in the Philippines. India is known for several exotic cultivars, and the latest varieties released by the Indian Agricultural Research Institute (IARI, New Delhi) include Mallika (Neelam × Dashehari) and Amrapali (Dashehari × Neelam).

CULTIVATION AND AGRONOMY

Mango is a tropical horticultural crop and grows up to an altitude of about 1400 m. The ideal climatic conditions include mean temperatures in the range of 24–27°C with less humidity during the flowering. Certain cultivars of mango grow at 45–48°C, and these higher temperatures are tolerated in the later part of fruit growth and development. The crop is primarily dependent on grafted varieties. The many advantages of grafted varieties include smaller size of plant conducive for mechanical harvesting besides scope for improved sensory attributes and disease resistance. The vegetative propagation of mango includes grafting, layering, and cutting. The effect of different climatic conditions and necessary factors involved in grafting had been researched widely (Singh and Srivastava 1982). Manuring and fertilization protocols were also described for various cultivars in different climatic zones (Chaddha et al. 1981). The usual physiological disorders associated with mango include malformation, clustering, fruit drop, internal breakdown, black tip, and alternate bearing. Mango crop is also highly susceptible for infestation by hoppers, mealy bugs, fruit flies, scale insects, and shoot borers. The preharvest diseases include powdery mildew, anthracnose, red rust, phoma blight, bacterial canker, and sooty molds. The remedial measures were discussed widely for effective control of infestation involving hygiene agro-practices and selection of disease-resistant germplasm by Chandrashekharan and others (1988).

SALIENT FEATURES OF MANGO FLAVOR

Mango fruits are known for exotic flavors, and the variance among different cultivars is significant. The storage practices also influence the flavor profile in a pronounced manner. Mango flavor constitutes hydrocarbons consisting of saturated and unsaturated components. The other volatiles contributing to the overall flavor include esters, alcohols, lactones, acetoins, carboxylic acids, furan compounds, and linalool oxides. The different constituents of each major component are listed in Table 19.1.

TABLE 19.1. Mango Flavor Compounds

Group	Compound
Hydrocarbons	Cyclopentane
	Limonene
	Myrcene
	cis-Ocimene
	trans-Ocimene
	β-Caryophyllene
	Humulene
	cis-Alloocimene
Alcohols	*n*-Butanol
	Linalool
	Isoamyl alcohol
	Terpinen-4-ol
	α-Terpineol
	2-Phenyl ethanol
Esters	Methyl pyruvate
	Ethyl acetate
	n-Butyl butyrate
	Isobutyl butyrate
	Isoamyl butyrate
	Ethyl laurate
	Ethyl decanoate
Lactones	Butyrolactone
	α-Methyl butyrolactone
	γ-Heptalactone
	γ-Hexalactone
	γ-Octalactone
	δ-Octalactone
	γ-Nonalactone
	γ-Decalactone
Others	Acetoin
	Acetic acid
	Furfural
	5-Methyl furfural
	2,5-Dimethyl-2-H-furan-3-one
	2-Acetyl furan
	2,5-Dimethyl-4-methoxy-2-H-furan-3-one
	β-Ionone
	cis-Linalool oxide (five-membered)
	trans-Linalool oxide (five-membered)

Source: Hunter and others (1974).

Alphonso mango is the cultivar that is widely accepted in global trade in fresh or processed forms, and the flavor profiles of the cultivar were investigated thoroughly. Among the hydrocarbons and *trans*-alloocimene was one of the earliest to be identified by gas chromatography (GC) technique coupled with infrared spectroscopy (Mitzner et al. 1965). At the same time, the other important hydrocarbons, that is, myrcene, limonene, caryophyllene, and humulene, were identified based on

mass spectroscopic studies (Hunter and Brogden 1965). Among the esters, acetates and butyrates occupy an important place, and the structural elucidation was made possible by mass spectroscopy with standards drawn from synthetic esters for validation against components derived from mango essence (Beynon et al. 1961).

Volatile constituents with lactone structures were also elucidated by mass spectroscopy and the lengths vary from 4 to 10 carbons, and the geometric isomers give a variety of flavor components (McFadden et al. 1965). The other important flavor components include six alcohols inclusive of α-terpineol and linalool. The four ketones listed include β-ionone. The furan compounds, that is, 2-5-dimethyl-4-methoxy-2-H-furan-3-one, are a major volatile in canned Alphonso mango, and similar compounds had also been identified in processed pineapple and strawberries (Willhalm and Thomas 1969).

Other cultivars of mango were also subjected to intensive studies. Pattabhiraman and others (1969) gave a detailed account of volatile constituents of several South Indian mango varieties. Gholap and Bandyopadhyay (1975a) gave a comparative account of aromatic principles of ripe Alphonso and Langra mangoes. The comparative flavor evaluation of Alphonso and Langra varieties had highlighted certain interesting features. Alphonso flavor was found to have shades of almond- and coconut-like aroma constituents, while Langra had camphor- and peach-like aroma notes. The flavor constituents of both cultivars were dominated by carbonyls, esters, alcohols, terpenes, and lactones. Kapur (1983) gave a detailed flavor profile of Dashehari mangoes. The flavor profile comprises of 30–40 components and the dominant constituents were ocimene, linalool, furfural, *cis*- and *trans*-alloocimene, myrcene, hexalactone, and heptalactone.

Extensive reports exist with regard to mango flavor profiles from different agro-climatic zones of the world. The flavor profiles significantly differ based on cultivars, seasons, agro-climatic zones, and mono/polyembryonic nature and also the origin of the cultivar with specific reference to the propagation methods. As a whole, mango flavor mostly consists of terpenes and esters. Saby and others (1999) characterized aroma components of sap from different Indian mango varieties. The following flavor profiles represent certain important agro-climatic zones of mango cultivation.

SRI LANKA MANGOES

MacLeod and Pieris (1984) reported flavor profiles of three cultivars of mango from Sri Lanka, that is, Jafna, Willard, and Parrot, with a total volatile content of 251, 422, and 628 μg/kg fresh weight. The monoterpene hydrocarbon contributed 50–63% (w/w) of the total volatiles, and the sesquiterpene hydrocarbons contributed 14–19%. Jafna mangoes showed *cis*-β-oscimene (38 %), whereas α-terpenolene was the major volatile of the other two cultivars. Esters were produced by all cultivars (2–16%).

FLORIDA MANGOES

Florida region in the United States is a major mango-growing area. Cultivars such as Tommy Atkins and Keitt were analyzed for their flavor profiles, which were

TABLE 19.2. Volatile Constituents of Tommy Atkins and Keitt Mangoes

Component	Odor Quality
α-Pinene	Pine, cedarwood
Ethanol	Sweet, yeasty
Z-methylpropan-l-ol	Pungent
Toluene	Caramel, solvent
α-Fenchene	Fruity
Camphene	Camphor, mothballs
Cyclohexane	Fruity, sweaty, buttery
Methylcyclohexane	
Dimethylcyclohexane	
Ethylcyclohexane	
Hexanal	Green grass, hexanal
Butan-1-ol	Sweet, silky
β-Pinene	Polish, varnish
Sabinene	Floral fragrant
ρ-Xylene	Cold meat fat
m-Xylene	Green, pungent, mango leaves
Car-3-ene	Fresh green grass
Myrcene	Estery
α-Phellandrene	Sweaty, valeric
3-Methylbutan-1-ol	Lemon like
Limonene	Flat, dull
γ-Terpinene	Fatty oily
β-Phellandrene	Herbal, minty
ρ-Cymene	Floral, fragrant
α-Terpinolene	Cold meat, gravy
2-Furfural	Green grass, fruity, hexanol
cis-Hex-3-en-1-ol	Slightly nutty, coconut
Ethyl octanoate	Floral, fragrant
Ethyl decanoate	Earthy, mango
α-Copaene	Sickly sweet, wallflowers
β-Caryophyllene	Fresh green, floral
α-Humulene	Slight mango
Ethyl dodecanoate	

Source: MacLeod and Snyder (1985).

largely dominated by monoterpene hydrocarbons (MacLeod and Snyder 1985). The component car-3-ene was found to be the most abundant (>60%) in both varieties. The different flavor constituents of Tommy Atkins and Keitt cultivars are attributed characteristic sensory attributes and are given in Table 19.2.

Tommy Atkins and Keitt varieties are unique in terms of relative percentage abundance of certain classes of aroma compounds. These cultivars predominantly have monoterpene hydrocarbons followed by car-3-ene and α-pinene. The comparative account of these varieties with other cultivars such as Alphonso could be highlighted by the lower presence of myrcene compounds in Tommy Atkins and Keitt cultivars compared with Alphonso mango, which has a myrcene content of 45.9% of the total volatiles (MacLeod and Gonzalez de Troconis 1982).

AUSTRALIAN MANGOES

Australia is known for a number of mango cultivars, and Kensington or Bowen variety is highly popular in the international trade. The following 58 constituents were reported in the flavor profile of Kensington mangoes. Monoterpene hydrocarbons were the major group of volatiles (49% w/w of total volatiles) with α-terpinolene (26%) as the most abundant constituent followed by esters (33%). The significantly higher presence of esters together with certain lactones could be an important feature of Kensington mango flavor producing a specific flavor profile (Table 19.3).

TABLE 19.3. Flavor Profile of Kensington Mango

Components	Amount (μg/g Fresh Tissue)
β-Caryophyllene	0.17
α-Copaene	0.07
β-Phellandrene	0.21
α-Terpinoline	3.42
α-Thujene	0.34
α-Humulene	0.16
α-Turpinene	0.42
γ-Turpinene	0.08
1,1-Diethoxyethane	0.01
2,6-Di-*tert*-butyl-4-ethylphenol	0.01
2-Acetylfuran	tr
2-Methylbutan-1-ol	tr
4-Isopropenyl-1-methylbenzene	0.1
4-Methylacetophenone	tr
5-Butyldihydro-3H-furan-2-one	0.17
5-Methylfurfural	tr
6-Pentyltetrahydro-2H-pyran-2-one	0.01
Acetaldehyde	tr
Butane-1-ol	tr
Butyl acetate	0.03
Butyl formate	tr
C_4-alkylbenzene	0.05
Camphene	0.12
Car-2-ene	0.13
Car-3-ene	0.96
Carveol	0.04
cis-Hex-3-en-1-ol	0.17
Cyclohexane	tr
Dihydro-5-hexyl-3H-furan-2-one	0.04
Dihydro-5-octyl-3H-furan-2-one	0.03
Dimethyl sulfite	0.27
Ethanol	0.05
Ethyl acetate	0.72
Ethyl but-2-enoate	0.61
Ethyl butanoate	2.18
Ethyl decanoate	0.04

TABLE 19.3. ***Continued***

Components	Amount (μg/g Fresh Tissue)
Ethyl dodecanoate	tr
Ethyl ester	tr
Ethyl hexadecanoate	0.08
Ethyl hexanoate plus *cis*-β-ocimene	0.05
Ethyl octanoate	0.12
Ethyl propanoate	0.04
Ethyl tetradecanoate	0.16
Furfural	0.04
Geranial	0.04
Hexadecanal	0.07
Hexadecyl acetate	0.17
Hexan-1-ol	0.09
Hexanal	0.08
Limonene	0.27
Methyl butanoate	0.03
Methylketone	tr
Myrcene	0.21
Octadecanal	0.01
p-Cymen-8-ol	0.18
p-Cymene	0.03
Pentadecanal	tr
Pentane-2,3-dione	0.01
Sabinene	0.14
Sequiterpene hydrocarbon	0.01
Sesquiterpene alcohol	0.01
Sesquiterpene hydrocarbon	0.05
Terpene	0.08
trans-Hex-2-enal	0.16
Unsaturated C_4-alkylbenzene	0.04
Unsaturated C_6 ester	0.10

tr, trace.
Source: MacLeod and Snyder (1988).

AFRICAN MANGOES

African mangoes were found to have predominantly monoterpene hydrocarbons as the major flavor constituents (Table 19.4). However, a number of glycosidically bound aroma compounds were also found (Adedeji et al. 1992). Interestingly, most of these components as given below do not conform with the free volatiles, and the trigger mechanism could be responsible for the release of these bound volatiles. The fatty acid profiles were also unique, which could play an important role in the overall flavor profile. Bandyopadhyay and Gholap (1973) suggested that the ratio of palmitic to palmitoleic acid determines the flavor quality of ripe mangoes as the ratio of >1 results in strong aroma and flavor. A strong presence of palmitic acid and 3-hydroxypropyl oleate in African mangoes could be attributed with a vital role in giving a strong flavor to the mangoes.

TABLE 19.4. Flavor Profile of African Mangoes

Compound	Estimated Conc. in Fruit Pulp (ppm)
Acetaldehyde	1.952
2-Butanol	0.389
Butyl 3-hydroxybutanoate	0.005
p-Cymen-8-ol	0.006
Propyl 3-hydroxybutanoate	0.030
Safranal	0.441
Ipsdienol	0.273
Isobutyl 3-hydroxybutanoate	0.069
Benzaldehyde	0.186
Benzyl alcohol	0.007
2-Phenylethyl alcohol	0.380
Epoxy-linalool	0.005
Butyric acid	0.459
Benzoic acid	0.003
Myrtenol	0.013
Epoxy-linalool	0.005
Butyric acid	0.459
p-Cymen-7-ol	0.004
9-Hydroxymegastigma-4,6-dien-3-one	0.023
Perillyl alcohol	0.005
Thymol	0.004
Ethyl benzaldehyde	0.033
3-Hydroxy-β-damascone	0.046
9-Hydroxymegastigma-4,7-dien-3-one	0.042
α,α-Dimethylbenzeneethanol	0.044
3-Isopropenyl-2,5-dimethyl-3,4-hexadien-2-ol	0.063
endo-Isocamphenone	0.018
Eugenol	0.028
Ethyl *o*-hydroxyphenyl acetate	0.006
trans-Verbanol	0.020
Palmitic acid	0.013
3-Hydroxypropyl oleate	0.026
9-Hydroxymegastigma-4,7-trien-3-one	0.062
Vomifoliol	0.041
Ferulic acid	0.019

Source: Adedeji and others (1992).

VENEZUELAN MANGOES

The areas surrounding the Gulf of Mexico and the Latin-American countries constitute a major mango-growing area, and the flavor profile of Venezuelan mangoes was thoroughly analyzed for the important constituents (MacLeod and Gonzalez de Troconis 1982). The authors reported the predominance of terpene hydrocarbons inclusive of eight monoterpenes and four sesquiterpenes. Important constituents included α-pinene, car-3-ene, limonene, γ-terpene, α-humulene, β-selinene, acetophenone, benzaldehyde, and dimethylstyrene. Limonene was found to an extent

of 3.67 μg/kg. The other dominating flavor constituents were furfurals (7.94 μg/kg), car-3-ene (15.88 μg/kg), and α-pinene (5.01 μg/kg).

BRAZILIAN MANGOES

Brazil is known for intensive mango cultivation, and the major varieties include Haden, Tommy Atkins, and Keitt (Franco et al. 2004). Monoterpene hydrocarbons were most abundant in the flavor profiles, and car-3-ene was the major component of Haden and Keitt, while car-3-ene and α-pinene predominated in Tommy Atkins mangoes. Other identified compounds include α-fenchene, α-camphene, *p*-cymene, β-myrcene, β-phellandrene, limonene, α-terpinolene, β-caryophyllene, and α-humulene.

THAILAND MANGOES

Several mango varieties including the cultivar Khieo Sawoei are grown extensively in the country. Flavor constituents such as γ-terpinene, (*E*)-beta-ocimene, (*E*)-2-hexenal, hexanal, and (*Z*)-3-hexane-l-ol were the major components detected differing from the major components of African, Florida, Indian, and Yellow Thai mangoes. Different volatile oils were extracted and identified from the pulp and peel having different odor unit values, which were helpful to generate odor spectra to characterize the aroma quality of Khieo Sawoei mangoes (Tamura et al. 2001).

CUBAN MANGOES

Cuba has a typical tropical climate and is known for several exotic varieties of mangoes including the cultivars Corazon, Bizcocheuelo, and Super Haden. A total number of 83 volatiles were identified. Corazon was attributed with 70 constituents, Bizcocheuelo with 39 constituents, and Super Haden with 58 constituents. The major principle volatiles include α-cubebene, β-maaliene, ethyl (*Z*)-9-hexadecanoate, ethyl (*Z*)(*Z*)-9,12-octadecadienoate, ethyl (*Z*)(*Z*)(*Z*)-6,9,12-octadecatrienoate, cucarvone, 2-methylpropan-2-ol, 3-methylpentan-l-ol, thymol, and carvacrol (Pino et al. 1989).

INDIAN MANGOES

India is known for more than 100 exotic mango cultivars. The major varieties include Alphonso, Langra, Dashehari, Amrapali, Mallika, Neelam, Badami, Chausa, Malta, Banganapalli, and Totapuri. Extensive work on the flavor profiles of Alphonso, Langra, and Deshehari was carried out. The higher content of myrcene is a novel feature of most of the Indian cultivars, and, in general, the constituents were found to be carbonyls, esters, alcohols, terpenes, and lactones. The cultivar Langra was

found to have more soily flavor notes compared with Alphonso mangoes. A certain coconut-like note is found in Alphonso mango due to the presence of γ-C5 to C10 lactones and δ-octalactone (Gholap and Bandyopadhyay 1975b). Dashehari mangoes were found to have myrcene, ocimene, and *cis*- and *trans*-alloocimene besides hexa- and heptalactones (Bandyopadhyay and Gholap 1973).

TECHNOLOGIES FOR THE EXTRACTION AND CONCENTRATION OF FRUIT FLAVORS

Fruit flavors, being highly delicate, need to have mild conditions during the extraction process to retain flavor with intact notes. The synthetic flavors are designed to obtain specific flavor profiles and the chances of abnormalities are less, ensuring the maintenance of critical flavor notes. However, in the case of natural flavors, utmost care needs to be taken as suppression of even a single critical note can make the flavor drastically different from the desired flavor profile. In fact, it is a common observation that elimination of a single critical component out of 40–60 notes could make certain fruit flavor taste like that of another commodity as in the case of mango versus orange flavors. Maintenance of low temperatures and restriction in oxidative deterioration of flavor with appropriate aroma recovery operations are very important in obtaining appropriate and intact fruit flavors for commercial use.

Mango flavor is one of the most difficult to define on a universal basis as there are too many cultivars and different agro-climatic zones causing extensive variation in the flavor profile (Singh et al. 2004). The taste may vary from very sweet to pulpy or very sour. The flavor is usually sweet, creamy, fruity, and floral with a canned peach character with a terpeny resinous note. The terpeny notes showed significant variance in different cultivars. The Indian Alphonso mangoes have a sweet and terpeny character with a sweet peachy overall taste. The Carabao mangoes from the Philippines have a terpenous top note in combination with fruit apricot-like flavor. Mangoes from Sri Lanka and Malaysia have green resinous terpeny note. The three basic cultivar types based on flavor notes can be summarized as follows:

1. ocimene type: ocimene together with β-caryophyllene giving a sweet, lemony, woody terpene notes as in the case of Alphonso mangoes;
2. carene type: δ-3-car-ene with β-caryophyllene giving a woody, parsley-like terpene note as in the case of Carabao mangoes; and
3. terpenolene type: α-terpenolene and β-selinene giving a pine needle-like terpene note as in the case of Malaysian mangoes.

These terpene notes are augmented by 3-hexanol, 2-hexanol, γ- and δ-lactones, and furan compounds to give the overall flavor perception. The esters impart the overall fruity character, and alcoholic compounds such as linalool, 2-phenyl ethanol, nerol, and citronellol give the overall floral top notes to the mango flavor. It can be understood from the above flavor attributes that the overall flavor perception is a synergistic phenomenon, and anything missing could significantly change the overall flavor perception.

FLAVOR EXTRACTION TECHNOLOGIES

Supercritical Fluid Extraction (SCFE)

SCFE is a favored aroma extraction unit operation. The reason could be the easy solubility of small lipophilic molecules (<400 μ) in CO_2 extracts such as mono- and sesquiterpenes, lactones, alcohols, and esters, and mango flavors mostly consists of these components. The solubility had been rated to an extent of 1–10% by weight, and the solubility as such decreases with increase in molecular weight and polarity. Irradiated mango seeds were subjected to SCFE for the isolation of lipid and 2-alkyl cyclobutanones (Stewart et al. 2001). Restriction of hydrolysis of terpene esters is an important characteristic of SCFE, which renders better quality to the flavor profile together with no loss of terpene alcohols that are otherwise adversely affected in steam distillation procedures.

Solid Phase Microextraction (SPME)

SPME is a state–of-the-art aroma extraction method from headspace. Though the method is used extensively for flavor profile analysis by gas chromatographic techniques, the procedure could be used for industrial application also. Carboxenpolydimethylsiloxane (PDMS) fibers are used for trapping the headspace volatiles and thermally eluted for instrumental analysis or further experiment in solvents of industrial importance (Bazemore et al. 1999). SPME methods for flavor extraction were applied for a number of fruits such as mango, raspberries, strawberries, and banana (Ibaner et al. 1998). The flavor profiles derived by SPME methods in the case of overripe fruits could highlight a number of constituents such as amyl acetate, 2-phenyl ethanol, phenyl ethyl acetate, acetic acid, and ethanol (Zhu et al. 2003).

The other extraction procedures widely used for mango flavor are osmotic distillation, liquid–liquid extraction, vacuum thermal evaporation, and aroma recovery methods.

Osmotic evaporation techniques are widely used for juice concentration. The tangential velocity, temperature, and concentration of solutions significantly influence the evaporation flux. Osmotic evaporation can also be conducted as a multistage procedure during which a constant evaporation flux is obtained during the osmo-concentration procedure (Vaillant et al. 2001). Kaswiga and others (2006) described a detailed liquid–liquid extraction method for mango flavor extraction (Fig. 19.1).

The other important aroma extraction or recovery methods include the use of spinning cone columns (SCCs) and centritherm evaporators. The SCC is a unique gentle distillation or steam stripping column that efficiently and cost-effectively separates volatile compounds such as aroma and flavors from liquids or thick viscous slurries. In the case of centritherm evaporators, a thin-film spinning cone evaporator functions to generate the thinnest film possible in any evaporator system. The product takes only a few seconds to pass over the contact surfaces. The unit is particularly suitable for the concentration of heat-sensitive viscous products such as mango pulp. The aroma is recovered in a suitable condensing unit and is usually added back to the pulp concentrate.

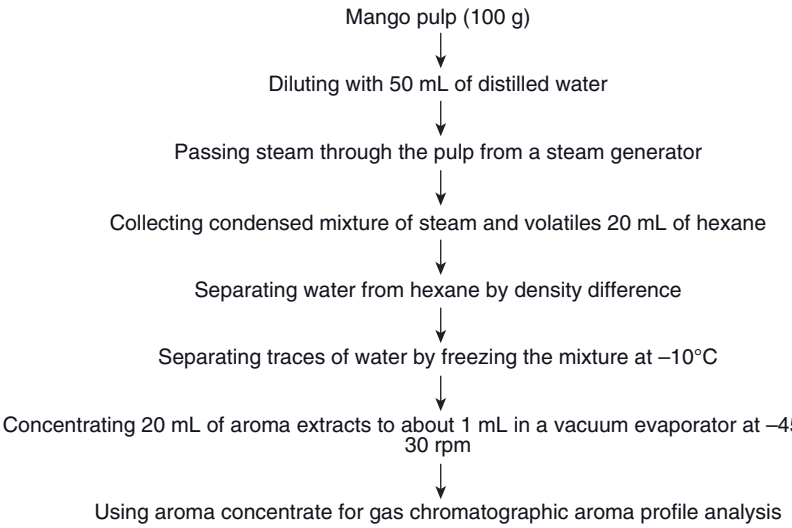

Figure 19.1. Liquid–liquid aroma extracting method for mango flavor.

ANALYTICAL METHODS

Mango flavor profiles were extensively researched by using gas chromatographs coupled with mass spectrometry (MS). Headspace analyses are carried out by using sealed vials containing known quantity of mango pulp, and the vials are usually frozen by immersing in liquid nitrogen and stored at –20°C until use. The flavor is injected into GC by an autosampler. The experimental conditions include flame ionization detector using duronax column and helium as a carrier gas. The detection parameters include programmed oven temperatures from 40 to 180°C at the rate of 6°C/min. The detector temperature is usually maintained at 250°C. Subsequently, mass spectral matches are made by comparison of mass spectra and retention time with those of authentic compounds. Concentration of volatile compounds after identification is usually carried out by using regression equations fitted to peak height calibration curves (Malundo et al. 1997). SPME using PDMS fibers is an authentic way of trapping headspace gases prior to gas chromatographic detection, and the trapped flavor constituents is eluted by raising the temperature of the fiber traps.

Noninvasive fractionation and identification of flavor profiles is a state-of-the-art methodology, and nuclear magnetic resonance (NMR) spectroscopy is an important feature to identify the flavor profiles of ripe mangoes. The improvised version of NMR such as magic angle spinning (MAS) NMR is widely being pursued for the molecular characterization of mango flavor constituents (Gil et al. 2000). The methodologies required for flavor extraction, identification, and characterization are mostly modern, and these methodologies are very much required to characterize the flavor profiles of newly bred cultivars of mango. Mango lines grown by micropropagation and biotechnological methods need authentic flavor profile analysis as a part of the process for germplasm evaluation. The flavor notes and their synergistic effects need to be well understood to develop exotic varieties with novel aroma profile features.

CHANGES IN MANGO FLAVOR DURING STORAGE

Fruit flavor is an important sensory attribute and takes note of the taste and aroma factors. Flavor is specific to the germplasm, and breeders are in continuous search for newer cultivars to develop fruits with exotic flavors. In a fruit like mango, the flavor component is distributed in different parts of the fruit in a characteristic manner (Lalel et al. 2003a). The peel and outer mesocarp contained higher concentrations of volatiles mostly consisting of monoterpenes than the other parts of the fruit. Sesquiterpenes were the second most abundant compound in the peel and the pulp followed by esters, aldehydes, alcohols, norisoprenoids, and lactones qualitatively. In terms of quantity, esters were the second most abundant compound in peel and pulp. The concentration of esters was found to be greater in the mesocarp compared with the other parts of the fruit. As such, the volatile aroma compound appeared to be concentrated in the outer and apical portions of the mango fruit. Maturity stage was also found to affect the overall flavor of mangoes (Lalel et al. 2003b). Kensington mangoes were analyzed for volatile compounds at four different stages of maturity; the fruits harvested at sprung green stage exhibited higher total volatile aroma compounds, monoterpenes, sesquiterpenes, and aromatic compounds. Fruits harvested at fully ripe stage had higher concentrations of esters, alkanes, and norisoprenoids. Polyembryonic and monoembryonic cultivars also showed significant variance in terms of flavor profile (Olle et al. 1998).

Storage conditions were found to have a significant role in the development and maintenance of flavor profiles of mangoes. Mango being a climacteric fruit has a characteristic ripening behavior, and the effect of exogenous ethylene application is pronounced in the preclimacteric phase. In addition, color and flavor are developed during the ripening as a culmination of specific biochemical reactions characteristic of climacteric fruit ripening. Appropriate maintenance of storage conditions, that is, temperature, ventilation, and humidity, is extremely important to obtain optimally ripened fruits with desired extent of flavor component. Immature fruits, defective fruits in terms of mechanical bruises, and microbial infections ultimately lead toward irregular flavor profiles. Hence, appropriate quality control of mangoes by invasive and noninvasive means is of vital importance in ensuring the development of optimal flavors in the ripened fruits. Noninvasive quality control measures involving acoustic resonance (Raju et al. 2006) and X-ray imaging (Thomas et al. 1993) have ample role in the detection of appropriate maturity stages or the presence of internal infestation and also formation of spongy tissue in cultivars such as "Alphonso" mangoes.

Use of ripening agents such as calcium carbide, smoking practices, ethrel/ethylene use on immature fruits could accelerate ripening, but such fruits could be deficient in the flavor component. The storage temperature of mango is usually in the range of 12–14°C, and maintenance of temperature below the threshold level could cause severe chilling injury leading toward significant changes in flavor profiles causing off-flavor (Nair et al. 2003). In "Kensington Pride" mangoes, storage temperatures below 15°C caused chilling injury to different extents, and the optimal flavor profiles were observed in fruits stored at 20°C. With chilling injury, fruits showed a significant reduction in total aroma compounds, monoterpenes, sesquiterpenes, hydrocarbon esters, decanol, and β-ionones.

Mangoes are traditionally known to have higher tolerance toward elevated CO_2 concentrations. The increase in CO_2 concentration in the ambient atmosphere to an extent of 25% was found to cause depletion in the flavor, while an increase up to 10% CO_2 coupled with low O_2 atmosphere did not show significant effects on the production of terpene hydrocarbons in Florida mangoes (Bender 1998).

The nature of ripening on mango flavor profile had also been thoroughly investigated. With the advancement in ripening, the intensity of flavor was found to be enhanced (MacRae et al. 1989), and the duration of ripening has also enhanced the magnitude of flavor (Subramanyam et al. 1976). The extent of volatile compounds was found to increase with the advancement in ripening (Gomez-Lim 1997; MacLeod and Snyder 1985). Tressel and others (1975) reported the maximum flavor of mangoes in the postclimacteric phase. The type of ripening showed interesting results as the source used for smoke generation was found to be critical (Kaswija et al. 2006). Natural sources such as mango leaves for smoke generation were found to cause normal development of flavor. The flavor profiles change with the type of ripening in terms of constitution to certain extent as benzaldehyde and ethyl acetate were found to be predominant in ethylene-induced pit ripening, whereas acetaldehyde was found in room temperature or untreated pit ripening methods. As such, if the fruits are mature enough, ethylene induction had no negative effects in terms of flavor profile.

Irradiation of mangoes (0.25 kGy) was found to have no significant effect on the aroma profiles. However, the treatment was found to induce lipolysis with emphasis on oleic acid and linoleic acids (Gholap et al. 1990). Packaging of mangoes is also crucial in obtaining optimal flavor. The fruits need to be ventilated well within the packages, and packing of the fruits in polyethylene-lined cardboard boxes often results in off-flavor development due to accumulation of CO_2 and suboptimal decrease in the oxygen concentration leading toward anaerobiosis. Modified atmosphere films are usually custom-made depending on the genetic lines and respiratory behavior of the cultivar concerned. Biodegradable packaging films such as chitosan were found to be ideal compared with low density polyethylene (LDPE) films in retaining the overall flavor quality of the mangoes (Srinivasa et al. 2002).

Traditionally, calcium infusion under vacuum conditions was extensively used for shelf-life enhancement of mangoes. However, the reports suggest that calcium salt infusion in the form of chlorides, sulfates, and nitrates could result in the development of poor flavor in spite of the textural and anti-senescence effects. As a whole, it is important to practice standard operating procedures in the preclimacteric and postclimacteric stages to obtain optimal flavor upon ripening of mangoes (Anjum and Ali 2004). The usual culprits could be immature fruits, mechanical and microbial damages, anaerobiosis, suboptimal storage temperatures, and chilling injury. A healthy fruit gives normal and optimal flavor in spite of the influence of genetic lines concerned.

EFFECTS OF PROCESSING ON MANGO FLAVOR

Processing of mango has vital influence on the quantity and quality of mango flavor. The usual process of mango involves extensive thermal process in the form of canned products. The products include mango puree and sliced mango halves. As

mango is an acid product, the thermal processing required is limited. However, most of the industries tend to overprocess the products without taking into consideration the differences in acidity of different cultivars for the calculation of thermal process duration and intensities. The canning industry often resorts to processing of mangoes by using mangoes of lower quality. Most of the fruits are either damaged in a culled state or the harvest indices practiced are suboptimal. As a result, the flavor profile of the raw material is often below the optimal levels, and the process further brings down the quality. The maceration techniques are usually mechanical, and the introduction of enzymatic technique was found to enhance the release of certain glycosidic aroma compounds to influence the overall flavor quality of the macerate (Sakho et al. 1998).

The processing industry also involves postharvest handling of mango fruits by using anti-senescence measures such as use of aminovinyl glycine (AVG) or monocyclopropane (MCP) for the extension of shelf life of mangoes prior to processing. Ethephon treatment was found to enhance the flavor profile of mangoes, while AVG and MCP treatments were found to result in suppression of mango flavors (Lalel et al. 2003c). The process duration during enzymatic liquefaction of mango needs to be optimized for different cultivars as overprocessing was found to decrease the monoterpenes and δ-3-carene compounds in the finished product (Singh et al. 2000). Varietal differences were thoroughly investigated in the quality evaluation of canned mango pulp. The susceptibility toward flavor losses was found to be linked not only to process intensity but also to the genetic lines. Alphonso mangoes scored highest for flavor profiles during thermal processing (Doreyappa Gowda and Huddar 2004). However, blending of pulps was recommended to enhance the acceptability of low-cost pulps. Despite the existing studies on the suitability of mango cultivars toward thermal processing, there is a need for deeper studies in understanding the controlling parameters, which render resistance against flavor degradation during thermal processing. Certain preclimacteric factors such as fertigation, manuring, micronutrient availability, and other agronomical factors were also known to contribute to this effect (Singh et al. 1993). It is imperative to study the kinetics of thermal process thoroughly for each cultivar to avoid overprocessing and off-flavor development so that standard operating procedures could be established for optimal thermal process for each cultivar (Argaiz and Lopez Malo 1996).

Stability of aroma during storage of processed mango products attracted attention as the presence of heavy metal ions could bring about significant reduction in the aroma profile of several fruit juice concentrates (Sulc 1984). Oxidative degeneration of flavor is a major problem for flavors more so in the products rather than in essence forms. Ramteke and Eipeson (1997) reported a more pronounced effect of storage temperature on the stability of mango flavor in stored juice concentrates compared with the effects of antioxidant additives such as glucose, glucose oxidase and catalase system, and ascorbic acid. The same authors reported a higher antioxidant potential of sodium sulfite as a scavenger for molecular oxygen to restrict oxidative deterioration of mango flavor.

Nonthermal processing of mango products by microfiltration and reverse osmosis was found to cause greater retention of flavor compounds. Among the retention constituents, terpene hydrocarbons were found to be the major constituent along with the oxygenated terpene derivatives. C13 norisoprenoids and phenolics were also found to increase substantially (Olle et al. 1997). Sreenath et al. (1995) reported

aroma losses in clear mango juice obtained by filtration through a cheesecloth using enzymatically liquefied purees. However, microfiltration techniques and reverse osmosis as such were found to have significant retention of monoterpenes. It is interesting to note that certain flavor alterations could take place even under nonthermal conditions due to acid-catalyzed transformation (Teisseire 1986). In several mango products such as mango bars, color retention was studied in conjunction with flavor profiles, and the losses in carotenoids are usually associated with flavor losses (Mir and Nath 1993). However, it is essential to study whether nonoxidative parameters such as water activity, solute mobility, and catalysis play an important role in flavor retention in addition to oxidative deterioration. It is a common observation that a uniform and well-colored product shows equally good flavor profile, indicating that the degradation products of color could adversely influence the flavor profile (Stillman 1993). Roasting of mango seeds was found to reveal a variety of odor compounds including 2-acetyl-1-pyrolene, butane-2-3-dione, pentone-2-3-dione, and pyrazine compounds.

Nonthermal processes such as high-pressure processing are yet to be explored to a greater extent for the processing of mango. Initial studies carried out by Boynton and others (2002) at 300–600 MPa for 1 min using vacuum-sealed mango slices of Tommy Atkins and Keitt varieties showed slight reduction in flavor and increased sweetness. However, more studies are required to be carried out to access the efficacy of high-pressure processing on the flavor profile of mangoes. Freezing of mango pulp is an important process, and MacLeod and Snyder (1988) gave a detailed account of flavor profile of deep-frozen mango slices. It is interesting to note that the flavor profiles were almost similar in the fresh and frozen products. Car-3-ene was slightly reduced on freezing, but certain additional volatile constituents were induced during the frozen storage along with adipate-based migratory substance from the packaging material. Sagar and Khurdiya (1999) described a dehydration process for mango slices and a pretreatment in sugar syrup (70°Brix) along with sulfitation, and the temperature of dehydration at 58°C was also found to promote better flavor retention.

Mango, being a delicious fruit, had been extensively subjected to value addition to manufacture a variety of products such as bottled beverages, fruit bars, osmodehydrated products, and high moisture and stabilized slices besides minimally processed products. Mango puree is routinely used as a flavoring agent in a number of products instead of mango as such, which is also used widely in flavoring many soft drink formulations, confectionery, and dairy products. The effect of processing with the emphasis on thermal processing as the flavor profile and also heat-sensitive constituents such as carotenoids had been widely studied. However, mango flavor being an intensive flavor, minute changes in the flavor do not adversely affect the quality of the product. Pott and others (2003) described the quantitative and qualitative changes of β-carotene stereoiosomers in solar dehydrated mangoes. The type of dehydration influenced the isomerization pattern. Conventional hot air drying caused the presence of higher extent of 13-cis β-carotene, and solar dehydration could result in higher extent of 9-cis isomers. The mango cultivars play an important role in obtaining the flavors during processing. Flavors extracted from mango purees and also the synthetic flavors dominate the market for various uses in the processing sector. The natural flavors need to be endowed with suitable additives to restrict oxidative degeneration of the flavor during storage. Stability of fruit flavors had

traditionally attracted the attention of several workers as the flavors are highly susceptible to temperature and oxidative stress during the storage. The effects of temperature on the storage stability of apple (Guadagni et al. 1967) and orange (Guadagni et al. 1970) highlighted the need of temperature regulation to stabilize the flavors concerned during the storage. Ramteke and Eipeson (1997) described the effect of different antioxidative additives such as glucose + glucose oxidase + catalase system, sodium sulfite and ascorbic acid to restrict the oxidative degeneration of mango flavor concentrate.

Several types of mango beverages are marketed extensively, and most of the beverages are nectars in bottled or canned forms. Mango juice is also used in the preparation of fruit juice cocktails and punches to render a typical mango flavor to the product. Ready-to-drink beverages such as coconut water blended with mango juice have extensive market circulation in the Indian subcontinent and also in Southeast Asian countries. Mango bars are delicious mango products as the fruit pulp is blended with sugar and dehydrated in layers by conventional hot air drying (Mir and Nath 1993). The quality of mango bars could be further enhanced by the addition of texturing and anti-crystallizing agents. However, the use of mango flavor in encapsulated form need to be further encouraged in the case of dry formulations such as spray-dried or freeze-dehydrated powders. Freeze dehydration was known to keep the flavor profile intact, and the resultant beverage formulation was known to possess excellent flavor quality as the heat-induced flavor losses are minimal. Minimally processed mango slices devoid of thermal process were reported to have excellent flavor attributes, and the modified atmosphere packaging further enhanced flavor retention of mango slices (Martinez et al. 2002). Chauhan and others (2006) reported higher retention of flavor when pretreated mango slices were subjected to modified atmosphere packaging in active and passive modes. These authors also reported the positive effects of controlled atmosphere storage on the magnitude of flavor retention during the storage of minimally processed mango slices. As such, the process durations and intensities need to be restricted for optimal retention of mango flavor. The process strategies could resort to combination processing techniques using the hurdle concept to minimize mango flavor losses for the development of high-moisture mango products (Garcia et al. 1998).

FLAVOR COMMERCIALIZATION REGULATION

Flavors are of three types, that is, natural, nature identical, and artificial. The natural flavors are once again classified into naturally occurring, heat induced, and enzymatically generated flavors. Nature identical flavors are those where the individual flavor components are identified and synthetic analogues are formulated as per the original natural flavor profiles. Artificial flavors include flavor constituents that have not been found in nature but synthesized to develop unique flavor formulations. In the case of mango, natural and also nature identical flavors are available for commercial use. In the United States, nature identical flavors are not separately distinguished from artificial flavors, which otherwise are identified separately in European countries. Consumers tend to link natural flavors with purity and safety. They often prefer foods with natural flavors to foods with synthetic or artificial flavors. As a result, the sales of natural flavors have steadily increased (Unger 1981).

The major steps in flavor development involve certain essential steps, and the selection of samples with a target flavor profile has significant importance. In the case of mango flavor, selection of target flavor has maximum importance because of vast diversity in the flavor profiles. Many of the mango cultivars are yet to be subjected to orientation toward specific flavor development as most of the commercial flavors are of general nature without being specific to any cultivar-dependent flavor. The steps involved in flavor development can be summarized as follows: selection of sample containing target flavor → isolation, concentration, and preliminary fractionation → final separation → identification → synthesis of authentic compounds → confirmation of identification → sensory evaluation → data interpretation (Mermelstein 1989).

In the case of mango, more than 40 flavor constituents were identified, making mango flavor one of the most complex profiles, and the key compounds that are also known as character-impact compounds are several inclusive of hydrocarbons, alcohols, esters, and lactones. The most common of them include car-3-ene, ocimene, myrcene, caryophyllene, limonene, linalool, and terpenol. Therefore, it is vital to take the varietal differences to derive full advantage of the varietal variance in terms of flavor profiles (MacLeod and Pieris 1984).

Manley and Ahmedi (1995) gave a comprehensive view of developing process flavors with emphasis on regulatory and labeling issues. Regulation and labeling of food flavor additives are extremely complex and vary from country to country. In the United States, food flavors are evaluated by a number of organizations including the Food and Drug Administration (FDA) and the Flavor and Extracts Manufacturers Association (FEMA). In general, the FDA takes the Delaney clause [Section 409 (c) (S) (A)], which states that "no additive deemed to be safe if it is found to induce cancer when ingested by man or animal." It is mandatory to subject flavors to premarket safety evaluation and clearance by the FDA. The generally recognized as safe (GRAS) status can be used judiciously to separate the flavor ingredients of natural origin from the requirements of daily food intake; FEMA gave a detailed account of GRAS flavors and the aspect include a detailed dietary analysis, recommended additives, and solvents used for extraction (Smith et al. 2001). The flavor profile of mango has specific building blocks identified, and the database could be used for the generation of several flavor formulations with specific sensory attributes.

There is a need for harmonizing the GRAS flavors with respect to the regulation followed by other countries. Simplification of the classification by removal of origin of flavors as a criterion can make the flavors more cost-effective (Stofberg 1992) (Table 19.5).

TABLE 19.5. Key Flavor Compounds and Their Perception

Perception	Key Compounds
Fresh	Hexyl butanoate *cis*-3-hexenol, acetaldehyde
Floral	Linalool, nerol, linalyl acetate
Sweet	Γ-Octalactone, γ-decalactone, γ-ionone, nerol
Citrus	Linalool, citronellol, geraniol, nerol
Cooked/juice	4-Hydroxy-2,5-dimethyl-3(2H)-furanone
Tropical/sulfury	Dimethyl sulfide

Commercial mango flavors in the form of essence are widely used in various processed foods. The other forms of mango flavor, such as semiliquid flavors and powdered flavors inclusive of spray-dried, surface-adsorbed, microencapsulated, and co-crystallized forms, need to be popularized to derive the vast commercial utility of mango flavor. The regulation of flavor quality at national level needs further inroads keeping in view the native requirements and cultivars available within different agro-climatic zones.

BREEDING AND BIOTECHNOLOGICAL APPROACHES FOR FLAVOR IMPROVEMENTS

The development of mango varieties is based on vegetative propagation and also breeding. These conventional techniques could give rise to a number of exotic varieties, and the modern selection methods were responsible for the development of varieties such as "Haden," "Keitt," "Kent," and "Tommy Atkins," which were identified from openly pollinated seedlings in Florida (Pua and Davey 2007). Mango hybrids such as "Jawahar" and "Sabri" are known to possess excellent flavor profiles besides high nutritional content (Naresh 1998). However, the modern biotechnological methods give a greater opportunity to develop gene sequences to obtain specific flavor profiles. Krishna and Singh (2007) emphasized the importance of DNA fingerprinting and marker-assisted characterization of gene sequences of mango to improve mango cultivars for disease resistance and also sensory attributes including flavor. Knight (1993) identified flavor improvement as one of the major criteria for evaluating mango germplasm. Bally and others (1996) studied the genetic diversity of "Kensington" mangoes in Australia within the various selections. The phenotypic variants and the effects of genotypes of the morphological features were investigated to prepare a gene database for "Kensington" mangoes. Such a database generation for the gene pool under various agro-climatic zones may spell a bright future for mango breeding by biotechnological methods to improve the yields, adaptability, and also sensory attributes in terms of flavor. Modern tissue culture techniques are also at the anvil of developing differentiated tissues of mango supported by scaled up tissue culture procedures. These differentiated tissues have a bright future for several future applications including indoor cultivation and long-distance space travels.

MARKETING OF MANGO FLAVORS

The commercialization of mango flavors is in infancy compared with other fruits such as oranges, pineapples, grapes, and strawberries. The product range is less compared with other commercially important flavors based on fruits. The commercial potential is vast, and the product range can be enhanced further to include natural and artificial flavors in several forms. Encapsulated mango flavors using spray-drying techniques were successful; however, the products are yet to see realization of commercial utility. There is a need to develop mango flavors in various forms inclusive of semiliquid and solid forms with emphasis on restriction of flavor losses on storage. The global market of mango and mango products is expanding

rapidly, and the United Kingdom itself is a major market to several tropical fruits such as mango and papaya (Weaver 1999). An increase in mango market ultimately results in familiarization of flavor, and demands for flavors grow when the consumers appreciate the commodity and related products. The future of the flavor industry is bright in the 21st century as flavors are likely to compensate for reduced fat, sugar, and cholesterol in foods as an important ingredient of convenience foods (van Berge and Evenhuis 1998). Widespread consumer awareness programs to improve quality of mango flavor in terms of sensory attributes were carried out to generate database, which can be used by flavor chemists for the development of exotic mango flavors (Malundo 1996). The usage of mango pulp and juice concentrates for flavoring various beverages is an established practice. However, the use of pulp or juice concentrates may not be equated as use of flavoring agent as such and the products can be termed as blended forms rather than flavored products. Chauhan and others (2007) reported the development of coconut water blended with mango pulp as a ready-to-serve beverage. The use of pulp and juice concentrates in several products including confectionery, ice creams, and sweetmeats such as puddings is in practice and is preferred over colored flavor analogues of mango flavor. Mango flavor by the direct fermentation of peels and stones for the production of vinegar was found to result in mango-flavored vinegar (Ethiraj and Suresh 1992). Mango drinks based on co-crystallized flavors also have ample potential as the products are cost-effective with refreshing taste and aroma. The Persian Gulf countries use a number of mango-flavored products inclusive of cookies, toffees, candies, ice creams, sweetmeats, yogurt, beverages such as nectars, squashes and ready-to-serve drinks, and sherbets. The products that use mango pulp to a specific level can also be subjected to flavor fortification by the use of added flavors. The demand for mango products and also mango flavor is gaining popularity over the years in the Middle East as well as the Oriental and Western countries. Higher emphasis need to be laid on natural flavor products using cost-effective technologies inclusive of vacuum thermal evaporation techniques. The benefits of supercritical-CO_2-extracted mango flavors can be maximized by improving the plant capacities and also adoption of flavor grading as per the requirement of the end use of the flavor. Co-crystallized sugar and β-cyclodextrins can make the products cost-effective besides offering better convenience for various culinary applications.

CONCLUSIONS

Mango is an exotic tropical fruit and is grown in 87 countries. Hundreds of cultivars are available due to intensive selection, grafting, and breeding practices. Monoembryonic as well as polyembryonic cultivars are available in different agro-climatic zones. A number of countries such as India, Indonesia, Pakistan, the United States, and Australia are known for some of the best cultivars such as Alphonso, Keitt, Tommy Atkins, Carabao, and Kensington. Mango requires a typical tropical climate and grows up to an altitude of about 1400 m. Mango flavor primarily constitutes hydrocarbons, alcohols, esters, and lactones. The major constituents include ocimene, myrcene, limonene, linalool, ethyl acetate, heptalactone, butyrolactone, acetoin, and furfurals. Mango flavor closely resembles orange flavor, and changes in certain delicate flavor notes during processing may change the flavor

profile drastically. The notes specific to mango could be car-3-ene, myrcene, and ocimene, and the synergistic effects of the constituents give a characteristic mango flavor. The synergistic components include limonene, linalool, pinene, hexanol, camphene, and furfurals. Mango flavors can be classified in to three types, that is, ocimene, carene, and terpinolene types. The flavor extraction technologies include SCFE methods, SPME, and solid liquid extractions. Modern analytic techniques such as GC-MS using SPME methods and also NMR spectroscopy played a vital role in characterizing different mango flavors leading toward a range of mango-flavored products. Mango flavors are available as natural, natural like, and synthetic products for use in different products to render specific mango flavor to the products. Mango flavor as such is influenced by the genetic lines, harvest maturity, and also the storage conditions. The process conditions also play a major role in restricting flavor losses during the process. Oxidative degeneration of flavor is a major problem concerned with the storage of flavor products, and the remedial measures include scavenging of molecular oxygen and use of antioxidant additives. Nonthermal processing of mango products inclusive of membrane processing, high-pressure, and pulsed electric fields are the latest in the technological arena to restrict flavor changes and losses during processing. Since the marketing of natural flavor is on the increase, the extraction technologies have a major role to play in the mango flavor industry, which has a greater future due to the increasing demand. The modern biotechnological methods may lead toward the development of a wider range of exotic mango flavors for the benefit of the food industry.

REFERENCES

Adedeji J, Hartman TG, Lech J, Ho C-T. 1992. Characterization of glycosidically bound aroma compounds in the African mango (*Mangifera indica* L.). *J Agric Food Chem* 40:659–61.

Anjum MA, Ali H. 2004. Effect of various calcium salts on ripening of mango fruits. *J Res (Sci)* 15(1):45–52.

Argaiz A, Lopez Malo A. 1996. Kinetics of first change on flavor, cooked flavor development and pectin esterase inactivation on mango and papaya nectars and purees. *Fruit Process* 6(4):145:148–50.

Bally ISE, Graham GC, Herry RJ. 1996. Genetic diversity of Kensington mango in *Australia. Aust J Exp Agric* 36(2):243–7.

Bandyopadhyay C, Gholap AS. 1973. Relationship of aroma and flavor characteristics of mango (*Mangifera indica* L.) to fatty acid composition. *J Sci Food Agric* 24:1497–503.

Bazemore R, Goodner K, Rouseff R. 1999. Volatiles from unpasteurized and excessively heated orange juice analyzed with solid phase micro extraction and GC olfactometry. *J Food Sci* 64(5):800–3.

Bender RJ. 1998. Elevated CO_2 in controlled atmosphere storage and regulation of mango ripening. *Dissertation Abstracts International. B. Thesis Publ. 1996* 58(7):3384.

Beynon JH, Saunders RA, Willams AE. 1961. The high resolution mass spectra of aliphatic esters. *Anal Chem* 33:221–323.

Boynton BB, Sims CA, Sargent S, Balaban MO, Marshall MR. 2002.Quality and stability of precut mangoes and carambolas subjected to high pressure processing. *J Food Sci* 67(1):409–15.

Chaddha KL, Thakur RS, Rajput MS, Samra JS. 1981. Leaf analysis as a guide to nitrogen fertilization in mango. *Indian J Hortic* 38:153–5.

Chandrashekharan SS, Kuttaim SS, Irulappan I. 1988. Field evaluation of certain insecticides against mango hopper *Idiscpus spp*. *Leth. Sarth. Indian Hortic* 36(3):146–8.

Chauhan OP, Raju PS, Dasgupta DK, Shylaja R, Rao S, Bawa AS. 2006. Modified/controlled atmosphere storage of minimally processed mango slices (var. *Arka Anmol*). *Am J Food Technol* 1(2):105–16.

Chauhan OP, Singh A, Raju PS, Bawa AS. 2007. Development of fruit flavored tender coconut water RTS beverage. Abstract No. TC08. In-ICFOST, December 31, 2007–January 3, 2008, IIT Kharagpur, India.

Doreyappa Gowda IN, Huddar AG. 2004. Investigations on processing quality of some mango varieties, hybrids and their blends. *J Food Sci Technol* 41(27):154–9.

Ethiraj S, Suresh ER. 1992. Studies on the utilization of mango processing wastes for production of vinegar. *J Food Sci Technol* 29(1):48–50.

FAO. 1990. *Food and Agriculture Production Year Book*. Rome, Italy: FAO.

Franco MRB, Rodrignez Amaya D, Lancas FM. 2004. Volatile composition of three cultivars of mango (*Mangifera indica* L.). *Ciencia-e-Tecnologia-de-Alimentos* 24(2):165–9.

Garcia Y, Cerezal P, Pinera RM, Castro D. 1998. Diced and sliced mangoes preserved by combined methods. Choice of alternatives. *Alimentaria* 289:75–81.

Gholap AS, Bandyopadhyay C. 1975a. Comparative assessment of aromatic principles of ripe Alphonso and Langra mango. *J Food Sci Technol* 12:262–3.

Gholap AS, Bandyopadhyay C. 1975b. Contribution of lipid to aroma of ripening mango (*Mangifera indica* L.). *J Am Oil Chem Soc* 52(12):514–6.

Gholap AS, Bandyopadhyay C, Nair PM. 1990. Lipid composition and flavor changes in irradiated mango (var. Alphonso). *J Food Sci* 55(6):1579–80.

Gil AM, Duarte IF, Delgadillo I, Colquhoun IJ, Casuselli F, Humpfer E, Spraul M. 2000. Study of the compositional changes of mango during ripening by use of nuclear magnetic resonance spectroscopy. *J Agric Food Chem* 48:1524–36.

Gomez-Lim MA. 1997. Post harvest physiology. In Litz RE (ed.), *The Mango—Botany, Production and Uses*. Oxfordshire, UK: CAB International, pp. 425–46.

Guadagni DG, Bomleu JL, Mannheim HG. 1970. Effect of temperature on stability of orange aroma solution. *J Food Sci* 35:279–81.

Guadagni DG, Okano S, Harris JG. 1967. Effect of temperature on storage stability of apple essence obtained from different varieties. *Food Technol* 21:665–8.

Hunter GLK, Brogden WB Jr. 1965. Terpenes and sesquiterpenes in cold pressed orange oil. *J Food Sci* 30:1–3.

Hunter GLK, Bucek WA, Radford T. 1974. Volatile components of canned Alphonso mango. *J Food Sci* 39:900–3.

Ibaner E, Sebastian SL, Ramos E, Tabera J, Reglero G. 1998. Analysis of volatile fruit components by hand space solid phase micro extraction. *Food Chem* 63(2):281–6.

Kalra SK, Tandon DK, Singh BP. 1995. Mango. In Salunkhe DK, Kadam SS (eds.), *Handbook of Fruit Science and Technology. Production, Composition, Storage and Processing*. New York: Marcel Dekker, pp. 123–70.

Kapur KL. 1983. Volatile components of Dashehari mango. *J Food Sci Technol* 20:242–3.

Kaswija M, Peter M, Leonard F. 2006. Sensory attributes, microbial quality and aroma profiles of off vine ripened mango (*Mangifera indica* L.) fruit. *Afr J Biotechnol* 5(2):201–5.

Knight RJ. 1993. Evaluating important fruit characters in mango germplasm. *Fruit Vars J* 47(1):25–31.

Krishna H, Singh SK. 2007. Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement—A review. *Biotechnol Adv* 25(3):223–43.

Lalel HJD, Singh Z, Tan SC. 2003a. Distribution of aroma volatile compounds in different parts of mango fruit. *J Hortic Sci Biotechnol* 78(2):131–8.

Lalel HJD, Singh Z, Tan SC. 2003b. Maturity stage at harvest affects fruit ripening, quality and biosynthesis of aroma volatile compounds in 'Kensington Pride' mango. *J Hortic Sci Biotechnol* 78(2):225–33.

Lalel HJD, Singh Z, Tan SC. 2003c. The role of ethylene in mango fruit aroma volatile biosynthesis. *J Hortic Sci Biotechnol* 78(4):485–94.

MacLeod AJ, Gonzalez de Troconis NG. 1982. Volatile flavor components of mango fruit. *Phytochemistry* 21:2523–6.

MacLeod AJ, Macleod G, Snyder CH. 1988. Volatile aroma constituents of mango (cv. Kensington). *Phytochemistry* 27(7):2189–93.

MacLeod AJ, Pieris NM. 1984. Comparison of the volatile components of some mango cultivars. *Phytochemistry* 23(2):361–6.

MacLeod AJ, Snyder CH. 1985. Volatile components of two cultivars of mango from Florida. *J Agric Food Chem* 33:380–4.

MacLeod AJ, Snyder CH. 1988. Volatile components of mango preserved by deep freezing. *J Agric Food Chem* 30(1):137–9.33

MacRae EA, Bowen JH, Stec MGH. 1989. Maturation of kiwifruit (*Actinidia deliclosa* cv. Hayward) from two orchards: Difference in composition of the tissue zones. *J Sci Food Agric* 47:401–16.

Majumdar PK, Sharma DK. 1990. Mango. In Bose TK, Mitra SK (eds.), *Fruits: Tropical and Subtropical*. Calcutta, India: NayaPrakash, pp. 1–9.

Malundo TMM. 1996. Application of the quality enhancement (QE) approach to mango (*Mangifera indica* L.) flavor research. *Dissertation Abstracts International B* 57(3):134; 1523 order (DA 9624067).

Malundo TMM, Baldwin EA, Moshonas MG, Baker RA, Shewfelt RL. 1997. Methods for the rapid headspace analysis of mango (*Mangifera indica* L.) homogenate volatiles constituents and factors affecting quantitative results. *J Agric Food Chem* 45:2187–94.

Manley CH, Ahmedi S. 1995. The development of process flavors. *Trends Food Sci Technol* 6(2):46–51.

Martinez FM, Harper C, Perez-Munoz F, Chaparro M. 2002. Modified atmosphere packaging of minimally processed mango and pineapple fruits. *J Food Sci* 67(9):3365–71.

McFadden WH, Day EA, Diamond MJ. 1965. Correlations and anomalies in mass spectra. *Anal Chem* 37:89–92.

Mermelstein NH. 1989. Food flavors. *Food Technol* 43(2):99–106.

Mir MA, Nath N. 1993. Storage changes in fortified mango bars. *J Food Sci Technol* 30(4):279–82.

Mitzner BM, Theimer ET, Freeman SK. 1965. The infrared spectra of monoterpenes and related compounds. *Appl Spectrosc* 19:178–9.

Nair S, Singh Z, Tan SC. 2003. Aroma volatiles emission in relation to chilling injury in 'Kensington Pride' mango fruit. *J Hortic Sci Biotechnol* 78(67):866–73.

Naresh K. 1998. A comparative study of mango hybrid Jawahar and Sabri with their patents. *Haryana J Hort Sci* 27(2):84–91.

Olle D, Baron A, Lozano YF, Sznaper C, Baumes R, Bayonove C, Brillouet JM. 1997. Microfiltration and reverse osmosis affect recovery of mango puree flavor compounds. *J Food Sci* 62(6):1116–19.

Olle D, Baumes RL, Bayonove CL, Lozano YF, Sznaper C, Brillouet JM. 1998. Comparison of free and glycosidically linked volatile components from polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *J Agric Food Chem* 46(3):1094–100.

Pattabhiraman TR, Sastry LVL, Abraham KD. 1969. Preparation of odor concentrates and identification of odorous ingredients in mango and guava. Part II. *Perfum Essent Oil Rec* 60:233–5.

Pino J, Rosado A, Souchez R. 1989. Volatile components of three cultivars of mango from Cuba. *Nahrung* 33(8):709–15.

Pott I, Marx M, Neidhart S, Muhlbauer W, Carle R. 2003. Quantitative determination of beta carotene stereoisomers in fresh, dried and solar dried mangoes (*Mangifera indica* L.). *J Agric Food Chem* 51(16):4527–31.

Pua E-C, Davey MR. 2007. Transgenic crop V. In Nagara T, Lorz H, Widholm JM (eds.), *Biotechnology in Agriculture and Forestry*, vol. 60. Berlin: Springer, pp. 51–71.

Raju PS, Jagannath JH, Ashok N, Dasgupta DK, Bawa AS. 2006. Nondestructive monitoring of ripeness in mango cultivars by acoustic resonance spectroscopy. *Int J Food Prop* 9:487–501.

Ramteke RS, Eipeson WE. 1997. Effect of additives on the stability of mango aroma concentrate during storage. *J Food Sci Technol* 34(3):195–9.

Saby JK, Mohan RO, Jagan L, Bhat SG, Prasada Rao UJS. 1999. Characterization of aroma components of sap from different Indian mango varieties. *Phytochemistry* 52(5):891–4.

Sagar VS, Khurdiya DS. 1999. Studies on dehydration of Dashehari mango slices. *Indian Food Packer* 53(1):5–9.

Sakho M, Chassagne D, Jaus A, Chiarazzo E, Crouzet J. 1998. Enzymatic maceration: Effects on volatile components of mango pulp. *J Food Sci* 63(6):975–8.

Singh NGI, Dhuique MC, Lozano Y. 2000. Physico-chemical changes during enzymatic liquefaction of mango pulp (cv. Keitt). *J Food Process Preserv* 24:73–85.

Singh NP, Srivastava RP. 1982. Studies on various factors involved in softwood grafting of mango. *Progressive Hort* 14:117–20.

Singh RV, Tewari JD, Chauhan BBS. 1993. Effect of combination of nitrogen, phosphorous and potassium fertilizers on the quality of fresh and canned mangoes. *J Food Sci Technol* 30(6):468–70.

Singh Z, Lalel HJD, Suresh N. 2004. A review of mango fruit aroma volatile compounds—State of the art research. *Acta Hortic* 645:519–27.

Smith RL, Doul J, Feron VJ, Goodman JI, Munro IC, Newberne PM, Portoghese PS, Woddell WJ, Wagner BM, Adams TB, McGowen MM. 2001. GRAS flavoring substances. *Food Technol* 55(12):34–6, 38, 40, 42, 44–8.

Sreenath HK, Sudarshana K, Santhanam K. 1995. Enzymatic liquefaction of some varieties of mango pulp. *Lebensm Wiss Technol* 28:196–200.

Srinivasa P, Baskara R, Ramesh M, Prashanth KH, Tharanathan R. 2002. Storage studies of mango packed using biodegradable chitosan film. *Eur Food Res Technol* 215(6):504–8.

Stewart EM, McRoberts WC, Hamittan JTG, Graham WD. 2001. Isolation of lipid and 2-alkylcyclobutones from irradiated foods by supercritical fluid extraction. *J AOAC Int* 84(3):976–86.

Stillman JA. 1993. Color influence flavor identification in fruit flavored beverages. *J Food Sci* 58(4):810–2.

Stofberg J. 1992. The significance of GRAS and nature identical for worldwide harmonization of flavor regulation. *Perfumer and Flavorist* 17(5):111–4.

Subramanyam H, Gouri S, Krishnamurthy S. 1976. Ripening behavior of mango fruits graded on specific gravity basis. *J Food Sci Technol* 13(2):84–6.

Sulc D. 1984. Fruit juice concentration and aroma separation. *Confructa* 28:258–318.

Tamura H, Boonbumnung S, Yoshizawa T, Varanyanond W. 2001. The volatile constituents in the peel and pulp of a green Thai mango, Khiev Sawoei cultivar (*Mangifera indica* L.). *Food Sci Technol Res* 7(1):72–7.

Teisseire P. 1986. Les gradides voies de Synthese des terpenoides aliphatiques. In Jonlain D (ed.), *Progress in Terpene Chemistry*. Gif sur Yvette: Editions Frontiers, pp. 1–39.

Thomas P, Saxena SC, Chandra R, Rao R, Bhatia CR. 1993. X-ray imaging for detecting spongy tissue, an internal disorder in fruits of Alphonso mango (*Mangifera indica* L.). *J Hortic Sci* 68(5):803–6.

Tressel R, Holzer M, Apertz M. 1975. Biosynthesis of volatiles. In Maare H, Groenen PJ (eds.), *International Symposium on Aroma Research*. Zeist, The Netherlands: Central Institute for Nutrition and Food Research TNO, pp. 41–62.

Unger L. 1981. US market of flavors and fragrances. *Perfumer and Flavorist* 6:37–8.

Vaillant F, Jeanton E, Dornier M, O'Brien GM, Reynes M, Decloux M. 2001. Concentration of passion fruit juice on an industrial pilot scale using osmotic evaporation. *J Food Eng* 43(3):195–202.

van Berge P, Evenhuis B. 1998. Flavors into the 21st century. *Perfumer and Flavorist* 23(3):1–2.

Weaver D. 1999. Mango market moves ahead as UK volumes grow steadily. *Fresh Produce J* 12:22–3.

Willhalm B, Thomas AF. 1969. Hydrofurans as strawberry like flavorings for food stuff and beverages. U.S. Patent 3,455,702.

Zhu J, Park KC, Baker TC. 2003. Identification of odors from over ripe mango that attract vinegar flies, *Drosophila melanogaster*. *J Chem Ecol* 29(4):899–909.

CHAPTER 20

Passion Fruit

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INTRODUCTION

The passion fruit belongs to the family of Passifloraceae, which is represented by 14 genera. The genus *Passiflora* is the principal representative of the family and constitutes nearly 580 species distributed in the tropical and subtropical regions in the world (Silva and São José 1994). More than 150 species are native of Brazil out of which about 60 are known to bear edible fruits (Hoehne 1946), but only a few are of commercial importance (Martin and Nakasone 1970).

The term passion fruit exclusively represents the species *Passiflora edulis* Sims, which contains two forms—purple (*P. edulis* Sims) and yellow (*P. edulis* Sims f. *flavicarpa* Degener), and these are the important commercial varieties. The yellow passion fruit is 6–12 cm long and 4–7 cm in diameter, and it has a hard and thick yellow rind and brown seeds and possesses an acidic pulp, which has a strong aromatic flavor. The purple passion fruit is relatively smaller in size (4–9 cm long and 3.5–7 cm in diameter) and has purple skin and black seeds (Bora and Narain 1997). The fruits of both varieties contain pulp, which varies from yellow to orange in color. Due to its pleasant flavor, the purple passion fruit is preferred for consuming as fresh juice, while yellow passion fruit is considered suitable for processing. In the last two decades, several hybrids have been developed based on mutagenic crossing of the two prominent varieties, and some of these have gained commerce locally in some countries such as India (Singh et al. 1991) and Taiwan (Chen et al. 1982). A promising purple × yellow hybrid, Kaveri, developed in India had 100% more juice yield and quality comparable to the better parent variety of purple fruit (Singh et al. 1991).

The passion fruit should be completely mature at the time of harvest when the rind is of full yellow/orange or purple color. Fruits with green rind possess woody off-flavor and have inferior commercial value even when they are internally mature. Harvesting of the fruit is done in two ways and each has its own purpose. Fruits

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destined to be processed are collected from the ground after their natural fall from the plant resulting from the maturation or picking directly from the plants when sold to the market for consumption in fresh form as they have better appearance and keeping qualities (Bora and Narain 1997).

In earlier publications on flavor evaluation, the volatile compounds in yellow passion fruit were reported by Hiu and Scheuer (1961), Winter and Kloti (1972), and Engel and Tressl (1983), while Parliament (1972), Murray and others (1972, 1973), and Casimir and others (1981) identified the volatile components in purple passion fruit. Quantitative comparison in the volatile compounds between yellow and purple variety was reported by Chen and others (1982), Tressl and Engel (1983), and Yamaguchi and others (1983). In most of these studies, the techniques used for the extraction of volatile compounds were either that of simultaneous distillation–extraction (SDE) technique using a modified Likens–Nickerson apparatus or simple solvent extraction or headspace isolation procedures. The main compounds reported were esters of aliphatic and aromatic classes substantiated by the presence of terpenoids.

In one of the earlier studies undertaken on yellow passion fruit juice by Hiu and Scheuer (1961), the main esters involved in the formation of passion fruit aroma were ethyl butanoate, ethyl hexanoate, hexyl butanoate, and hexyl hexanoate. These four esters constituted about 95% of total volatiles extracted by SDE technique, and hexyl hexanoate was the main compound (74%) followed by hexyl butanoate (15%). Belitz and Grosch (1987) reported the presence of 2-methyl-4-propyl-1,3-oxathiane, which contributes to the aroma of yellow passion fruit juice. The difference in composition of these classes of compounds was attributed to the aroma quality of yellow and purple passion fruit. Engel and Tressl (1983) reported that purple variety contained the key character-impact compounds belonging to the esters of 2-heptyl, 2-nonyl, (*Z*)-3-hexenyl, and (*Z*)-3-octenyl acetate, geranyl, and citronellyl compounds. Winter and others (1976) working with the yellow variety of fruit identified two sulfur-containing compounds viz., 3-methylthiohexanol and 2-methyl-4-propyl 1,3-oxathienes, which were mainly responsible for its characteristic aroma.

Several reviews on the chemical composition of volatile compounds were published earlier (Casimir et al. 1981; Chan 1980; Pino 1997; Shibamoto and Tang 1990; Winterhalter 1990). However, during the last decade, there has been substantial new data generated on identification, quantification, and aroma characterization of volatile compounds in passion fruit of the two varieties by applying new volatile extraction and capture techniques. Thus, this chapter will focus on recent findings on the volatile compounds found in passion fruit juice.

FLAVOR CHARACTERISTICS

The moment fruit is harvested, a series of physiological processes gets initiated, which continue until the death of the fruit. Maturation, respiration, and transpiration are part of these processes. During this period, substantial changes in color, flavor, aroma, and consistency of fruit occur. Production of volatile compounds, which contribute to aroma and flavor, changes dramatically with the ripening of the fruit. Passion fruit, if harvested at an immature stage, produces many of the flavor

compounds at a slow pace, hardly attaining its characteristic flavor as generation of most of the volatile substances occur during the process of maturation of the fruit. Preharvest factors, which influence the flavor quality of the fruit, have been reviewed by Mattheis and Fellman (1999).

The acidity of the fruit decreases with maturity, while total sugars and Brix/acid ratio increase. The physicochemical and chemical characteristics at different stages of maturity change according to respiration behavior after harvest of the fruit. The pH of the yellow passion fruit juice varies from 2.8 to 3.4, while that of purple passion fruit juice lies between 2.6 and 3.2. The juice contains acetic, ascorbic, citric, malic, lactic, malonic, and succinic acids. Citric acid is the most predominant acid present in both purple and yellow varieties. In yellow passion fruit, malic acid is the second dominant acid, while in purple variety, lactic and malonic acids are the dominant acids. The total soluble solid contents of yellow passion fruit juice is related to the stage of maturity of the fruit, and it increases from 6.6 for mature green fruits to 11.0 for half-ripe fruits and finally attaining a value of about 12.5 for ripe fruits. The Brix/acid ratio increases from 1.4 for mature green stage to 3.2 for ripe fruits (Bora and Narain 1997). Fresh passion fruit juice is usually considered a concentrate due to its high acidity and strong aroma, and it requires further dilution before consumption.

VOLATILE COMPOUNDS

Tables 20.1–20.7 list the volatile compounds found in yellow and purple passion fruit juice and essence reported by various researchers. The data are assembled from the publications since the year 1998 and organized according to main organic classes of compounds (Brat et al. 2000; Carasek and Pawliszyn 2006; Jordan et al. 2002; Narain et al. 2004; Werkhoff et al. 1998) wherein various forms of volatile extraction/capture techniques have been employed. These include the conventional SDE, headspace (vacuum headspace [VHS]; dynamic headspace [DHS]), and the usage of different fibers by solid phase microextraction (SPME) technique. In most of the earlier reports, some solvent was usually employed for extracting volatiles that generated artifacts, formation of which increased by thermal treatment necessary for the capture of volatiles of higher boiling points. Of late, the use of DHS and SPME techniques are able to identify the authentic compounds present in the sample, although these too have their own inherent limitations. The tables assemble the identification of a total of 406 volatile compounds, which include 7 acids, 62 alcohols, 24 aldehydes, 143 esters, 8 furans, 42 ketones, 8 lactones, 54 sulfur-containing compounds, 49 terpenes, and 11 other compounds.

Among the several volatile extraction techniques tried by Werkhoff and others (1998), the flavor of the VHS sample was found to be of particular sensory interest and was generally recognized as superior to those obtained by other classical isolation techniques. The odor of the VHS extract was representative of the fruit and was described as fresh, juicy, tropical, grassy, fruity, green, sulfury, and estery odor notes reminiscent of honey, pineapples, melons, and grapefruits. The VHS concentrate contained more high boiling components. The DHS sample represented mostly the very highly volatile compounds, while the SDE extracts are known to possess thermally generated compounds creating artifacts, and thus, some compounds

TABLE 20.1. Volatile Acids and Alcohols Found in Passion Fruit Juice and Essence

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)	(Compd. Identified)	
Acids													
Acetic acid		<0.1	<0.1	<0.1	<0.1								*Acidic, *fruity, *plastic, *pungent, *stinging sour odor, *unpleasant
Butanoic acid				<0.1	<0.1								Unpleasant odor and acrid taste
2- and 3-Methylbutanoic acid				<0.1									Strong pungent cheesy or sweaty smell
2-Methylhexyl butanoic acid									0.58	nd			Green, waxy, unripe fruity, apple and banana
Hexanoic acid		<0.1		0.1	<0.1				26.78	tr			*Pesticide, *pungent, *oily, *acrid-acid, *fatty-rancid odor
Nonanoic acid		<0.1											Mildly nut-like, fatty, and acid odor of good tenacity
Octanoic acid				0.2					1.98	5			Oily-rancid, sweat like, repulsive to most people
Alcohols													
Ethanol											0.51		Strong, alcoholic
1-Propanol		<0.1	<0.1	<0.1	<0.1						0.03		Alcoholic, nauseating
2-Methyl-1-propanol		0.1	0.2	<0.1	<0.1				109.81	nd			Pungent
1-Butanol		0.2	0.1	0.2	0.3				10.22	nd	0.04		Highly alcoholic aroma
3-Methyl-1-butanol									17.3	0.33			Onion, gasoline, rubber, burnt oil
2- and 3-Methyl-1-butanol		0.2	<0.1	0.1	0.2								*Onion, *gasoline, *rubber, *burnt oil

3-Methyl-2-buten-1-ol		<0.1		<0.1	<0.1							*Cooked nut
2-Butanol		<0.1	0.1	<0.1	<0.1							Pleasant aromas
2-Methyl 2-butanol				<0.1								Green, oily
1-Pentanol				<0.1						0.06		Sweet, alcoholic, ethereal
2-Pentanol		<0.1			<0.1				5.3	nd	0.09	Ethereal, wine like
3-Pentanol		<0.1	<0.1		<0.1							Strong smell, sharp burning taste
(*Z*)-2-penten-1-ol		<0.1	<0.1	<0.1	<0.1							Green, plastic, rubber
3-Penten-2-ol									1.47	0.75		Fruity, acrid, pungent
1-Hexanol		8	3.3	2.5	5.4	70	90	40	9.78	1.56	2.23	x	*Herbal, *fruity, slightly fatty odor, green
(*E*)-3-hexen-1-ol		0.6	0.1	0.2	0.3				6.98	nd	0.02		Intensely green, bitter-foliage-like, fatty odor
(*Z*)-3-hexen-1-ol		2.5	0.6	1	1.7	50	60	20	1.7	nd	0.02	x	*Green, *herbal, *unripe banana, *grassy odor
1-Heptanol		<0.1			<0.1						0.29		Fresh and light, green-fatty, winey, and sap-like odor of poor tenacity
1-Hepten-3-ol	x												Oily green metallic acrylate tomato spicy
2-Heptanol			<0.1		<0.1	10	10	10	1.87	nd	0.02		*Mashed potato, *fried, *oily, lemon-like, grassy-herbaceous odor, sweet-floral undertone
3-Heptanol	x												Powerful, herbaceous, warm-and-cool odor of poor tenacity, balsamic
6-Methyl 5-hepten-2-ol	x												Flowery, green, mushroom like, honey like
1-Octanol		2.3	0.4	0.3	0.8	20	30	tr	67.47	nd	0.32	x	*Skin potato, *burnt matches, *fatty, *bitter almond, powerful, orange-rose-like, waxy, and sweet odor
(*E*)-3-octen-1-ol	x												Mushroom
(*Z*)-5-octen-1-ol	x												Green, juicy, fruity, sweet, melon like, pear like
2-Nonanol	x												Coconut like

TABLE 20.1. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)	(Compd. Identified)	
3-Nonanol	x												Herbaceous, mildly spicy-earthy but overall sweet and oily odor of moderate to poor tenacity
1-Decanol		0.1		<0.1	0.1								Sweet, fatty-oily odor, waxy, and floral, mainly rose, lily, and orange blossoms
(*E*)-3-decenol	x												Green leafy note
(*Z*)-3-decenol	x												Green leafy note, soft mold-ripened cheese
(*Z*)-4-decenol	x												Fruity, melon like, cucumber like
2,3-Butanediol									nd	0.55			Faint herbal
(*E*)-4, (*Z*)-7-decadienol	x												Oily, fresh and citrusy odor
(*Z*)-4, (*Z*)-7-decadienol	x												Oily, vegetable like
(*E*)-isopiperitenol	x												
(*Z*)-isopiperitenol	x												
2-Methoxy-4-vinylphenol				0.1									Powerful, spicy, clove-like odor, warm, tarry, and undertone
Cyclopentanol		<0.1	<0.1	<0.1	<0.1								Odor of amyl alcohol
2-Phenylethanol		<0.1		0.1	<0.1				1.28	1.57			Rose like, perfumy
3-Phenyl-1-propanol		0.2											Warm, floral, balsamic
3,4-Dihydro-β-ionol	x												Sweet, berry like
7,8-Dihydro-β-ionol	x												Sweet, berry like
8-Hydroxymenthol	x												Cool sensation

β-Citronellol	x											Fresh rose odor, variable according to purity, overall sweet
Benzyl alcohol		2.8	<0.1	0.3	0.2				1.35	4.74	2.93	*Sunflower seeds, *herbal, *moldy, *roasted breed
β-Ionol	x											Sweet, oily-herbaceous, warm odor with floral-balsamic undertones
Borneol	x											Camphoraceous odor
Cinnamic alcohol	x											Warm, balsamic, floral, sweet odor
Chavicol	x											Phenolic odor, penetrating, betel
Eugenol		<0.1		0.1								*Spicy, *fruity, clove
(*E*)-isoeugenol				<0.1								Mild, sweet, deep-floral, nutmeg
Furaneol									nd	tr		*Caramel, *passion fruit
Furfuryl alcohol				0.1								Very mild, warm-oily, burnt odor
Geranyl acetol		0.1										
Isomyrcenol	x											Fresh, floral, lime-like odor
Isopulegol	x											Minty-herbaceous, bittersweet odor
Nerol		0.1		0.3	0.2	tr	5	tr				Sweet rosy, refreshing, and wet seashore odor
Neryl acetol		0.1										
p-1(7),2-menthadienol-8	x											
p-1,5-menthadienol-8	x											
p-1,8-menthadienol-4	x											
p-cymenol-8		<0.1		<0.1	<0.1							Citrus, must

[a]Use of * citation within this column refers to the odor descriptors cited by Jordan and others (2002), while non-asterisk odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure; LLE, liquid–liquid extraction; CF/SPME, cold fiber/solid phase microextraction; nd, not detected; tr, traces.

TABLE 20.2. Volatile Aldehydes Found in Passion Fruit Juice and Essence

Compound	Werkhoff and Others (1998) (Yellow P. Fruit) Juice VHS Compd. Identified	Werkhoff and Others (1998) (Yellow P. Fruit) Juice VHS Area (%)	Werkhoff and Others (1998) (Yellow P. Fruit) Juice DHS Area (%)	Werkhoff and Others (1998) (Yellow P. Fruit) Juice SDE Area (%)	Werkhoff and Others (1998) (Yellow P. Fruit) Juice SDEV Area (%)	Brat and Others (2000) (Purple P. Fruit) Juice LLE Concn (µg/100 g)	Brat and Others (2000) (Purple P. Fruit) Juice SDE Concn (µg/100 g)	Brat and Others (2000) (Purple P. Fruit) Juice VHS Concn (µg/100 g)	Jordan and Others (2002) (Yellow P. Fruit) Essence SDE Concn (ppm)	Jordan and Others (2002) (Yellow P. Fruit) Juice SDE Concn (ppm)	Narain and Others (2004) (Yellow P. Fruit) Juice DHS Area (%)	Carasek and Pawliszyn (2006) (Yellow P. Fruit) Juice CF/SPME (Compd. Identified)	Odor Descriptors[a]
(*E*)-2-butenal				<0.1	<0.1								
2- and 3-Methylbutanal				0.4									Suffocating, malt
2-Methyl-(*E*)-2-butenal				<0.1									
3-Methyl-2-butenal		<0.1			<0.1								
Pentanal				<0.1									Fruity, pungent
Hexanal		0.1	0.1	0.1	0.2	20	40	5			5.12		Very powerful, penetrating, fatty, green, grassy odor
(*E*)-2-hexenal		0.1		0.4	0.4								Powerful green-fruity, pungent vegetable-like odor, fruity and fresh-green in dilutions
(*E*)-3-hexenal		<0.1		<0.1	<0.1								Herbaceous, fatty, tomato-like
(*Z*)-3-hexenal				<0.1	<0.1								Green, herbaceous, leafy, sweet, passion fruit like, apple like
Octanal		<0.1		<0.1	<0.1								Powerful and, in undiluted state, harsh-fatty, penetration odor, orange like
(*E*)-2-octenal					<0.1								Fatty, nutty
Nonanal												x	Citrus, fatty, aldehydic, soapy, floral

Decanal	x											x	Green, waxy
(*E*)-2-decenal	x												Very powerful, waxy-orange-like, sweet-aldehydic odor
Undecanal												x	Pleasant waxy-floral, refreshing odor with a discrete fruity overtone
(*E*)-2, (*E*)-4-heptadienal	x												
Benzaldehyde		1.9	<0.1	1.9	2.9	10	5	30	25.35	3.06	6.94	x	*Roasted pepper, *green almond
Phenylacetaldehyde				0.8	<0.1	10	40	10				x	Very powerful and penetration, pungent-green, floral, and sweet odor of passion fruit
Salicyladehyde	x												Pungent, irritating odor of benzaldehyde, acetophenone, and nitrobenzene
(*E*)-cinnamaldehyde	x												Spice, warm-spicy-balsamic, sweet
Furfural		<0.1	<0.1	2.3	<0.1				nd	0.42	3.17		*Fresh garlic, *rubbery, *moldy
5-Methylfurfural				0.1							0.04		*Roasted garlic, *spicy, *metallic
5-Hydroxymethyl furfural									nd	tr			Warm-herbaceous, winey-ethereal odor
β-Cyclocitral	x												Green notes, flower-shop odor

[a]Use of * citation within this column refers to the odor descriptors cited by Jordan and others (2002), while non-asterisk odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure; LLE, liquid–liquid extraction; CF/SPME, cold fiber/solid phase microextraction; nd, not detected; tr, traces.

TABLE 20.3. Volatile Esters Found in Passion Fruit Juice and Essence

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Ethyl acetate		0.7	3	1.4	2						0.16		*Fruity, *acidic, ethereal-fruity, brandy-like odor
Propyl acetate		<0.1	<0.1		<0.1				1.19	nd			Diffusive and fresh, sweet, fruity-ethereal, pear-like odor of poor tenacity
Butyl acetate		0.1	0.6	<0.1	0.1				2.35	nd	0.34		Pungent, pear, banana, strawberry
Isobutyl acetate		<0.1	0.1	<0.1	<0.1				1.12	nd			*Herbal, *plastic, *solvent, very diffusive, ethereal odor resembling rum
2- and 3-Methylbutyl acetate		<0.1	0.3	<0.1	<0.1								Pear like, banana like
Hexyl acetate		2.0	7.9	0.7	1.6	70	40	30	21.1	0.98	0.45	x	*Banana, *fruity, *cherry, sweet fruity berry, and pear-like odor; milder than amyl acetate, less natural, slightly floral and green
(*E*)-2-hexenyl acetate		<0.1											Powerful and fresh-green, sweet, and fruity
(*E*)-3-hexenyl acetate		1.6	4.2	0.5	1.3								Fruity, green
(*Z*)-3-hexenyl acetate		<0.1	<0.1	0.7	<0.1	90	60	60	6.63	nd	0.13		*Fruity, *candy, *banana, intensely green, sharp-fruity, and very diffusive odor

(*E*)-2-heptenyl acetate	x												
(*Z*)-4-heptenyl acetate	x												
Heptyl acetate	x					10	5	10					Fruity, fatty-green and slightly floral odor with pleasant, leafy undertones
Octyl acetate	x					10	10	5	3.35	0.24			*Woody, *tar, *burnt *plastic, fruity, slightly fatty, waxy-floral odor, green apple like
3-Octenyl acetate	x												
(*Z*)-5-octenyl acetate	x												
Decyl acetate	x												Sweet fatty-fruity odor of pineapple, rosy waxy undertone
9-Decen-1-yl acetate						220	160	90					Pleasant, oily-rosy, rose-petal-like odor of diffusive power
(*Z*)-5-tangerinol (6,10-dimethyl-(*Z*)-5,9-undecadienyl 2-acetate)	x												
(*E*)-5-tangerinol (6,10-dimethyl-(*E*)-5,9-undecadienyl 2-acetate)	x												
Benzyl acetate		1.0	0.1	0.2	0.3				5.64	0.23	0.29	x	*Menthol, *woody, *honey, *rain, *pear
Phenyl acetate						40	20	10					Very sweet, rosy-fruity, honey like
Methyl phenylacetate	x												Powerful and quite diffusive honey-musky odor with traces of jasmin-floral notes
Ethyl (methylthio) acetate		<0.1											Not sulfury, ester like, pineapple like

TABLE 20.3. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)	(Compd. Identified)	
3-Mercaptohexyl acetate				<0.1									
Citronellyl acetate	x												Fresh, fruity, and sweet odor
Cyclohexyl acetate	x												Sweet-fruity candy like
3-(Methylthio) propyl acetate	x												Herbaceous, vegetable like
3-Phenylpropyl acetate		<0.1											Sweet, floral-fruity, warm, and mildly balsamic odor
Phenylethyl acetate	x	0.1		0.1	0.1				1.09	nd			Very sweet, rosy-fruity, honey-like odor
Styryl acetate	x												
Ethyl acetoacetate									4.34	nd			Fruity, ethereal-fruity, sweet and rum-like odor
Ethyl propanoate		<0.1	<0.1	0.1	<0.1				43.22	nd	5.86		Very volatile, ethereal, sweet, fruity, rum-like odor
Hexyl propanoate		<0.1		<0.1	<0.1								Peculiar sweetish-sour, almost “earthy” odor, resembling the odor of overripe fruit on the ground, with sweet, earthy-herbaceous notes
Neryl 2-methylpropanoate		<0.1			<0.1								

(*Z*)-3-hexenyl propanoate	x												Sweet, powerful, intensely green, somewhat vegetable-like but also slightly fatty, oily odor
Ethyl 3-(methylthio) propanoate		<0.1	<0.1	<0.1	<0.1				1.97	nd			*Eucalyptol, *menthol, *caramel, *medicinal
Benzyl propanoate	x												Sweet, floral
Ethyl 3-phenylpropanoate		0.5											Sweet, very light, fruity, honey-like odor
Hexyl 2-methylpropanoate		<0.1										x	Sweet, green, fruity, apple pear, wine, grape
Ethyl 3-(methylthio)-(*E*)-2-propenoate		<0.1	<0.1		0.1								
Methyl butanoate		<0.1	<0.1	<0.1	<0.1				2.65	nd	0.64		*Fruity, *floral, *roasted nut, very diffusive and penetrating, sweet, ethereal, fruity odor
Ethyl butanoate		4.7	23.0	7.9	9.1	980	440	470	215.4	3.86	8.27	x	Powerful, *fruity, *sweet, *strawberry candy, ethereal, fruity odor
Isopropyl butanoate	x												Powerful and diffusive, pungent, pineapple-strawberry-fruit type odor of poor tenacity
Propyl butanoate		<0.1	<0.1	<0.1	<0.1						0.57		Sweet, heavy-fruity, banana, pineapple-like ethereal-pungent odor of poor tenacity
Butyl butanoate		<0.1	<0.1		<0.1	140	60	60	0.48	nd	0.39	x	Apple, banana, pear, nut, peach
Isobutyl butanoate		<0.1	<0.1		<0.1								Ethereal fruity, somewhat pungent odor, reminiscent of pear, pineapple, and banana

TABLE 20.3. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Butyl 2-methyl butanoate						10	20	tr					Green
1-Methylbutyl butanoate	x												Heavy sweet, banana like
Pentyl butanoate	x												Pear or apricot
Isopentyl butanoate	x												Fruity, green, apricot, pear, banana-green
Cyclopentyl butanoate	x												
Hexyl butanoate		8.9	15.5	2.9	8.7	1740	1650	1150	20.55	1.17	14.83	x	*Toothpaste, *fresh, *medicinal, *faint citrus like, powerful, fruity, heavy odor
(*E*)-3-hexenyl butanoate		0.3	0.3	0.1	0.2								Fruity, cognac like
(*Z*)-3-hexenyl butanoate		2.1	2.2	0.5	1.3	600	620	460			0.32		Powerful, fruity-winey, green, cognac-like or brandy-like, slightly buttery-oily odor
1-Methylhexyl butanoate	x												
Heptyl butanoate	x					30	20	10					Sweet-green, fresh, and slightly tea-like odor
Isopentyl 2-methylbutanoate	x												
2-heptyl butanoate						400	430	230					Sweet-green, fresh, tea-like odor

2- and 3-Methylbutyl butanoate		<0.1	<0.1									
Octyl butanoate		<0.1			0.2	360	420	190				Peculiar, green-herbaceous, remotely orange-like, and mildly fatty-floral odor
Ethyl 3-hydroxybutanoate		1.6	<0.1	0.2	<0.1	50	10	5	9.89	6.62		*Pesticide, *roasted nut, *medicinal
(*Z*)-3-hexenyl 3-hydroxybutanoate	x											
Hexyl 3-hydroxybutanoate	x											
Isobutyl 3-hydroxybutanoate	x											
Ethyl 2-methylbutanoate		<0.1	<0.1	<0.1	<0.1							Powerful and diffusive, green-fruity, pungent odor
Hexyl 2-methylbutanoate		0.1	0.1	<0.1	0.1						x	Powerful, sweet, fresh-green fruity odor
Hexyl 3-methylbutanoate					<0.1							
3-methylthio hexyl butanoate		<0.1			<0.1							
Decyl butanoate	x											Mild-fruity, somewhat brandy-like, apricot-type odor, sweet, oily-fruity
Benzyl butanoate		0.3		0.1	0.1				1.37	nd		*Sweet, *peachy, fruity-floral odor
Geranyl butanoate		<0.1										Sweet, fresh, fruity, rose, waxy, raspberry
Neryl butanoate	x											Floral, fruity orange-like, and very sweet odor
Phenylmethyl butanoate						120	100	80				
3-Phenylpropyl butanoate		0.1										

TABLE 20.3. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Phenethyl butanoate		<0.1											Rosy, warm, floral-fruity
Ethyl 3-acetoxybutanoate	x												
Ethyl 2-butenoate		<0.1	<0.1	<0.1	<0.1								Sour, caramel, fruity
Hexyl 2-butenoate		<0.1	<0.1		<0.1								Sweet, fruity, green apple
(*Z*)-3-hexenyl (*E*)-2-butenoate	x												Powerful, fruity-winey, green, cognac-like or brandy-like, slightly buttery, oily odor
Benzyl (*E*)-2-butenoate	x												
Ethyl pentanoate		<0.1	<0.1	<0.1	<0.1						0.02	x	*Dry fish, *nutty, *herbal
Butyl pentanoate	x												Sweet fruity, pineapple, green, raspberry, tropical
(*Z*)-3-hexenyl pentanoate	x												
4-Hydroxy-4-methyl-2-pentanoate						40	5	tr					
3-Mercaptohexyl pentanoate	x												Sulfury, passion fruit character
Methyl hexanoate		<0.1	<0.1	<0.1		tr	5	tr	0.69	nd			Pineapple-like, apricot like
Ethyl hexanoate		5.2	7.2	2.0	5.8	270	140	130	183.21	5.08	2.97	x	*Anise, *fruit overripe
Propyl hexanoate		<0.1	<0.1			140	50	20					*Petrol, *roasted garlic, *skin potato, *pesticide
Isopropyl hexanoate	x					20	5	tr					

Butyl hexanoate		<0.1	<0.1	0.1	<0.1	200	410	140			0.54		Fruity, berry, pineapple, wine
Isobutyl hexanoate	x								0.56	nd		x	*Plastic, *pesticide, *spicy, *green
Pentyl hexanoate						50	20	10			3.98	x	
Isopentyl hexanoate												x	
Hexyl hexanoate		26.7	7.6	10.3	24.4	2120	2550	1100	77.64	9.9	10.76	x	*Peachy, *plum
(*E*)-3-hexenyl hexanoate				0.2									Powerful fruity-green, diffusive odor
(*Z*)-3-hexenyl hexanoate		4.7	1.2	1.6	3.7	810	950	530	11.91	1.25	3.67	x	Strong fruity, green
(*Z*)-3-hexenyl (*E*)-2-hexanoate	x												
2-Heptyl hexanoate						510	580	220					Fresh-green, foliage, and vegetable-like odor
1-Methylbutyl (*E*)-3-hexanoate	x												
2-Methylbutyl hexanoate	x												*Sharp sweat, passion fruit like
3-Mercaptohexyl hexanoate		<0.1			0.1								
Heptyl hexanoate						80	10	5					
Octyl hexanoate		<0.1	<0.1	<0.1	<0.1								Fruity, herbaceous
Isoamyl hexanoate									2.7	nd			*Anise, *spicy, *fruity
Ethyl 3-hydroxyhexanoate		0.2		0.1	0.1	40	10	10	5.04	2.63			*Floral, *passion fruit, *sharp herbal, *fruity, *powerful
Ethyl 5-hydroxyhexanoate		<0.1											
Hexyl 3-hydroxyhexanoate	x												
Benzyl hexanoate		0.3		0.1	0.1								Mild fruity, apricot like
Neryl hexanoate	x												Refreshing, sweet-rosy, fruity-herbaceous

TABLE 20.3. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Phenylethyl hexanoate		0.1			<0.1								Fruity-green, rosy, fresh-pineapple-like, banana like odor
Methyl 3-hydroxyhexanoate						5	10	5					Oily-ethereal, powerful, fruity-winey odor
Geranyl hexanoate	x												Fruity, geranium like
Ethyl (*E*)-2-hexenoate		<0.1	<0.1		<0.1				3.61	nd			
Ethyl (*E*)-3-hexenoate		<0.1	<0.1	<0.1	0.1								
Ethyl heptanoate	x												Powerful, fruity-winey-like, brandy, and berry-like odor with an oily-sharp undertone
Ethyl octanoate		0.3	0.1	0.1	0.3	20	20	50	18.84	1.01	4.86	x	Apple like, sweet, fruity
1-Methylbutyl octanoate	x												
Pentyl octanoate						70	110	40					
Isopentyl octanoate	x					40	10	10					
Hexyl octanoate		0.3	<0.1	0.4	0.6								Oily-herbaceous, slightly green, condiments-like odor
(*Z*)-3-hexenyl octanoate		0.1			<0.1								
1-Methylhexyl octanoate	x												
Heptyl octanoate						240	320	80					Oily-fruity undertone, green potatoes or peanuts

Hexyl decanoate	x										
Ethyl 3-hydroxyoctanoate	x										
Ethyl (*Z*)-5-octenoate	x										
Ethyl benzoate	x										Sweet, warm, floral-fruity, somewhat heavy odor
Benzyl benzoate	x										Faint, sweet-balsamic odor, floral
Hexyl benzoate		0.1									Woody, green, piney-balsamic odor with sweet-herbaceous undertones
Methyl 2-hydroxybenzoate		0.2	<0.1	<0.1	0.1						
Ethyl 2-hydroxybenzoate		<0.1		<0.1							
Diethyl carbonate						3.87	nd	0.19			
Diethyl malonate						0.43	nd				Sweet, soft and pleasant fruity-green, slightly balsamic odor
Diethyl succinate		0.3		<0.1	0.2	nd	0.8				*Fabric, *floral, *cotton, faint, pleasant odor
Ethyl 2-furoate						0.73	nd				Warm, fruity-floral odor slightly, very pleasant or plum-rabble-like undertones
Methyl cinnamate	x										Powerful, yet very tenacious, fruity-balsamic odor, in extreme dilution more fruity, strawberry like
(*E*) and (*Z*) ethyl cinnamates		0.8		0.3	0.3					x	Sweet-balsamic, fruity-honey-like odor, undertones of orange or grape-like character

TABLE 20.3. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Ethyl crotonate									4.41	0.03			Powerful and diffusive, *faint tropical fruity, caramelic-fruity odor
Ethyl furanoate													Warm, fruity-floral odor slightly more pungent than that of ethyl benzoate
Ethyl lactate									4.77	0.33			*Onion, *pungent, *rubbery
Ethyl salicylate	x												Heavy, sweet, floral-fruity odor
Hexyl furoate	x												Peculiar fruity-earthy, yet sweet and pear-like odor with a trace of fungus-like undertone, sweet, herbaceous-fruity, mushroon-like flavor in extreme dilution
S-methyl acetothioate				<0.1									

[a]Use of * citation within this column refers to the odor descriptors cited by Jordan and others (2002), while non-asterisk odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure; LLE, liquid–liquid extraction; CF/SPME, cold fiber/solid phase microextraction; nd, not detected; tr, traces.

TABLE 20.4. Volatile Ketones and Lactones Found in Passion Fruit Juice and Essence

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Ketones													
2-Butanone		0.3	1.3	0.3									
3-Hydroxy-2-butanone		0.4	1.3	0.4	0.7				2.5	27.87			Fruity, moldy, woody, fresh, butter like
3-Methyl-2-butanone									nd	1.56			
2-Pentanone											0.34		Sweet, fruity ketone, acetone like
3-pentanone		<0.1	0.1	0.1	0.1				3.53	nd	10.26		*Burnt, *plastic, *pungent
Cyclopentanone		0.2	0.1	<0.1	0.1				10.74	0.54	0.05		Grassy, woody, musty odor
3-Penten-2-one				<0.1	<0.1								Ethereal
4-Methyl-3-penten-2-one		<0.1		<0.1	<0.1								
2-Cyclopenten-1-one		<0.1	<0.1	0.3	<0.1				0.5	nd			
2-Heptanone		<0.1	0.1	<0.1	<0.1	50	10	10			1.02		Blue cheese, cinnamonbarck like, pear drops, fruity, musty
3-Heptanone		<0.1	<0.1	<0.1	<0.1								Powerful, green, fatty, fruity
3-Hexanone									0.62	nd			Ethereal, grape, wine like
Cyclohexanone	x												Unpleasant, solvent-like odor

TABLE 20.4. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)	(Compd. Identified)	
1-Hexen-3-one				<0.1	<0.1								
3-Methyl 2-cyclohexenone	x												
2-Nonanone											0.02		Green, fatty, ketonic, fruity, musty, blue cheese
3-Nonanone		<0.1	<0.1		<0.1						0.01		Powerful and somewhat sharp or pungent grassy-herbal odor with green-fruity undertones
2-Tridecanone	x												Rancid, fruity, tallowy, fruity, green
2-Tetradecanone													Bear like
2-Pentadecanone	x												Fresh, jasmine, celery
(*E*)-α-ionone	x												Warm-woody, balsamic-floral odor of sweetness, resemblance to the odor of violet flowers
β-Ionone		0.1		0.1	0.2	370	440	260			0.19	x	Warm, woody, fruity undertone
(*E*)-3, (*E*)-5-pseudoionone		0.2		<0.1	<0.1								Oily-balsamic, warm-floral odor, jasmin-violet character

(*E*)-β-damascone	x						Fruity, floral, plum, rose, honey, tobacco
2,3-Butanedione				<0.1			Creamy, buttery, sickly, penetrating, quinonic, chlorine like
2,3-Pentanedione		<0.1	<0.1	<0.1	<0.1		Estery apple, almond, malty, butter, sickly, burnt, grain, burnt butter, oily buttery, pungent
3,6-Octanedione	x						Roasted nuts
4,(*Z*)6,(*E*)8-megastigmatrienone	x						
Acetophenone	x						Pungent-sweet odor, in dilution resembling that of harsh orange-blossom type
3-Methylacetophenone	x						Bitter almond like
4-Methylacetophenone	x						Pungent, almost harsh, but warm, sweet floral odor
3-Ethylacetophenone	x						Sweet, warm, somewhat pungent-floral odor with a herbaceous-balsamic undertone
Isopiperitenone	x						Diffusive, sweeter, minty
Menthone	x						Minty-refreshing, slightly woody, dry
Neryl acetone	x						Fatty, metallic
Geranyl acetone						x	Fresh-floral, light, sweet-rosy
p-3-Menthen-2-one	x						
Piperitone	x						Fresh, minty, camphoraceous
Pulegone	x						Herbaceous, lavender
Verbanone	x						

TABLE 20.4. ***Continued***

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Lactones													
γ-Butyrolactone									nd	1.26			*Toasted nut, *cheesy, sweet, caramellic taste
α–Angelica lactone				<0.1									Sweet-herbaceous
(*E*)-marmelolactone	x												Strongly fruity, floral, quince like
(*E*)-γ-jasmin lactone		<0.1											Sweet, creamy, oily, flowery, fruity
(*Z*)-γ-jasmin lactone		<0.1											Powerful and fruity, reminiscent of peach and apricot, sweet odor of gardenia flowers
δ-Jasmin lactone	X												Creamy, milky, buttery, fruity
δ-Hexalactone	X												Sweet, coumarin like
3-Methyl-δ - hexalactone	X												

[a]Use of * citation within this column refers to the odor descriptors cited by Jordan and others (2002), while non-asterisk odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure; LLE, liquid–liquid extraction; CF/SPME, cold fiber/solid phase microextraction; nd, not detected.

TABLE 20.5. Volatile Terpenes and Norisoprenoids Found in Passion Fruit Juice and Essence

Compound	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	Odor Descriptors[a]
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME (Compd. Identified)	
	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)		
Myrcene		0.2	4.4	0.7	1.2				4.37	0.58	0.04	x	*Fruity, *herbal, *spicy
β-Ocimene									1.06	0.69		x	*Floral, *rose, *spicy, *carnation
(*E*)-β-ocimene		0.3	4.1	0.7	1	30	5	5					Sweetness, almost floral
(*Z*)-β-ocimene			<0.1	<0.1									Citrus, green, lime
Allo-ocimene												x	Anise
α-Pinene		<0.1	0.3	<0.1	<0.1	10	tr	tr				x	Turpentine like
β-Pinene			<0.1	<0.1	<0.1							x	Dry-woody, resinous-piney
p-Cymene						10	60	0					Gassy, kerosene like, citrusy
α-Phellandrene			<0.1	<0.1	0.1								Citrus, spice
β-Phellandrene			<0.1		0.2								Peppery-minty, refreshing, slightly
α-Terpinene			0.1	<0.1	0.2							x	Refreshing, lemony-citrusy
γ-Terpinene		0.1	0.3	0.1	0.2							x	Herbaceous, gasoline like, ethereal
Caryophyllene												x	Woody, spicy
3-Carene		<0.1	<0.1		<0.1							x	Sweet, diffusive
Camphor	x												Ethereal-diffusive, bitter warm
Camphene						tr	tr	tr					Mild, oily, camphoraceous
Limonene		0.2	1.8	0.4	0.7	1070	30	5	6.59	0.5	0.54	x	*Herbal, *mild fruit, *pesticide, pine, orange, lemon like
α-Terpinolene						tr	tr	tr				x	Fresh, woody, sweet, pine, citrus
β-Farnesene	x					20	20	20					Very mild, sweet, warm
(*E*)-3, (*E*)-6-farnesene		0.1		<0.1	0.5							x	Green apple odor

TABLE 20.5. ***Continued***

	Werkhoff and Others (1998) (Yellow P. Fruit)					Brat and Others (2000) (Purple P. Fruit)			Jordan and Others (2002) (Yellow P. Fruit)		Narain and Others (2004) (Yellow P. Fruit)	Carasek and Pawliszyn (2006) (Yellow P. Fruit)	
	Juice					Juice			Essence	Juice	Juice	Juice	
	VHS	VHS	DHS	SDE	SDEV	LLE	SDE	VHS	SDE		DHS	CF/SPME	
Compound	Compd. Identified	Area (%)				Concn (μg/100 g)			Concn (ppm)		Area (%)	(Compd. Identified)	Odor Descriptors[a]
(*Z*)-3, (*E*)-6-farnesene		<0.1			0.1								Green apple odor
Theaspirane A		<0.1		<0.1	<0.1								Tea, herbal, green, wet, tobacco, woody, spicy
Theaspirane B		<0.1			<0.1								Tea, wet, tobacco, leaf, metallic, woody, spicy
Geranial	x												Green, floral, fruity, citrus like
Neral	x												Green, floral, fruity, citrus like, peely
(*E*)-ocimenol				0.4									Viscosity, camphoraceous-lime-like odor, floral undertone
(*Z*)-ocimenol				0.1									Camphoraceous, lime-like, floral
Thymol	x												Sweet-medicinal, herbaceous, warm
4-Terpineol		0.3		0.1	0.2				1.6	nd			Warm-peppery, mildy earthy, warm-herbaceous, camphoraceous
α-Terpineol		0.5	<0.1	2.0	1.7	260	300	250					Delicately floral odor and sweet of lilac type
γ-Terpineol		0.5	<0.1	2.0	1.7								Pleasant odor similar to lilac
3-[(*E*)-1-propenyl]-α-terpineol P2		0.2											
3-[(*E*)-1-propenyl]-α-terpineol P1		<0.1											

Hotrienol		<0.1		<0.1	<0.1								Sweet, tropical, fennel, ginger
α-Citronellol		0.1			0.1				2.24	nd			Fresh-rose odor, sweet
Geraniol		0.3		0.8	0.8	50	40	10	18.14	nd			*Tropical fruity, *passion fruit, *peachy
Linalool		0.5	0.3	4.6	4.9	60	80	20	18.18	nd	0.13	x	*Sweet, *fruity, *floral, *lemon
(*E*)-anhydrolinalool oxide				0.3									Sweet, woody, floral, earthy
(*Z*)-anhydrolinalool oxide				0.2									Sweet, woody, penetrating odor with floral-woody earthy undertone
(*E*)-linalool oxide (f)		0.2		1.2	0.4	10	10	tr	3.42	nd			Sweet, woody, floral-woody earthy
(*Z*)-linalool oxide (f)		<0.1		0.6	0.1								Powerful sweet-woody, penetrating odor with floral-woody-earthy undertones
Nerol oxide				<0.1									Weedy, floral, orange blossom, green, sweet
Carvacrol	x												Herbaceous, penetrating, dry-medicinal
7,8-Dihydro-β -ionone		0.2		0.1	0.2								Oily
Carvone	x												Caraway, spearmind-like
β-Damascenone		<0.1		<0.1	<0.1								Boiled apple like
1,8-Cineole		<0.1	<0.1	<0.1	<0.1								Sweet, fresh, camphoreceous, cool odor
(*E*)-α-bergamotene	x												Woody, warm, tea
β-Bisabolene	x												Pleasant, sweet, spicy, balsamic odor

[a]Use of * citation within this column refers to the odor descriptors cited by Jordan and others (2002), while non-asterisk odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure; LLE, liquid–liquid extraction; CF/SPME, cold fiber/solid phase microextraction; nd, not detected; tr, traces.

TABLE 20.6. Volatile Furans and Other Compounds Found in Passion Fruit Juice

	Werkhoff and Others (1998) (Yellow P. Fruit)					
	VHS—Compd Identified	VHS	DHS	SDE	SDEV	
Compound	Area (%)					Odor Descriptors[a]
Furans						
2-Ethylfuran				<0.1		Powerful and diffusive, sweet-ethereal "burnt" odor; in extreme dilution rather pleasant, warm, and sweet
2-Acetylfuran				0.4		Green, resinous, woody
2-Methyl-3(2H)-furanone				<0.1		
2,5-Dimethyl-3(2H)-furanone		<0.1		<0.1	<0.1	
2,5-Dimethyl-4–hydroxy 3-(2H)-furanone		0.1				Caramel like
2,5-Dimethyl-4-methoxy-3(2H)-furanone	x					
2,5-Dimethyl-4-acetoxy-3(2H)-furanone	x					Savory, roasty, caramel-like
Other miscellaneous compounds						
Perillene	x					Woody
(2-Nitroethyl) benzene		0.1				Sweet-floral, warm-spicy, tenacious odor
(*Z*)-edulan						
(*Z*)-rose oxide	x					Grassy-green, floral odor
1-Isopropenyl-4-methylbenzene		<0.1		<0.1	<0.1	
1-Nitro-2-phenylethane	x					Sweet-floral, warm-spicy
2,6,6-Trimethyl-2-vinyltetrahydropyran				0.4		Sharp, almost irritating, fresh-camphoraceous cineolic odor
Cycloionone (2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5H-1-benzopyran)	x					Fruity, sweet, floral, woody
Dill ether ((*Z*)-3,9-epoxy-*p*-menth-1-ene)	x					
Riesling acetal				<0.1		
Thymolmethyl ether (1-isopropenyl 2-methoxy-4-methylbenzene)	x					Warm-spicy, rooty, herbaceous

[a]Odor descriptors were collected from various references (Arctander 1969).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure.

TABLE 20.7. Volatile Sulfur Compounds Found in Passion Fruit Juice and Concentrates

	Engel and Tressl (1991) (Yellow P. Fruit)						Werkhoff and Others (1998) (Yellow P. Fruit)				Narain and Others (2004) (Yellow P. Fruit)	
	SDE from Concentrate Samples						VHS	VHS	SDE	SDEV	DHS	
	a	b	c	d	e	f						
Compound	Concentration (ppb)						Compd. Identified	Area (%)	Area (%)	Area (%)	Area (%)	Odor Descriptors[a]
Dimethyl disulfide											0.05	Sulfurous, sickly, cooked cabbage, intensively onion like, very diffusive, non-lachrymatory
2-Pentanethiol									<0.1			
3-(Methylthio) hexyl hexanoate							x	<0.1		<0.1		
3-Methylthio hexanol									<0.1			
Diethyl disulfide							x				0.03	Sulfury, rubbery, carbide like, sweet, garlic, burnt rubber, hydrogen sulfide (in beer)
Diisopropyl disulfide							x					Sulfury, oniony, roasted onion, tropical fruits, durian like
Methional										<0.1		Musty tomato, musty potato, earthy, vegetable, creamy
p-1-Menthenal-9							X		<0.1	<0.1		
Methyl 2-methylbutyl disulfide							x					Sulfury, rubbery, onion-like
Diisopropyl trisulfide							x					Oniony, leek like, durian like, sulfury, roasted onion, metallic
1,1-Bis(methylthio) 2-methyl propane							x					Roasty, coffee like, shiitake, oniony, metallic
(*Z*)-2-methyl 4-*n*-propyl 1,3-oxathiane							x	<0.1	<0.1	<0.1		Fruity, tropical fruity note, green, passion fruit
(*E*)-2-methyl 4-*n*-propyl 1,3-oxathiane							x	<0.1	<0.1	<0.1		Sulfury, tropical fruit note, herbaceous, mango, passion fruits
4-Methyl 5-vinylthiazole							x					Fatty, roasty, nutty, roasted peanut, bread crust like, popcorn, cocoa

TABLE 20.7. ***Continued***

Compound	Engel and Tressl (1991) (Yellow P. Fruit) SDE from Concentrate Samples a	b	c	d	e	f	Werkhoff and Others (1998) (Yellow P. Fruit) VHS	VHS	SDE	SDEV	Narain and Others (2004) (Yellow P. Fruit) DHS	Odor Descriptors[a]
	Concentration (ppb)						Compd. Identified	Area (%)	Area (%)	Area (%)	Area (%)	
3-Mercaptohexanol	195	61	109	47	22	1	x	0.1	0.2	0.2		Juicy, tropical fruits, grapefruit, black currant, mango
3-Mercaptohexyl acetate	1	2	2	0.4	0.5	0.1	x					Grapefruit, black currant, mango, passion fruit
3-Mercaptohexyl butanoate	2	7	2	1	1	0.5	x					Fruity, grapefruit, black currant, tropical fruits, mango
3-Mercaptohexyl pentanoate							x					Tropical fruit, passion fruit
3-Mercaptohexyl hexanoate	1	4	1	2	1	0.5	x					Grapefruit, black currant, tropical fruits, passion fruit
3-(Methylthio) hexanol	16	19	3	21	1	1	x					Fruity, juicy, melon like, black currant, passion fruits
3-(Methylthio) hexyl acetate	0.1	0.3	–	–	–	–	x					Fatty, fruity, sweet, mango, passion fruit, guava, durian like
3-(Methylthio) hexyl butanoate	0.2	1	–	–	–	–	x					Sulfury, fruity, caramel like, rhubarb, carbide like, caraway like
3-(Methylthio) hexyl hexanoate	0.3	1	–	–	–	–	x					Faintly fruity, green, tropical fruit note
2-(Methylthio) ethyl acetate							x					Sulfury, rotten, creamy, cauliflower, kohlrabi
3-(Methylthio) propyl acetate							x					Herbaceous, mushroom like, cabbage, asparagus, potato
3-(Methylthio) propyl butanoate							x					Sulfury, cheese like, mushroom-like
3-(Methylthio) propyl hexanoate							x					Tropical fruit note, methional like, canned pineapple
Ethyl (methylthio) acetate							x					Fruity, sweet, juicy, radish like
Methyl 3-(methylthio) propanoate							x					Sulfury, tropical fruit note, radish like, cabbage
Ethyl 3-(methylthio) propanoate							x					Fruity, herbaceous, sulfury, milk like, cheese like

Propyl 3-(methylthio) propanoate	x	Vegetable like, mushroom like, cabbage
Butyl 3-(methylthio) propanoate	x	Sulfury, rubbery, fruity, mushroom like, kohlrabi, radish like
Isobutyl 3-(methylthio) propanoate	x	Rubbery, fruity, mushroom like
Secbutyl 3-(methylthio) propanoate	x	Sulfury, not fruity, weak cabbage character
Pentyl 3-(methylthio) propanoate	x	Fruity, somewhat pineapple like, milk like, green, radish like
2-Methylbutyl 3-(methylthio) propanoate	x	Faintly fruity
3-Methylbutyl 3-(methylthio) propanoate	x	Fruity, estery, pineapple like
Hexyl 3-(methylthio) propanoate	x	Fruity, tropical fruits, mango, passion fruits, guava, geranium like
(*Z*)-3-hexenyl 3-(methythio) propanoate	x	Sulfury, sweet, green, carbide like, leek like
Methyl 3-(methylthio) (*E*)-2-propenoate	x	Cheesy, yoghurt like, pineapple like, weak caramel character
Methyl 3-(methylthio) (*Z*)-2-propenoate	x	Sulfury, faintly cabbage like, cauliflower
Ethyl 3-(methylthio) (*E*)-2-propenoate	x	Sulfury, sweet, metallic
Ethyl 3-(methylthio) (*Z*)-2-propenoate	x	Not fruity or pleasant, rotten, sour
Propyl 3-(methylthio) (*E*)-2-propenoate	x	Faintly fruity, sweet
Hexyl 3-(methylthio) (*E*)-2-propenoate	x	Sulfury, fruity, green, estery, rotten, faintly passion fruit like
3-(1-Hydroxy-3-hexyldithio) hexanol	x	
3-(1-Hydroxy-3-hexyldithio) hexyl acetate	x	
3-(1-Hydroxy-3-hexyldithio) hexyl butanoate	x	
3-(1-Acetoxy-3-hexyldithio) hexyl acetate	x	
3-(1-Acetoxy-3-hexyldithio) hexyl butanoate	x	
3-(1-Acetoxy-3-hexyldithio) hexyl hexanoate	x	
3-(1-Butyryloxy-3-hexylthio) hexyl butanoate	x	
3-(1-Butyryloxy-3-hexylthio) hexyl hexanoate	x	

[a]Citation within this column refers to the odor descriptors cited by Werkhoff and others (1998).

Abbreviations used for the volatiles extraction techniques in the table are VHS, vacuum headspace; DHS, dynamic headspace; SDE, simultaneous distillation and extraction at atmospheric pressure; SDEV, simultaneous distillation and extraction under reduced pressure.

identified in these extracts are not considered as authentic compounds present in the juice. Each isolation method causes alterations in overall aroma composition of passion fruit and formation of new compounds known as artifacts can happen during analysis, depending on several parameters viz., solvent choice, temperature and duration of extracting volatiles, concentration of the dilute solutions, or extracts using a small quantity of solvent. Moreover, SDE technique is a rather complex procedure in which volatile compounds distilled with water can be collected and hence may result in distortion of aroma profile.

Acids and Alcohols

Table 20.1 shows the volatile acids and alcohols found in passion fruit juice. Among the volatile acids identified in yellow passion fruit juice, octanoic and hexanoic were the principal compounds (Jordan et al. 2002; Werkhoff et al. 1998). However, a large number of acids such as (*Z*)-oct-3-enoic, (*E*)-oct-3-enoic, benzoic, phenylacetic, cinnamic, furoic, and a series of aliphatic (heptanoic, nonanoic, decanoic, dodecanoic, tetradecanoic, pentadecanoic, and hexadecanoic) acids were reported in yellow passion fruit juice earlier (Winter and Kloti 1972). Most of the low-carbon-chain-containing acids contribute to the acidic flavor of passion fruit juice, as the flavor of organic acids, which have higher molecular weight than that of nonanoic acid, is quite mild, and thus these acids do not contribute to the acidic flavor of passion fruit juice. The free acids such as butanoic, hexanoic, and benzoic in passion fruit could be formed from the metabolism of lipids. Yamaguchi and others (1983) also reported that the aliphatic acids varying from C_2–C_8 influence the characteristic aroma of passion fruit. The prominent acids that contribute to the acidic flavor of passion fruit juice are acetic acid, 2- and 3- methyl butanoic acid, 2-methylhexyl butanoic acid, and nonanoic acid. Hexanoic acid was found to be present in canned passion fruit juice from Taiwan to the extent of 13% (Yamaguchi et al. 1983).

A large number (62) of alcohols were reported in the passion fruit juice (Table 20.1). In analyzing the volatiles of the yellow fruit juice, Werkhoff and others (1998) used several techniques of extraction (VHS, DHS, SDE, simultaneous distillation and extraction under reduced pressure [SDEV]) and identified the presence of 58 alcohols, most of them found in the extracts obtained by VHS technique. The three alcohols viz., 1-hexanol, (*Z*)-hexen-1-o, 2-heptanol, and 1-octanol were found to be present in all the extracts and volatiles captured by all techniques including SPME in both yellow and purple passion fruit juices (Table 20.1). Hexanol possesses herbal, fatty, and fruity aroma; (*Z*)-3-hexenol characterizes for green, herbal, and unripe banana aroma; 2-heptanol for fried and oily aroma; and octanol is known for fatty, buttery, and almond-like aroma (Brat et al. 2000; Carasek and Pawliszyn 2006; Jordan et al. 2002; Narain et al. 2004; Werkhoff et al. 1998).

It is noteworthy that a series of 1- and 2-alcohols starting from C_2 to C_{10} were present in yellow passion fruit juice. Most of these alcohols possess pleasant aroma of sweet, fruity, and pungent notes. Higher concentration of benzyl alcohol was also found in yellow passion fruit juice. In purple passion fruit, 1-hexanol, (*Z*)-3-hexen-1-ol, 2-heptanol, 1-octanol, and nerol, which posses sweet rosy, refreshing, and wet seashore odor, were found to be present (Table 20.1). The quantity of (*Z*)-3-hexenol present in purple passion fruit is higher (475 µg/kg) than in yellow passion fruit

(72 µg/kg), while the reverse was true for 1-octanol (being 1235 µg/kg in yellow and 239 µg/kg in purple passion fruit) (Chassagne et al. 1999).

In the yellow passion fruit juice, the other prominent alcohols were 1-butanol, 1-decanol, 2-methyl 1-propanol, 2-pentanol, 2-phenylethanol, (*E*)-3-hexenol, (*Z*)-2-penten-1-ol, 1-decanol, 3-methyl 1-butanol, 2-phenylethanol, cyclopentanol, and other terpenic alcohols such as linalool, α-terpineol, and *p*-cymenol, while in purple passion fruit juice, only a few alcohols have been identified and prominent of these are linalool, nerol, and α-terpineol (Brat et al. 2000). Bound aromatic alcohols were found in purple and yellow passion fruits, whereas phenolic compounds can be considered as characteristic of purple variety (Chassagne et al. 1999).

Several phenolic compounds such as 4-hydroxy-ionol; 4-oxo-ionol; 4-hydroxy-7,8-dihydro-ionol; 4-oxo-7,8-dihydro-ionol; 3-oxo-ionol; isomeric 3-oxo retro-ionols; 3-oxo-7,8-dihydro-ionol; 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene vomifoliol and dehydrovomifoliol (Winterhalter 1990); terpene alcohols linalool and terpeneol (Challier et al. 1990); terpene diols (*E*) and (*Z*)-2,6-dimethyl-octa-2,7-diene-1,6-diol; 2,6-dimethyl-octa-3,7-dien-2,6-diol; 2,6-dimethyl-1,8-octanediol; 2,6-dimethyl-octa-1,7-diene-3,6-diol; ionol derivatives oxygenated in position 3; and 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (furaneol) have been identified (Chassagne et al. 1999).

In an earlier work on passion fruit, methanol was found to be present in purple variety of the fruit from Australia (Murray et al. 1972). Ethanol was the major volatile component of the purple passion fruit from Australia, while only a small quantity in traces was found in the fruit juice from Taiwan. Chen and others (1982) reported that the ethanol recuperation could be due to poor retention capacity of the adsorbent Tenax-GC. It is surprising to observe that none of the researchers (Brat et al. 2000; Carasek and Pawliszyn 2006; Jordan et al. 2002; Werkhoff et al. 1998) reported the presence of ethanol in yellow or purple passion fruit juice (Table 20.1).

Werkhoff and others (1998) reported (*Z*)-5-octen-1-ol to be of interest to yellow passion fruit flavor. Unsaturated alcohols have relatively low threshold values, and hence compounds such as 3-decenol or 4,7-decadienol, which have a vegetable-like odor with a green, leafy note, may play an important part in the fruity flavor of passion fruit juice. Geranyl acetol was shown to have a sweet, fruity, and floral flavor note, which was associated with honey-like and peach-like odors, while the aroma of neryl acetol is known to be fruity, sweet, floral, mango like, and rose like. Citronellol, which was identified in yellow passion fruit juice, has been described by sensory panelists as blueberry like.

In yellow passion fruit juice, Werkhoff and others (1998) also reported the presence of phenols such as eugenol, isoeugenol, and chavicol, which possess characteristic flavors of cloves, nutmeg, and betel oily notes, respectively.

The aromatic fraction of the commercial passion fruit juice essence consisted of 2-methyl-1-propanol and octanol as the key components found in higher concentrations, while in the juice extracts, benzyl alcohol was the most abundant compound. 3-Penten-2-ol was present in both samples (juice and essence). Only benzyl alcohol and 2-phenylethanol were quantified in greater concentrations in the juice than in the essence (Jordan et al. 2002). Higher concentration of 2-methyl-1-propanol in passion fruit essence was found; however, this compound was not detected in passion fruit juice, which contained furaneol representing passion fruit flavor. Ironically, furaneol was not detected in passion fruit essence.

Aldehydes

Among the various techniques of volatile extraction tried by Werkhoff and others (1998), the aldehydes were mostly identified in the extracts obtained by SDE (Table 20.2). However, in the volatiles captured by VHS, benzaldehyde, hexanal, (*E*)-2-hexenal, (*E*)-3-hexenal, octanal, 3-methyl 2-butenal, and furfural were detected, while in the DHS, higher concentrations of benzaldehyde and hexanal were found.

The prominent aldehydes reported in yellow passion fruit were benzaldehyde, hexanal, octanal, phenylacetaldehyde, (*E*)-2-hexenal, and furfural. In the SPME technique used for the volatile capture, Carasek and Pawliszyn (2006) reported the presence of 1-nonanal and 1-decanal. Higher concentrations of benzaldehyde, hexanal, and furfural were reported by Narain and others (2004). Benzaldehyde is known for its characteristics almond flavor note. The precursors of benzaldehyde formation could be cyanogenic glycoside, which is hydrolyzed by β-glucosidase involving the evolution of HCN, which gets vaporized at low pH (Shoseyov et al. 1990).

According to Werkhoff and others (1998), in general, the aldehydes such as (*E*)-2-hexenal (green, fruity, juicy, sweet, leafy, apple like, banana like), (*Z*)-3-hexenal (green, herbaceous, leafy, sweet, bean like, apple like, tomato like, passion fruit like), and (*E*)-3-hexenal (herbaceous, fatty, aldehyde like, tomato like) as well as neral, geranial, β-cyclocitral, and phenylacetaldehyde contribute mainly the green, floral, and fruity aroma impressions to yellow passion fruit flavor. The odor threshold of alkanals varies from 0.04 to 1 mg/kg, while that of 2-alkenals falls between 0.04 and 2.5 mg/kg (Palm 2002). Moreover, most of the straight-chain and branched aliphatic aldehydes listed in the table accompany also their corresponding alcohol.

Hexanal, which characterizes for green flavor of fresh grass type was found in the juice obtained from passion fruit of the green stage of maturity (Parliament 1972). The author reported that with the advance in the maturation of the fruit, hexanal concentration decreases.

Yamaguchi and others (1983) found higher concentration (21%) of furfural in canned juice from Taiwan, but it was not found in freshly extracted juice. In yellow passion fruit juice, Narain and others (2004) reported the presence of furfural and methyl furfural, which could be produced by thermal treatment of the juice and are formed by the Amadori rearrangement involving the carbonyl compounds such as the reducing sugars reacting with free amino groups.

Esters

A class of compounds, which is of special importance for passion fruit aroma, is that of esters, which constitute the largest number of volatile compounds present in passion fruit juice. Table 20.3 shows the recent data on esters (143 compounds) reported in passion fruits juice and essence. The main esters found in the passion fruit juice are the compounds belonging to acetates, butanoates, and hexanoates. The VHS concentrate contained more high boiling components and in which both alkenyl alkanoates and alkyl alkenoates were detected. The dominant alkenyl groups were hexenyl, heptenyl, and octenyl with double bonds at different positions.

The prominent esters found in both varieties of passion fruit juice are hexyl hexanoate, (*Z*)-3-hexenyl acetate, hexyl butanoate, hexyl acetate, methyl hexanoate, ethyl hexanoate, ethyl butanoate, (*Z*)-3-hexenyl hexanoate, ethyl octanoate, ethyl 3-hydroxy butanoate, (*Z*)-3-hexenyl butanoate, ethyl 3-hydroxy hexanoate, butyl hexanoate, and butyl butanoate (Table 20.3). The principal compounds identified by different extraction techniques in yellow passion fruit juice are ethyl acetate, propyl acetate, butyl acetate, isobutyl acetate, 2- and 3-methylbutyl acetate, hexyl acetate, (*Z*)-3-hexenyl acetate, benzyl acetate, phenylethyl acetate, ethyl propanoate, ethyl butanoate, methyl butanoate, propyl butanoate, hexyl butanoate, (*Z*)-3-hexenyl butanoate, ethyl 3-hydroxy butanoate, benzyl butanoate, ethyl pentanoate, methyl hexanoate, ethyl hexanoate, propyl hexanoate, butyl hexanoate, hexyl hexanoate, (*Z*)-3-hexenylhexanoate, and ethyl octanoate (Werkhoff et al. 1998). The identification of esters such as ethyl propanoate, methyl butanoate, propyl butanoate, ethyl pentanoate, and butyl hexanoate in the yellow variety of passion fruit was reported for the first time by Narain and others (2004). Quantitatively, the major components were hexyl butanoate, hexyl hexanoate, ethyl 3-hydroxybutanoate, and (*Z*)-3-hexenyl hexanoate.

In purple fruit juice, a higher concentration (varying from 90 to 220 μg/100 g) of 9-decen-1-yl acetate possessing pleasant rose petal-like odor was found in all the volatile extracts by Brat and others (2000) while this compound was not identified in yellow passion fruit juice. Similarly, higher concentrations of 2-heptyl butanoate (230–400 μg/100 g), phenylmethyl butanoate (80–120 μg/100 g), 2-heptyl hexanoate (220–510 μg/100 g), pentyl octanoate (40–110 μg/100 g), and heptyl octanoate (80–240 μg/100 g) were also reported while these compounds were not identified in yellow passion fruit juice (Table 20.3). Very high concentrations of sweet, green, and fruity odor compounds such as ethyl butanoate (440–980 μg/100 g), hexyl butanoate (1150–1740 μg/100 g), (*Z*)-3-hexenyl butanoate (460–600 μg/100 g), octyl butanoate (190–360 μg/100 g), hexyl hexanoate (1100–2120 μg/100 g), and (*Z*)-3-hexenyl hexanoate (530–950 μg/100 g) were found in purple passion fruit juice. The difference in this composition and concentration of esters could be attributed to the flavor differences in the two varieties of passion fruit.

Werkhoff and others (1998) studied the aromatic profile of yellow passion fruit and reported that this fruit is characterized by an exotic estery aroma with a sharp sulfury note. Among the esters possessing fruity aroma, higher concentrations of ethyl butanoate, hexyl hexanoate, ethyl hexanoate, (*Z*)-hexenyl hexanoate, (*E*)-3-hexenyl acetate, hexyl butanoate, ethyl (3)-hydroxy hexanoate, ethyl octanoate, benzyl hexanoate, and ethyl cinnamate were found in volatile extracts of passion fruit juice. Furaneol acetate produces a typical roasty, caramel-like, chocolate-like, and vanilla-like sensory impression substantiated by characteristic creamy, milky, and malty notes, reminiscent of strawberries.

Parliament (1972) suggested that the flavor differences between the yellow and purple varieties of passion fruit could be related to the reverse order of abundance of the four esters, ethyl butanoate, ethyl hexanoate, hexyl butanoate, and hexyl hexanoate. In the freshly extracted yellow passion fruit juice analyzed on the same day of the fruit's fall to the ground, Narain and others (2004) did not find a higher concentration of hexyl hexanoate.

In an earlier work, Chen and others (1982) identified 33 esters in the headspace of passion fruit from Taiwan. They found a concentration of 267, 205, and 160 μg of

ethyl hexanoate, ethyl butanoate, and hexyl hexanoate, respectively, obtained from 85 g of yellow passion fruit juice. However, in the purple variety, Murray and others (1972) reported higher concentration (35%) of ethyl butanoate as compared with hexyl hexanoate (2%).

Ethyl butanoate, which possesses the sweet and typical fruit aroma and is a key odorant for orange flavor having an odor threshold of 0.13 ppb, was identified as a major component in volatiles of passion fruit. Ethyl hexanoate possessing a similar aroma note was also found in passion fruit juice. Other esters such as butyl acetate, butyl butanoate, hexyl butanoate, and hexyl hexanoate, which have mixed aroma notes of banana, pineapple, and pear fruits, were identified in all varieties of passion fruit. The esters of saturated and unsaturated acids belonging to the C_2–C_6 chain result in typical aroma of passion fruit juice.

In headspace analysis of yellow passion fruit juice, Narain (1993) reported the presence of hexyl butanoate (14.8%), hexyl hexanoate (10.7%), ethyl butanoate (8.3%), benzaldehyde (6.9%), ethyl propanoate (5.9%), and ethyl octanoate (4.9%). In an analysis of dilution of the aroma extract obtained from the yellow passion fruit juice and essence, Jordan and others (2002) verified that the presence of more potent compounds such as 2-methylbutyl hexanoate, ethyl propanoate, ethyl butanoate, ethyl hexanoate, and hexyl hexanoate were found in commercial essence, while in the juice extracts, 2-methylbutyl hexanoate, 3-hydroxy butanoate, and ethyl hexanoate were found in fresh passion fruit juice. Ethyl butanoate, followed by ethyl hexanoate and hexyl hexanoate, was the ester found in greatest concentration in the essence. In the juice, the order of abundance was hexyl hexanoate, followed by ethyl 3-hydroxy-butanoate, ethyl hexanoate, and ethyl butanoate. The concentration of the ethyl propanoate decreased with the advance in the maturity of the fruit.

In organoleptic evaluation of yellow passion fruit juice, Jordan and others (2002) concluded the importance of 2-methylbutyl hexanoate for the characteristic aroma of the fresh juice as this compound was perceived by all the panelists in all the replications. In relation to the yellow passion fruit essence, 54 components contributed for the characteristic aroma. Among the esters identified in these components were isobutyl acetate, ethyl butanoate, ethyl lactate, ethyl hexanoate, 2-methylbutyl hexanoate and benzyl acetate as all of these compounds were perceived by panelists in all the replications. However, other esters that were perceived by panelists in commercial essence but not recognized in all the replications were ethyl pentanoate, ethyl 3-hydroxybutanoate, and propyl hexanoate. The esters that contributed for the passion fruit juice aroma and that were described by all panelists were ethyl 3-(methylthio) propanoate, benzyl acetate, hexyl butanoate, and isoamyl hexanoate.

In the commercial essence, two components that appeared to be the most intense odorants were 2-methylbutyl hexanoate, contributing to a sharp sweat odor, and hexyl hexanoate, contributing to a peach- and plum-like odor. In general, based on the comparative study between the aromatic profile of commercial essence and the juice, Jordan and others (2002) concluded that the esters with the higher volatility were not detected in the juice. Diethyl malonate was found only in passion fruit essence and was not detected in fruit juice, while ethyl lactate was found only in the juice and was not present in the passion fruit essence.

Ketones and Lactones

Table 20.4 presents the list of ketones and lactone compounds identified on passion fruit juice and essence. In the aromatic fraction of the passion fruit essence, higher concentrations of cyclopentanone (10.7 ppm), which has a grassy and musty odor; 3-pentanone (3.5 ppm), which possesses a burnt and pungent odor; and 3-hydroxy-2-butanone (2.5 ppm), characteristic of fruity and butter-like odor, were found (Jordan et al. 2002). However, in the juice extracts, the most abundant ketone was 3-hydroxy-2-butanone (27.8 ppm), while 3-pentanone was not detected. In purple passion fruit juice, Brat and others (2000) found higher concentrations of β-ionone in all the extracts obtained by liquid–liquid extraction (LLE), SDE, and VHS, the concentration being 370, 440, and 260 μg/100 g, respectively.

3-Pentanone, which is known to impart a prominent pungent odor, was present in all the extracts (Werkhoff et al. 1998) in yellow passion fruit juice. This compound was not identified earlier in juice obtained from passion fruit of green stage of maturity but was reported to be the main compound in ripe fruit (Parliament 1972). Jordan and others (2002) did not detect this compound in the passion fruit juice, while it was identified in passion fruit essence at the concentration of 3.5 ppm.

Geranyl acetone, which possesses a floral and sweet rosy aroma, was identified by SPME technique in yellow passion fruit juice (Carasek and Pawliszyn 2006), while this compound was not found in the extracts obtained by SDE, DHS, and VHS extraction techniques.

Werkhoff and others (1998) reported for the first time the presence of *cis*- and *trans*-γ-jasmin lactone, δ-jasmin lactone, and *trans*-marmelolactone. The presence of γ-hexa-, γ-hepta-, γ-octa-, γ-nona-, γ-deca-, and γ-dodecalactones was reported in passion fruit by Nitz and others (1990), while four alkylated γ-lactones were found by Bernreuther and others (1989).

In earlier works on passion fruit aroma, several γ-lactones and δ-lactones that possess fatty, creamy and fruity flavors were found to contribute substantially for passion fruit flavor. However, in recent data, only eight lactones, as shown in Table 20.4, have been identified. Marmelolactone was reported for the first time in quince by Tsuneya and others (1980) and later in the bound fraction of peach flavor by Krammer and others (1991). Marmelolactone possesses a strong fruity, floral, and quince-like aroma and is also associated with creamy, buttery, and coconut-like odors.

Terpenes and Norisoprenoids

These classes of compounds play a major role in passion fruit aroma. Forty-nine compounds classified as terpenes and isoterpenes in free and glycosylated form were found in several volatile extractions (Table 20.5). The main terpenes such as myrcene, limonene, α-pinene were found in almost all the volatiles analyzed by various researchers. These compounds have characteristic fruity and woody aroma notes. In an earlier work, α-pinene was reported to be present in only yellow passion fruit juice (Winter and Kloti 1972). However, Brat and others (2000) reported a concentration of 10 μg/100 g of this compound in LLE extracts of purple passion fruit juice. In other extracts of SDE and VHS, the presence of this compound was reported in

traces in purple passion fruit juice. Very high concentration (1070 µg/100 g) of limonene was found in LLE extract of purple passion fruit juice (Brat et al. 2000).

Other terpenes that could contribute for passion fruit floral aroma note could be ocimene, cymene, phellandrene, terpinene, 3-carene, α-terpinolene, β-farnesene, cis and trans isomers of 6-farnesene and theaspirene (Table 20.5). These terpenes are known for their characteristic green aroma notes of apple and pome fruits. These monoterpene hydrocarbons are used in the preparation of several fruit flavors and fragrances. The presence of terpenic aldehydes such as geranial and neral is interesting to note as their alcoholic derivatives were also prominent.

A large number of terpenic derivatives were alcohols and their oxides: α-terpineol, geraniol, α-citronellol, linalool, and cis and trans isomers of linalool oxides, which are known to possess sweet, pleasant, fruity aroma contributing for the characteristic passion fruit flavor. In purple passion fruit juice, higher concentration of α-terpineol (300 µg/100 g), linalool (80 µg/100 g), geraniol (40 µg/100 g), and (*E*)-linalool oxide (10 µg/100 g) were found (Brat et al. 2000). Several terpene diols such as 2,6-dimethyl-1,8-octanediol; (*E*)- and (*Z*)-2,6-dimethylocta-2,7-diene-1,6-diol; 2,6-dimethylocta-3,7-dien-2,6-diol; and 2,6-dimethylocta-1,7-dien-3,6-diol have been identified in both purple and yellow passion fruits (Chassagne et al. 1999).

α-Ionol derivatives oxygenated in position 3 seem to be characteristic of purple passion fruit, while β-ionol compounds oxygenated in position 3 are the major nor-isoprenoids identified as the aglycone in yellow passion fruit (Chassagne et al. 1999).

Furans and Other Compounds

Table 20.6 presents the information on furans and other miscellaneous compounds identified in various volatile extracts obtained by the usage of different isolation techniques from yellow passion fruit juice. A characteristic property of the spectrum of passion fruit volatiles is the presence of numerous furanones with double bonds in position 3, which are methyl-, dimethyl-, and acetoxy esters. 2,5-Dimethyl-4-hydroxy-3-(2H) furanone (furaneol) was identified for the first time in bound form in purple (236 µg/kg) and in yellow (534 µg/kg) passion fruits (Chassagne et al. 1999). Besides 2-ethyl- and 2-acetyl-furans, 2,5-dimethyl-3(2H)-furanone and its several derivatives were found. 2,5-Dimethyl-4-hydroxy-3-(2H)-furanone, which possesses a caramel-like flavor, is known to be a prominent key odorant in strawberry fruit (Zabetakis and Holden 1997).

The organoleptic properties of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol) and 4-methoxy-2,5-dimethyl-3(2H)-furanone (mesifuran) as well as their importance for fruit flavors are well documented in the literature (Schieberle and Hofmann 1997). Both compounds probably influence the aroma profile of passion fruit flavor due to their low odor thresholds.

Among the other compounds listed in the Table 20.6, the importance of cis and trans edulan, and of rose oxide for the characteristic passion fruit aroma, is very well documented (Whitfield et al. 1974).

Sulfur Compounds

There has been a great emphasis on the role of sulfur compounds in passion fruit aroma, and these compounds are the most interesting aroma character-impact

components from an organoleptic point of view for passion fruit flavor. Table 20.7 lists 54 sulfur compounds reported in recent publications (Engel and Tressl 1991; Narain et al. 2004; Werkhoff et al. 1998). Engel and Tressl (1991) quantified sulfur compounds in six different passion fruit concentrates obtained from the market. Werkhoff and others (1998) used different volatile isolation techniques (VHS, SDE, SDEV), and the extracts were identified for the composition of sulfur compounds, which were also evaluated for their characteristic odor descriptors by preparation of several dilutions of the compounds. Narain and others (2004) analyzed the volatile compounds in yellow passion fruit juice by DHS technique.

Werkhoff and others (1998) reported the analysis of aroma fractions obtained by VHS, which revealed the presence of a large number of sulfur compounds, most of which were present in low concentrations. They reported about 180 components for the first time in this fruit and emphasized that the attractive tropical flavor note of the ripe yellow passion fruits was associated with trace levels of sulfur compounds. These sulfur-containing components possess high odor intensities and low threshold values. The researchers reported the presence of 47 new sulfur-containing flavor components in yellow passion fruits. Of these compounds, 23 were never described before in food flavors at all. The sensorial importance of 3-mercaptohexanol and 3-(methylthio) hexanol as well as their acetates, butanoates, and hexanoates and their sulfur-substituted alcohols for the aroma of yellow passion fruits was very significant. Due to their high odor values and their olfactive profiles, these sulfur substances are the key ingredients of the yellow variety of passion fruit.

In an earlier report, Winter and others (1976) described the presence of 3-(methylthio) hexanol, a mixture of (*Z*) and (*E*)-2-methyl-4-propyl-1,3-oxathiane, and (*Z*) and (*E*)-2-methyl-4-propyl-1,3-oxathiane-3-oxide, as key odorants in the aroma of the yellow passion fruit. The presence of these components, which impact the aromatic character in the fruit, was reported by several researchers. Engel and Tressl (1991) described for the first time the presence of 3-mercaptohexanol and their acetates, butanoates, and hexanoates of both 3-mercaptohexanol and 3-(methylthio) hexanol in yellow passion fruit concentrates. These compounds and their sulfur-substituted alcohols were considered as the character-impact compounds for yellow passion fruit aroma.

Jordan and others (2002) analyzed the aromatic profile of an aqueous essence as well as that of passion fruit juice by the application of gas chromatography (GC)–olfactometry. Of these, 3-methylthio-hexanol, which possesses green and fatty sulfur note and a mixture of (*Z*)- and (*E*)-2-methyl-4-propyl-1,3-oxathianes, which possess green and a little burnt odor, were characterized as the principal compounds responsible for the typical passion fruit flavor. A strong influence of chiral configuration of these volatiles was demonstrated in the organoleptic properties of the purple passion fruit juice.

Tominaga and Dubourdieu (2000) reported the presence of 3-mercapto-3-methylbutan-1-ol and its acetate, citing the precursors of 3-mercapto-hexen-1-ol being *S*-(3-hexan-1-ol)-L-cysteine. In their earlier publication, Tominaga and others (1998) related that the tasters of some grape varieties such as Sauvignon Blanc in France relate the aroma of these grapes with that of passion fruit, and this analogy was validated by the presence of compounds such as 3-mercaptohenxen-1-ol and its acetate in wine made from this grape variety.

Among the sulfur compounds, methyl and ethyl 3-(methylthio) propanoate dominated in the VHS extract of yellow passion fruits (Table 20.7). Both compounds have previously been found in pineapple juice (Nijssen et al. 1996). 3-(Methylthio) propyl acetate (methionyl acetate) possesses herbaceous odor impressions and a typical vegetable-like character and has been described in the literature in numerous flavor systems (Nijssen et al. 1996). In addition, methionyl butanoate and methionyl hexanoate, which have threshold concentrations of 10–20 ppb and 500 ppb, respectively, were also identified in the VHS extract obtained from the juice of the yellow variety.

Werkhoff and others (1998) revealed the presence of 3-(methylthio) esters of propanoic acid in the yellow passion fruit variety. The aroma properties of the 3-(methylthio) esters of propanoic acid are not very interesting with the exception of the hexyl derivative. In general, the 3-(methylthio) propanoic acid esters have a sulfury, vegetable-like odor, and only hexyl 3-(methylthio) propanoate with its fruity and geranium-like odor note may contribute to the overall olfactory impression of the passion fruits.

The spectrum of esters comprised of the whole series of propyl, butyl, isobutyl, sec-butyl, pentyl, 2-methylbutyl, 3-methylbutyl, hexyl, (*Z*)-3-hexenyl esters of 3-(methylthio) propanoic acid and 3-mercaptohexyl pentanoate in yellow passion fruit. These compounds possess the characteristic passion fruit flavor having sulfur note.

The biogeneration of sulfur esters has been discussed in the literature (Homatidou et al. 1992; Wyllie and Leach 1990; Wyllie et al. 1994, 1995). According to various hypotheses put forward, 3-(methylthio) propanol, 3-(methylthio) propanoic acid, and 3-(methylthio)-(*E*)-2-propenoic acid are the most important intermediates for the formation of sulfur-containing volatiles in fruits. The amino acid methionine is considered as a common precursor of 3-(methylthio) propanol and 3-(methylthio) propanoic acid formed by a series of biochemical transformations, which occur during the process of ripening in the passion fruit.

The aromatic quality of passion fruit is also influenced by chirality of the substances. In all cases, sulfur-containing passion fruit volatiles were found to be present at high optical purity favoring the S-configured enantiomer. The enantiomers of chiral sulfur-containing volatiles often show clear differences in their sensorial properties. The enantiomers of 3-mercaptohexanol and 3-(methylthio) hexanol, which possess S configuration, contribute to characteristic aroma, and these are found interesting for tropical and exotic fruits. However, the R forms of these substances are distinguishably mild and present only herbaceous notes (Werkhoff et al. 1998). Comparing the two forms (S and R) of enantiomers, the authors concluded the methyl esters with S configurations present more intense odor to that of R configurations compounds. Weber and others (1995) determined the enantiomeric distribution of *cis*- and *trans*-2-methyl-4-propyl-1,3-oxanthiane and 3-mercaptohexyl acetate and butanoate in passion fruit extracts. They reported that the stereoisomeric distribution of the sulfur-containing volatiles is not influenced by processing conditions of passion fruit juice, which could serve to differentiate between naturally occurring flavor compounds and the synthetic racemates added to passion fruit product.

Effects of Storage and Processing of Fruit

A total of seven volatile markers, which included ethyl acetate, ethyl butanoate, ethyl hexanoate, hexyl butanoate, 1-hexanol, 2-heptanone, and benzaldehyde, were

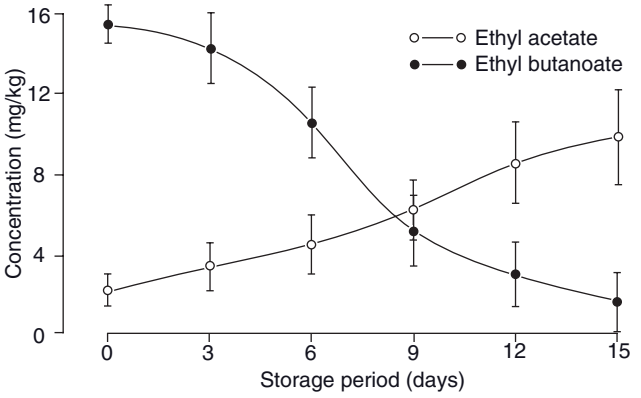

Figure 20.1. Changes in ethyl acetate and ethyl butanoate content during postharvest storage of yellow passion fruit.

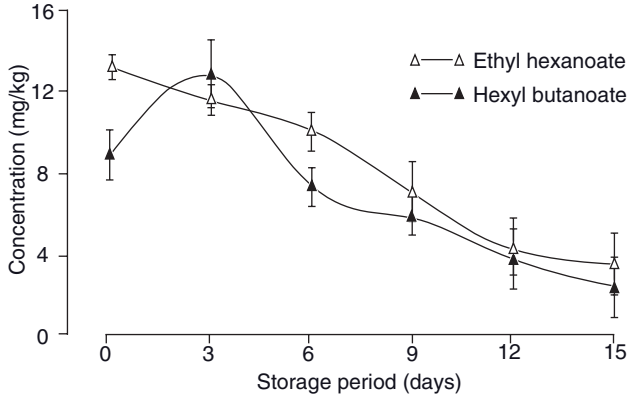

Figure 20.2. Changes in ethyl hexanoate and hexyl butanoate content during postharvest storage of yellow passion fruit.

monitored during postharvest storage of naturally fallen, ripe yellow passion fruit for a period of 15 days by Narain and Bora (1992). The results are shown in Figures 20.1–20.3. Based on the sweet aroma of ethyl butanoate and ethyl hexanaote substantiated by nutty stone fruit aroma of benzaldehyde and an undesirable increase in the ethyl acetate, they concluded that ripe yellow passion fruit stored at ambient temperature (29 ± 2°C) retains its fresh characteristic estery aroma until 3 days after harvest. The authors reported the concentration of 14 ppb of ethyl butanoate, which possesses sweet, fruity, strawberry-like odor and 11 ppb of ethyl hexanoate characterizing anise and overripe fruit flavor in the pulp after 3 days of storage of the yellow passion fruit at ambient temperature (29 ± 2°C).

In pasteurized yellow passion fruit juice, a higher concentration of 5-methylfurfural (13.9%), followed by furfural (12.5%) and esters, hexyl hexanoate, and ethyl butanoate, were found, which represented a 7.5% of total area. The sensorial evaluation of the thermally treated juice demonstrated that the juice does not maintain

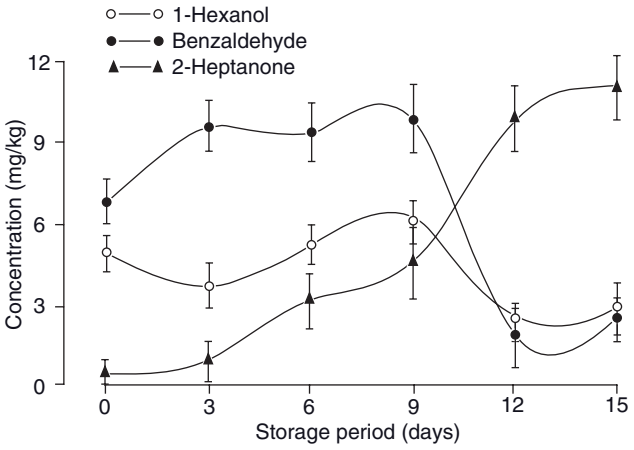

Figure 20.3. Changes in 1-hexanol, benzaldehyde, and 2-heptanone content during postharvest storage of yellow passion fruit.

the characteristic flavor of the fresh fruit juice. Several ketones were not found in pasteurized juice such as 2-pentanone, 2-nonanone, and 3-nonanone, which possess characteristic fruit aroma (Narain 1993).

Sandi and others (2004) evaluated the quality of yellow passion fruit juice pasteurized at 75°C/60 s, 80°C/41 s, or 85°C/27 s, and the juices were stored at room temperature (25 ± 5°C) and refrigeration (5 ± 1°C) for 120 days They monitored four volatile compounds such as ethyl butanoate, ethyl hexanoate, hexyl butanoate, and hexyl hexanoate besides furfural by SPME technique. They reported that the volatile compound concentration decreased over time, and the pasteurization undertaken at 85°C/27 s resulted in minimal changes in the studied passion fruit characteristics, while pasteurization realized at 75°C/60 s was the most harmful. The concentrations of the prominent compounds in passion fruit juice reported after 2 months of storage were ethyl hexanoate (35%) followed by hexyl hexanoate (32.6%), hexyl butanaote (19.8%), and ethyl butanoate (12.6%). Ethyl hexanoate was the compound that decreased to a maximum extent during storage, indicating that this ester was the most sensitive to heat treatment and storage.

SUMMARY

This chapter summarizes the current data on volatile compounds identified and quantified in passion fruit juice. The main volatile compounds that contribute to the characteristic flavor of the fruit belong to the classes of esters, terpenes, and sulfur compounds. A very large number of compounds belong to the esters, alcohols, terpenes, and sulfur compounds. The prominent compounds that could contribute to the aroma of passion fruit juice are discussed, and the discussion is substantiated by detailing the differences in the composition and quantification of volatile compounds present in the two most prominent varieties of yellow and purple passion fruits.

ACKNOWLEDGMENT

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REFERENCES

Arctander S. 1969. *Perfume and Flavor Chemicals (Aroma Chemicals)*. Carol Stream, IL: Allured Publishing.

Belitz HD, Grosch W. 1987. *Food Chemistry*. Berlin: Springer Verlag, pp. 578–621.

Bernreuther A, Christoph N, Schrier P. 1989. Determination of the enantiomeric composition of gamma-lactones in complex natural matrices using multidimensional capillary gas chromatography. *J Chromatogr* 481:363–7.

Bora PS, Narain N. 1997. Passion fruit. In Mitra S (ed.), *Postharvest Physiology and Storage of Tropical and Subtropical Fruits*. New York: CAB International, pp. 375–86.

Brat P, Brillouet JM, Reynes M, Cogat PO, Ollé D. 2000. Free volatile components of passion fruit puree obtained by flash vacuum-expansion. *J Agric Food Chem* 48:6210–4.

Carasek E, Pawliszyn J. 2006. Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. *J Agric Food Chem* 54:8688–96.

Casimir DJ, Kefford JF, Whitfield FB. 1981. Technology and flavour chemistry of passion fruit juice and concentrate. *Adv Food Res* 27:243–93.

Challier P, Koulibaly AA, Fontivielle MJ, Crouzet J. 1990. Fruit glycosides as aroma precursors. *Wissenschafts-Technikhistoriker Kommunikations* 21:349–54.

Chan, HT. 1980. Passion fruit. In Nagy S; Shaw PE (eds.), *Tropical and Subtropical Fruits*. Westport, CT: Avi Publishing, pp. 300–15.

Chassagne D, Boulanger R, Crouzet J. 1999. Enzymatic hydrolysis of edible *Passiflora* fruit glycosides. *Food Chem* 66:281–8.

Chen CC, Kuo MC, Hwang LS, Wu JSB, Wu CM. 1982. Headspace components of passion fruit juice. *J Agric Food Chem* 30:1211–5.

Engel HK, Tressl R. 1983. Formation of aroma components from non-volatile precursors in passion fruit. *J Agric Food Chem* 31:998–1002.

Engel KH, Tressl R. 1991. Identification of new sulfur-containing volatiles in yellow passion fruits (*Passiflora edulis* f. flavicarpa). *J Agric Food Chem* 39:2249–52.

Hiu DN, Scheuer PJ. 1961. The volatile constituents of passion fruit juice. *J Food Sci* 26:557–63.

Hoehne FC. 1946. *Frutas Indígenas*. São Paulo, Brazil: Secretaria de Agricultura, Indústria e Comercio, Instituto Botanica (in Portuguese).

Homatidou V, Karvouni S, Dourtoglou V, Poulos CN. 1992. Determination of total volatile components of *Cucumis melo* L. variety Cantaloupensis. *J Agric Food Chem* 40:1385–8.

Jordan MJ, Goodner KL, Shaw PE. 2002. Characterization of the aromatic profile in aqueous essence and fruit juice of yellow passion fruit (*Passiflora edulis* Sims F. *Flavicarpa degner*) by GC-MS and GC/O. *J Agric Food Chem* 50:1523–8.

Krammer G, Winterhalter P, Schwab M, Schreier P. 1991. Glycosidically bound aroma compounds in the fruits of *Prunus* species: Apricot (*P. armeniaca*, L.), peach (*P. persica*, L.), yellow plum (*P. domestica*, L. ssp. *Syriaca*). *J Agric Food Chem* 39:778–81.

Martin FW, Nakasone HY. 1970. The edible species of *Passiflora*. *Econ Bot* 24:333–43.

Mattheis JP, Fellman JK. 1999. Preharvest factors influencing flavor of fresh fruit and vegetables. *Posthasvest Biol Technol* 15:227–32.

Murray KE, Shipton J, Whitfield FB. 1972. Volatile constituents of passion fruit *(Passiflora edulis)*. *Aust J Chem* 25:1920–33.

Murray KE, Shipton J, Whitfield FB. 1973. The flavour of purple passion fruit. *Food Technol Aust* 25:446–8.

Narain N. 1993. Headspace Análises de Constituintes Voláteis do Maracujá (*Passiflora edulis* forma *flavicarpa*). Tese do Concurso Público de Professor Titular, Departamento de Nutrição, UFPE, Recife, p. 109.

Narain N, Almeida JN, Galvão MS, Madruga MS, Brito ES. 2004. Compostos voláteis dos frutos de maracujá (*Passiflora edulis* forma *flavicarpa*) e de cajá (*Spondias mombin* L.) obtidos pela técnica de headspace dinâmico. *Ciênc Tecnol Aliment* 24(2):212–6.

Narain N, Bora PS. 1992. Post-harvest changes in some volatile flavour constituents of yellow passion fruit (*Passiflora edulis* f. *flavicarpa*). *J Sci Food Agric* 60:529–30.

Nijssen LM, Visscher CA, Maarse H, Willemsens LC, Boelens MH. 1996. *Volatile Compounds in Food. Qualitative and Quantitative Data*, 7th Ed. Zeist, the Netherlands: TNO Nutrition and Food Research Institute.

Nitz S, Kollmannsberger H, Drawert F. 1990. Determination of non-natural flavors in sparkling fruit wines. Part 2. Enantiomeric gamma-lactones in passion fruit and passion fruit products. *Chemical and Microbial Technology Lebensmittel* 12:105–10.

Palm H. 2002. Detection and characterization of un-wanted off-flavors caused by rancidity through analysis of volatiles. *Malaysian Oil Sci Technol* 11:14–18.

Parliament TH. 1972. Some volatile constituents of passion fruit. *J Agric Food Chem* 20:1043–5.

Pino JA. 1997. The volatile constituents of passion fruit. Inst. de Investigaciones para la Industria Alimentaria, La Lisa, Havana, Cuba. *Alimentaria* (280):73–81.

Sandi D, Chaves JBP, Sousa ACG, Parreiras JFM, Silva MTC, Constant PBL. 2004. Hunter color dimensions, sugar content and volatile compounds in pasteurized yellow passion fruit juice (*Passiflora edulis* var. flavicarpa) during storage. *Braz Arch Biol Technol* 47:233–45.

Schieberle P, Hofmann T. 1997. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J Agric Food Chem* 45:227–32.

Shibamoto T, Tang CS. 1990. Minor tropical fruits—Mango, papaya, passion fruit and guava. In Morton ID, Macleod AJ (eds.), *Food Flavours Part C. The Flavour of Fruits*. Amsterdam: Elsevier, pp. 253–67.

Shoseyov O, Bravdo BA, Siegel D, Goldman A, Cohen S, Shoseyov L, Ikan R. 1990. Immobilized endo-b-glucosidase enriches flavor of wine and passion fruit juice. *J Agric Food Chem* 38:1387–90.

Silva AC, São José AR. 1994. Classificação Botanica de Maracujazeiro. In São José AR (ed.), *Maracujá: Produção e Mercado*. Vitória da Conquista, BA, Brazil: Universidade Estadual do Sudoeste da Bahia, Dept. de Filotécnica e Zootécnica, pp. 1–5.

Singh HP, Yadav IS, Jolikop SH. 1991. "Kaveri," a hybrid passion fruit. Indian Horticulture. Horticultural Experiment Station, Indian Institute of Horticultural Research, Cethalli, Karnataka, India.

Tominaga T, Dubourdieu D. 2000. Identification of cysteinylated aroma precursors of certain volatiles thiols in passion fruit juice. *J Agric Food Chem* 48:2874–6.

Tominaga T, Furrier A, Henry R, Dubourdieu D. 1998. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flav Frag J* 13:159–62.

Tressl R, Engel KH. 1983. Formation of esters and terpenoids in passion fruits and their importance to quality evaluation. In Charalambous G, Inglett G (eds.), *Instrumental Analysis of Foods Recent Progress*, Vol. 1. New York: Academic Press, pp. 153–82.

Tsuneya T, Ishihara M, Shiota H, Shiga M. 1980. Isolation and identification of novel terpene lactones from quince fruit (*Cydonia oblonga* Mill., Marmelo). *Agric Biol Chem* 44: 957–8.

Weber B, Maas B, Mosandi A. 1995. Stereoisomeric distribution of some chiral sulfur-containing trace components of yellow passion fruits. *J Agric Food Chem* 43:2438–41.

Werkhoff P, Guntert M, Krammer G, Sommer H, Kaulen J. 1998. Vacuum headspace method in aroma research: Flavor chemistry of yellow passion fruits. *J Agric Food Chem* 46: 1076–93.

Whitfield FB, Murray KE, Stanley G, Sugowdz G. 1974. Structures of edulan I and edulan II and their role in the aroma of the juice of the purple passion fruit. International Congress of Essential Oils, September 8–12, pp. 151–4.

Winter M, Furrer A, Willhalm B, Thommen W. 1976. Identification and synthesis of two new organic sulfur compounds from the yellow passion fruit (*Passiflora edulis* f *flavicarpa*). *Helv Chim Acta* 59:1613–20.

Winter M, Kloti R. 1972. The aroma of yellow passion fruit (*Passiflora edulis* f *flavicarpa*). *Helv Chim Acta* 55:1916–21.

Winterhalter P. 1990. Bound terpenoids in the juice of the purple passion fruit (*P. edulis* Sims). *J Agric Food Chem* 38:452–5.

Wyllie SG, Leach DN. 1990. Aroma volatiles of *Cucumis melo* cv. Golden Crispy. *J Agric Food Chem* 38:2042–4.

Wyllie SG, Leach DN, Wang Y, Shewfelt RL. 1994. Sulfur volatiles in *Cucumis melo* cv. Makdimon (muskmelon) aroma. Sensory evaluation by gas chromatography-olfactometry. In Mussinan CJ, Keelan ME (eds.), *Sulfur Compounds in Foods*. Washington, DC: American Chemical Society, pp. 36–48.

Wyllie SG, Leach DN, Wang Y, Shewfelt RL. 1995. Key aroma compounds in melons. In Rouseff RL, Leahy MM (eds.), *Fruit Flavors. Biogenesis, Characterization, and Authentication*. Washington, DC: American Chemical Society, pp. 248–57.

Yamaguchi K, Nishimura O, Toda H, Mihara S, Shibamoto T. 1983. Chemical studies on tropical fruits. In Charalambous G, Inglett G (eds.), *Instrumental Analysis of Foods*. New York: Recent Progress Academic, pp. 93–117.

Zabetakis I, Holden MA. 1997. Strawberry flavor: Analysis and biosynthesis. *J Sci Food Agric* 74:421–34.

CHAPTER 21

Pineapple (*Ananas comosus* [L.] Merril) Flavor

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University of Lleida

INTRODUCTION

Pineapple is one of the most popular tropical fruits. It is recognized as a very aromatic fruit, which can be found in just about any market around the world. It was first spread as juice or canned pineapple, and as the transportation resources, rapid distribution, and postharvest technology developed, it also became available as fresh fruit (Flath 1980; Umano et al. 1992). Studies on pineapple aroma have been made since many years ago using both fresh fruits from different cultivars (not always specified) and processed foods.

By 2005, nearly 380 volatile constituents had been recognized in pineapple flavor, including alcohols, aldehydes, esters, ketones, lactones, terpenes and terpenoids, hydrocarbons, lactones, and others (Paull 1993; Tokitomo et al. 2005; Umano et al. 1992); however, only some of them have been identified as pineapple flavor contributors. Aroma constituents may vary with season, cultivar, maturity, processing conditions, ethylene control, temperature, chemical treatments, modified atmosphere, and preharvest factors, such us carbon supply, water stress, light, temperature, and biotic stresses.

In this chapter, a review of the state of knowledge of pineapple aroma is presented, taking into consideration flavor changes due to stages of maturity, cultivars, as well as processing conditions, and finally a sensory characterization of pineapple flavor.

FLAVOR COMPONENTS OF PINEAPPLE

Flavor consists mainly of lipophilic volatile compounds, but low and nonvolatile materials also play an important part of the overall sensation. As with many other

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fruits and foods, pineapple flavor is a combination of volatile components perceived by the human olfactory system and nonvolatile components (sugars, acids) recognized by tongue sensors (Flath 1980). In fact, flavor is a combination of both taste and odor.

The flavor of pineapple is a blend of a number of volatile and nonvolatile compounds that are present in small amounts and in complex mixtures, with the nonvolatile compounds more difficult to analyze (Pickenhagen 1999). Many of these compounds have been identified and reported by several authors from fresh fruit, processed pineapple products, and pineapple essences (Badilla-Porras 2005; Berger et al. 1985; Brat et al. 2004; Elss et al. 2005; Haagen-Smit et al. 1945a; Umano et al. 1992; Wu et al. 1991). However, comparison among reported results is difficult; since different pineapple cultivars and pineapple products have been used, results are given in different units and bases, and separation techniques and analysis vary among works. A summary of pineapple constituents identified by several researchers from 1945 to 2005 is presented in Table 21.1.

One of the most important flavor compounds in fruits is 2,5-dimethyl-4-hydroxy-3(2H)-furanone, which is a relatively hydrophilic and not very stable molecule (Fig. 21.1). It has been found to be part of the aroma of pineapple when it was identified for the first time (Rodin et al. 1965). This compound is generally known under its trade name Furaneol®, DMHF, or pineapple furanone. Furaneol has also been identified in strawberries (Re et al. 1973), raspberries (Honkanen et al. 1980), mangoes (Pickenhagen et al. 1981), tomatoes (Buttery et al. 1995), and many other fruits. Its content increases as the fruit ripens, and it gives the characteristic caramel-like, sweet, floral, and fruity aroma (Miller et al. 1973; Perez et al. 1996; Tonsbeek et al. 1968). Also, it is extensively used as food flavoring due to its low odor thresholds and flavor-enhancing properties (Dahlen et al. 2001).

Flavor, however, also depends on the presence of small quantities of other volatiles with low threshold values. The characteristic pineapple aroma has been attributed to ethyl 3-(methylthio)propanoate and methyl 3-(methylthio)propanoate (Umano et al. 1992). Aliphatic esters, which often have fruity notes such as apple, banana, plum, or apricot, have also been reported (Flath 1980). Berger (1985), reported that esters such as 2-methylbutanoates and hexanoates give fruity notes to fresh pineapple as well as other fruits. Takeoka and others (1991) also identified many sulfur-containing esters among pineapple volatiles, but their concentrations were lower than their odor thresholds. In addition, two minor hydrocarbon compounds, 1-(*E*,*Z*)-3,5-undecatriene and 1-(*E*,*Z*,*Z*)-3,5,8-undecatetraene, have been identified as important contributors to fresh-cut pineapple aroma due to their low odor threshold values (Berger et al. 1985).

Other compounds have also been identified and considered important for pineapple aroma. Lactones can contribute to the pleasant coconut character in some cultivars. In fact, the coconut-like aroma often found in pineapple has been attributed to lactones, namely, γ-octalactone, δ-octalactone, and γ-nonalactone (Flath 1980).

The first studies on pineapple aroma, by Haagen-Smit and others (1945a,b), were done before gas–liquid chromatography techniques were available. These authors studied volatile flavor and odor constituents of fresh pineapple (Smooth Cayenne cultivar). They analyzed volatile components of summer and winter fruits grown in Hawaii in order to establish a correlation between flavor and these substances. They found differences among summer and winter fruits in both volatile extraction yield

TABLE 21.1. Summary of Volatile Compounds Identified in Pineapple Fruits and Its Processed Products from 1945 to 2005

Esters	Esters	Esters	Esters
1 1-pentyl hexanoate (5)	22 diisobutyl phthalate (9)	40 ethyl 2-methyl propanoate (5, 8)	54 ethyl 3-hydroxy pentanoate (9)
2 1-propyl formate (5)	23[a] dimethyl malonate (3, 4, 5, 6, 9, 10)	41 ethyl 2-propenoate (4)	55 ethyl 3-hydroxy-2-methyl butanoate (9)
3 2,3-butanediol diacetate (9)	24 dimethyl succinate (4)	42 ethyl 3 methyl butanoate (5)	56 ethyl 3-methyl butanoate (4, 5, 6)
4[a] 2-methyl-1-butyl acetate (4, 5, 6)	25 *erythro*-butane-2,3-diol diacetate (9)	43[a] ethyl 3-(methylthio) propanoate (2, 3, 4, 5, 6, 9)	57 ethyl 4-(methylthio) butanoate (7)
5 2-methyl-1-propyl acetate (5, 9)	26 ethyl (E)-2-butanoate (7)	44 ethyl 3-(methylthio)-(E)-2-propenoate (7)	58 ethyl 4-acetoxy butanoate (9)
6 2-methyl-1-propyl formate (5)	27 ethyl (E)-2-hexenoate (2)	45 ethyl 3-(methylthio)-(Z)-2-propenoate (7)	59 ethyl 4-acetoxy hexanoate (6, 9, 10)
7 2-phenylethyl acetate (4)	28 ethyl (E)-3-hexenoate (2, 4, 5, 9)	46 ethyl 3-acetoxy butanoate (4, 6, 7, 9)	60 ethyl 4-acetoxy octanoate (6, 9, 10)
8 2-propenyl hexanoate (2)	29 ethyl (E)-3-octenoate (5)	47[a] ethyl 3-acetoxy hexanoate (4, 5, 9, 10)	61 ethyl 4-acetoxy pentanoate (9)
9 2-propyl 2-methyl propanoate (5)	30 ethyl (methylthio) acetate (4, 5)	48 ethyl 3-acetoxy octanoate (9, 10)	62 ethyl 4-hydroxy hexanoate (6, 9)
10 2-propyl acetate (5)	31 ethyl (Z)-3-hexenoate (2, 9)	49 ethyl 3-acetoxy pentanoate (9)	63 ethyl 4-hydroxy octanoate (6, 9)
11 3-(methylthio) propyl acetate (4, 9)	32 ethyl (Z)-3-octenoate (4)	50 ethyl 3-acetoxy-2-methyl butanoate (6, 9)	64[a] ethyl 5-acetoxy hexanoate (4, 5, 6, 9, 10)
12 3-methyl-2-butenyl acetate (4, 9)	33[a] ethyl 2-(methylthio) acetate (2)	51 ethyl 3-hydroxy butanoate (9)	65 ethyl 5-acetoxy octanoate (4, 5, 6, 7, 9)
13 3-methylbut-3-enyl acetate (7)	34 ethyl 2-butenoate (4)	52[a] ethyl 3-hydroxy hexanoate (4, 5, 6, 9, 10)	66 ethyl 5-hexanoate (7)
14 3-methylbutyl acetate (5, 9)	35 ethyl 2-hydroxy hexanoate (4, 9)	53 ethyl 3-hydroxy octanoate (4, 6, 9)	67 ethyl 5-hydroxy hexanoate (9)
15 allyl isothiocyanate (9)	36 ethyl 2-hydroxy propanoate (3, 4, 5, 8, 9)		
16 butyl acetate (3, 4, 6)	37 ethyl 2-hydroxy-2-methyl butanoate (4, 6, 9)		
17 butyl formate (5)	38 ethyl 2-hydroxy-3-methyl butanoate (4, 9)		
18 dibutyl phthalate (9)	39[a] ethyl 2-methyl butanoate (3, 4, 5, 6, 7, 8, 9)		
19 diethyl carbonate (4, 5)			
20 diethyl malonate (2)			
21 diethyl succinate (9)			

TABLE 21.1. ***Continued***

Esters	Esters	Esters	Esters
68 ethyl 5-hydroxy octanoate (2, 9)	94 methyl (E)-2-butanoate (7)	110 methyl 2-acetoxy butanoate (6)	125 methyl 3-acetoxy-2-methyl butanoate (6, 9)
69 ethyl 5-oxohexanoate (5)	95 methyl (E)-2-hexenoate (2, 9)	111[a] methyl 2-hidroxy propanoate (4)	126 methyl 3-hexenoate (5, 10)
70 ethyl acetate (5, 6, 8, 9, 10)	96 methyl (E)-3-hexenoate (4, 5, 6, 9, 10)	112 methyl 2-hydroxy-2-methyl butanoate (3, 4, 6, 9)	127 methyl 3-hydroxy butanoate (3, 4, 5, 6, 9)
71 ethyl acrylate (5, 10)	97 methyl (E)-3-octenoate (4, 5)	113 methyl 2-hydroxy-3-methyl butanoate (4)	128[a] methyl 3-hydroxy hexanoate (3, 4, 5, 6, 9, 10)
72 ethyl benzoate (5)	98 methyl (E)-4-hexanoate (7)	114 methyl 2-hydroxy-hexanoate (6, 9)	129 methyl 3-hydroxy octanoate (4, 5, 9, 10)
73[a] ethyl butanoate (3, 4, 5, 6, 8, 9)	99 methyl (*E*,*E*)-2,4-hexadienoate (9)	115[a] methyl 2-methyl butanoate (3, 4, 5, 6, 8, 9, 10)	130 methyl 3-hydroxy pentanoate (9)
74 ethyl cinnamate (4, 7)	100 methyl (methylthio) acetate (4, 5, 9)	116 methyl 2-methyl propanoate (5, 8, 9)	131 methyl 3-hydroxy-2-methyl butanoate (6, 9)
75 ethyl cis-4-decenoate (5)	101 methyl (Z)-3-hexenoate (4, 6, 9)	117[a] methyl 2-methyl-3-oxobutanoate (3)	132 methyl 3-hydroxy-3-methyl butanoate (3, 4, 6, 9)
76 ethyl decanoate (4, 5, 9)	102 methyl (Z)-3-octenoate (4)	118 methyl 2-octenoate (4)	133 methyl 3-methyl butanoate (4, 5, 6, 8)
77 ethyl dodecanoate (2)	103 methyl (Z)-4-decenoate (4, 5)	119[a] methyl 3-(methylthio) propanoate (1, 3, 4, 5, 9)	134 methyl 4-(methylthio) butanoate (7)
78 ethyl formate (5)	104 methyl (Z)-4-hexenoate (6)	120 methyl 3-(methylthio)-(E)-2-propenoate (7)	135 methyl 4-acetoxy hexanoate (1, 6, 9, 10)
79 ethyl heptanoate (4, 5)	105 methyl (Z)-4-octenoate (5, 6)	121 methyl 3-(methylthio)-(Z)-2-propenoate (7)	136 methyl 4-acetoxy octanoate (6, 9, 10)
80 ethyl hexadecanoate (2, 9)	106 methyl (Z)-9-octadecenoate (2)	122[a] methyl 3-acetoxy butanoate (4, 6, 9)	137 methyl 4-hydroxy butanoate (3)
81[a] ethyl hexanoate (2, 3, 4, 5, 6, 8, 9, 10)	107 methyl (Z, Z)-9,12-octadecadienoate (2)	123[a] methyl 3-acetoxy hexanoate (3, 4, 5, 6, 9, 10)	138 methyl 4-hydroxy hexanoate (9)
82 ethyl methyl malonate (9)	108 methyl (Z, Z, Z)-octadecatrienoate (2)	124 methyl 3-acetoxy octanoate (5, 9, 10)	139 methyl 4-hydroxy octanoate (9)
83 ethyl methyl propanoate (4)	109 methyl 2,4-hexadienate (6)		
84 ethyl methyl succinate (9)			
85 ethyl nonanoate (5)			
86 ethyl octadecanoate (2)			
87 ethyl octanoate (2, 3, 4, 5, 9)			
88 ethyl pentanoate (4, 5, 9)			
89 ethyl phenylacetate (4, 9)			
90 ethyl propanoate (4, 5, 9)			
91[a] ethyl tetradecanoate (3)			
92 geranyl acetate (10)			
93 hexyl acetate (4)			

140 methyl 4-methyl pentanoate (5, 10)
141 methyl 5-acetoxy heptanoate (6, 9)
142[a] methyl 5-acetoxy hexanoate (3, 4, 5, 6, 9, 10)
143[a] methyl 5-acetoxy octanoate (3, 4, 5, 6, 9, 10)
144 methyl 5-hexenoate (7, 9)
145 methyl 5-hydroxy hexanoate (4, 6, 9)
146 methyl 5-hydroxy octanoate (6, 9)
147 methyl acetate (5, 6, 9)
148 methyl acrylate (5)
149 methyl benzoate (4, 6, 7)
150[a] methyl butanoate (3, 4, 5, 6, 9, 10)
151 methyl cinnamate (7)
152 methyl decanoate (4, 5)
153 methyl dodecanoate (2)
154 methyl heptanoate (4, 5)
155 methyl hexadecanoate (2)
156[a] methyl hexanoate (3, 4, 5, 6, 9)
157 methyl nicotinate (4)
158 methyl nonanoate (5)
159 methyl octadecanoate (2)
160 methyl octadienoate (2)
161 methyl octanoate (3, 4, 5, 6, 9, 10)
162 methyl pentanoate (3, 4, 5, 6, 9, 10)
163 methyl phenylacetate (9)
164 methyl propanoate (5)
165[a] propyl acetate (4, 5, 9, 10)
166 *threo*-butane-2,3-diol diacetate (9)
167 δ-heptanoate (10)

Alcohols and Phenols

201 (3-hydroxyphenyl) ethyl alcohol (10)
202 (E)-2-hexen-1-ol (4)
203 (Z)-3-hexen-1-ol (4, 9)
204 1-butanol (4, 9)
205 1-decanol (4)
206 1-dodecanol (2)
207 1-hexanol (4, 9, 10)
208 1-menthen-4-ol (5)
209 1-octen-3-ol (7, 9)
210 1-pentanol (4, 5)
211 1-penten-3-ol (4)
212 1-propanol (5)
213[a] 2,3-butanediol (4, 6)
214 2,3-dimethyl-2-butanol (5)
215 2-/3-methyl-1-butanol (4)
216 2-allylphenol (5, 6)
217 2-butoxy-ethanol (6, 10)
218 2-ethyl-1-hexanol (9)
219 2-hexanol (5)
220 2-methyl butan-1-ol (5)
221 2-methyl pentan-2-ol (5)
222[a] 2-methyl propan-1-ol (4, 5, 9)
223 2-methyl-2-butanol (9)
224 2-methyl-3-buten-2-ol (3, 4, 5, 9)
225 2-pentanol (4, 9, 10)
226 2-phenyl ethanol (4, 9)
227[a] 3-(methylthio)-1-propanol (4, 9)
228 3-hexanol (5)
229 3-methyl butan-1-ol (5, 9, 10)
230 3-methyl pentan-2-ol (9)
231 3-methyl pentan-3-ol (5)
232 3-methyl phenol (9)
233 3-methyl-2-butan-1-ol (4, 7, 9)
234 3-methyl-2-butenol (9)
235 3-methyl-3-butan-1-ol (7)
236 3-methyl-3-buten-2-ol (9)
237 3-pentanol (4)
238 4-allyl-2,6-dimethoxy phenol (10)
239 4-ethyl phenol (9)
240[a] 4-vinyl guaiacol (4, 6)
241[a] 4-vinyl phenol (4, 6)
242 benzyl alcohol (6, 9)
243 coniferilic alcohol (6)
244 *erytro*-3-acetoxy-2-butanol (9)
245 *erytro*-3-hydroxy-2-butanol (9)
246 ethanol (5)
247 eugenol (10)
248 furfuryl alcohol (4)
249 heptanol (7, 9)
250 menthol (9)
251 methanol (5)
252 methyl-3-buten-2-ol (5)
253 nonanol (7, 9)
254 *p*-allylphenol (chavicol) (5)
255 *p*-cymen-8-ol (4, 9)
256 pentyl alcohol (9)
257 phenethyl alcohol (9)
258 phenol (9, 10)
259 solerol (4)
260 tert-butanol (possible trace) (5)
261 *threo*-3-acetoxy-2-butanol (9)
262 α-terpineol (4, 5, 9)

TABLE 21.1. ***Continued***

Aldehydes	Aldehydes	Aldehydes	Aldehydes
301 (E)-2-hexenal (4)	305 5-(hydroxymethyl) furfural (4, 5, 9)	310 formaldehyde (5)	315[a] phenylacetaldehyde (4)
302 1,1-diethoxyethane (5)	306 5-methylfurfural (4)	311[a] furfural (4, 5, 6, 9)	316 *p*-hydroxybenzaldehyde (10)
303 2-butyl-2-octenal (9)	307 acetaldehyde (5)	312 hexanal (3, 4, 5, 9, 10)	317 propanal (5)
304 3-(methylthio)-propanal (4)	308 benzaldehyde (4, 5)	313 nonanal (2, 3, 4, 9)	318 syringaldehyde (10)
	309 decanal (3, 4)	314 octanal (8)	319 vanillin (8, 9, 10)
Ketones	**Ketones**	**Ketones**	**Ketones**
401 (Z)-1,5-octadien-3-one (8)	406 2-hexanone (5)	411[a] 3-hydroxy-2-butanone (4, 9)	415 acetone (5, 6, 9)
402 2,3-butanedione (4, 5, 8)	407 2-pentanone (4, 5, 10)	412 3-methyl-2-butanone (4)	416 acetoxyacetone (5)
403 2-acetylfuran(2-furylmethylketone) (4, 9)	408 3-acetoxy-2-butanone (9)	413 3-pentanone (5)	417 hydroxyacetone (9)
404 2-butanone (5)	409 3-hexanone (5)	414 4-hydroxy-4-methyl-2-pentanone (4, 6, 8)	418 methyl amyl ketone (6)
405 2-heptanone (4)	410[a] 3-hydroxy-(2H)-pyran-2-one (4)		419 β-damascenone (8)
Lactones	**Lactones**	**Lactones**	**Lactones**
501[a] 2,5-dimethyl-3(2H)-furanone (4, 5)	506 3,5-dimethyl-4-hydroxy-2,3-dihydroxyfuran-3-one (5)	512[a] solerone (4)	519[a] γ-octalactone (3, 4, 5, 6, 8, 9, 10)
502[a] 2,5-dimethyl-4-hydroxy-3(2H) furanone (1, 3, 4, 5, 6, 8, 9, 10)	507 3-hydroxy-2-methyl-(4H)-pyran-4-one (maltol) (4)	513[a] γ-butyrolactone (3, 4, 5, 6, 9)	520 γ-palmitolactone (5)
503[a] 2,5-dimethyl-4-methoxy-3(2H)-furanone (3, 4, 6, 8, 9)	508 3-hydroxy-4,5-dimethyyl-2(5H)-furanone (8)	514 γ-decalactone (4, 5, 6, 8, 9, 10)	521 γ-valerolactone (9)
504 2-methyl-2(3H)-furanone (4)	509 6-methyl-5-hepten-2-one (4)	515 γ-dodecalactone (4, 5, 8, 9)	522 δ-decalactone (4, 6, 8, 9)
505 2-methyltetrahydrofuran-3-one (9)	510 methyl tetrahydrofuran-3-one (9)	516 γ-heptalactone (4, 6, 9)	523 δ-dodecalactone (4)
	511 pantolactone (4)	517[a] γ-hexalactone (3, 4, 5, 6, 9, 10)	524 δ-heptalactone (4, 6)
		518 γ-nonalactone (4, 5, 8, 9, 10)	525[a] δ-hexalactone (3, 4, 6, 9, 10)
			526 δ-nonalactone (1)
			527[a] δ-octalactone (3, 4, 5, 6, 8, 9, 10)

Terpenes and Terpenoids	Terpenes and Terpenoids	Terpenes and Terpenoids	Terpenes and Terpenoids
601 (E)-β-caryophyllene (3)	606 1-(E,Z,Z)-3,5,8-undecatraene (2)	615 linalool (4, 5, 9)	623 α-patchoulene (2)
602[a] (E)-β-ocimene (3,7-dimethyl-1,3,6-octatriene) (3)	607 1,3,5,8-undecatetraene (8)	616 linalool oxide (E-furanoid) (4, 5, 7, 9)	624[a] α-pinene (3)
603 1-(E,E)-3,5-undecatriene (2)	608 1,4-cineol (5)	617 linalool oxide (Z-furanoid) (4, 7, 9)	625 α-zingiberene (3)
604 1-(E,E,Z)-3,5,8-undecatraene (2)	609 1,8-cineol (5)	618 *p*-cymene (3, 4, 9)	626[a] β-myrcene (3, 4)
605 1-(E,Z)-3,5-undecatriene (2, 8)	610 4-terpinenol (4, 9)	619 sabinene (3)	627[a] β-phellandrene (3)
	611 camphor (5, 9)	620 Z-ocimene (4)	628 β-pinene (3)
	612 geraniol (4, 7)	621[a] α-copaene (3, 5)	629[a] β-ylangene (2)
	613 germacrene D (2)	622 α-muurolene (2)	630 γ-eudesmol (2, 5)
	614 limonene (3, 4, 9)		631 γ-gurjunene (2)
			632 δ-cadinene (2, 3)
Miscellaneous	Miscellaneous	Miscellaneous	Miscellaneous
701 2-methylbutyric acid (9)	706 butanoic acid (4, 8)	711 hexadecanoic acid (palmitic acid) (6)	714 N,N-dimethylformamide (9)
702 3-hydroxy-2-methyl-4H-pyran-4-one (maltol) (4)	707 cinamic acid (10)	712[a] hexanoic acid (3, 6, 9, 10)	715[a] octanoic acid (3, 6, 9)
703 3-methylbutanoic acid (9)	708 decanoic acid (9)	713 methyl mercaptan (methanethiol) (5)	716 phenylacetic acid (8)
704[a] acetic acid (4, 5, 6, 9)	709 dimethyl disulfide (5)		717 propanoic acid (9)
705 benzene (5)	710 dimethyl trisulfide (7)		

1-Badilla-Porras (2005); 2-Berger and others (1983, 1985); 3-Brat and others (2004); 4-Elss and others (2005); 5-Flath (1980); 6-Sinuco and others (2004); 7-Takeoka and others (1991); 8-Tokitomo and others (2005); 9-Umano and others (1992); 10-Wu and others (1991).

[a]Volatile compounds composition shown in Figures 21.1, 21.2 or 21.3.

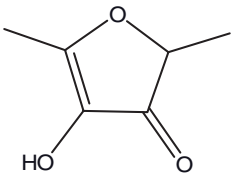


Figure 21.1. Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone).

and composition. Summer fruits had much greater volatile oil content than winter fruits (190 and 15.6 mg/kg, respectively). They reported ethanol and ethyl acetate as major components of summer fruits, with smaller quantities of acetaldehyde, ethyl acrylate, ethyl 3-methylbutanoate, ethyl hexanoate, methyl and ethyl esters of C_5 unsaturated acid, methyl 3-(methylthio)propanoate, and acetic acid. For winter fruits, they found ethyl acetate as the major component, followed by acetaldehyde, methyl 3-methylbutanoate, methyl pentanoate, methyl 4-methylpentanoate, methyl octanoate, and methyl 3-(methylthio)propanoate. Furthermore, they observed that summer fruits seemed to contain mostly ethyl esters, while winter fruits contained mostly methyl esters, even though this observation has not been confirmed yet in other reported results.

Studies from the 1960s and 1970s were summarized by Flath (1980), which included some studies with canned Malayan pineapple juice with paper chromatography made in 1964 by Mori in Hawaii and continued by Connell with fresh Australian pineapple. These were the first researchers to use gas chromatography techniques for pineapple aroma studies, and they were able to identify 16 new volatile components. In 1965, two researchers studied Smooth Cayenne fruit harvested during the winter season from Hawaii; they were able to identify 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (furanone) from the juice extracted from the fruit, using magnetic resonance, infrared, ultraviolet (UV), and mass spectra (Rodin et al. 1965; Silverstein et al. 1965). Later on, they worked with pineapple juice concentrate and identified *p*-allylphenol (chavicol) and γ-hexalactone and confirmed the presence of methyl and ethyl 3-(methylthio)propanoates, which were previously reported by Haagen-Smit and others since 1945. Flath and Forrey (1970) studied the essence extracted from Smooth Cayenne pineapple concentrated juice from Hawaii using the tubular gas chromatography–mass spectrometry technique, which simplified the identification of gas chromatography compatible components; they identified 44 volatile compounds, half of them previously identified as well as some others which could not be identified at that time.

Berger and others (1983) pointed out that volatile constituents of pineapple included aliphatic, hydroxyl, acetoxy, and carboxylic esters; γ-lactones; sulfur compounds; linalool oxide; 2,5dimethyl-4-hydroxy-3(2H)-furanone; monoterpene alcohols; and sesquiterpenoid structures. They isolated pineapple volatiles under enzyme inhibition with methanol to reduce the formation of secondary aroma compounds. These researchers also identified more than 20 sesquiterpenes with either bi- or tricyclic skeletons, terpenoids, fatty acid/amino acid derivatives, phenylpropanoids (furanol), and benzenoids (benzaldehyde), as well as N- and S-containing compounds. Other 19 volatile constituents were found in a later study by Berger and others (1985) with fresh whole ripe pineapple from the Ivory Coast (cultivar

was not reported); the newly identified compounds included four non-terpenoid hydrocarbons, carboxylic esters, and others; however, the authors found that mechanical damage during sample preparation or processing of the fruit tissue can cause a rapid decrease in the concentration of all undecaenes, which can be avoided by preventing enzymatic and oxidative degradation.

As reported by Wu and others (1991) aromatic components of fruits are present either in a free form or bound to sugar as glycosides. They prepared pineapple juice from fresh pineapple fruit from Costa Rica (nonspecified cultivar) and found free and glycosidically bound volatile compound in pineapple. Methyl 3-acetoxyhexanoate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and methyl 5-acetoxyhexanoate were the most abundant. But they also identified 2-pentanol, 2-butoxyethanol, hexanoic acid, phenol, *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde that were not reported before. They found out that free volatile fraction had fruity and pineapple-like aroma, while the glycosidically bound fraction had no odor, until it was released with enzymatic hydrolysis. Lactones and hydroxyl compounds were the main glycosidically bound volatiles; 2,5-dimethyl-4-hydroxy-3(2H)-furanone was the most abundant compound followed by δ-octalactone and ethyl 3-hydroxyhexanoate. Some lactones were only found as glycosidically bound, while others were free, or, in both forms. In addition, Sinuco and others (2004) identified 17 glycosidically bound aroma compounds (aglycones) in fresh Perola cultivar pineapple. Phenolic compound, carboxylic acids, and furanic compounds were the main aglycones identified, and coniferlilic alcohol, hexadecanoic acid, furaneol, and 4-vinylguaiacol were the most important. Table 21.2 summarizes the free and bound volatile compounds reported by Sinuco and others (2004) and Wu and others (1991).

TABLE 21.2. Free and Glycosidically Bound Compounds in Pineapple Aroma (μg/kg)

	Compound	Free[a]	Bound[a]	Freed from Glycosidic Extract[b]
23	dimethyl malonate	105	—	
47	ethyl 3-acetoxy hexanoate	101	—	
48	ethyl 3-acetoxy octanoate	13	—	
52	ethyl 3-hydroxy hexanoate	52	168	≤50
53	ethyl 3-hydroxy octanoate			≤50
59	ethyl 4-acetoxy hexanoate	76	—	
60	ethyl 4-acetoxy octanoate	42	—	
62	ethyl 4-hydroxy hexanoate			50–500
63	ethyl 4-hydroxy octanoate			50–500
64	ethyl 5-acetoxy hexanoate	52	—	
70	ethyl acetate	470	—	
81	ethyl hexanoate	37	—	
90	ethyl propanoate	10	—	
115	methyl 2-methyl butanoate	70	—	
122	methyl 3-acetoxy butanoate	210	—	
123	methyl 3-acetoxy hexanoate	—	—	
124	methyl 3-acetoxy octanoate	116	—	
126	methyl 3-hexenoate	5	—	
128	methyl 3-hydroxy hexanoate	12	—	
129	methyl 3-hydroxy octanoate	—	29	≤50

TABLE 21.2. ***Continued***

	Compound	Free[a]	Bound[a]	Freed from Glycosidic Extract[b]
131	methyl 3-hydroxy-2-methyl butanoate			50–500
135	methyl 4-acetoxy hexanoate	193	—	
136	methyl 4-acetoxy octanoate	6	—	
140	methyl 4-methyl pentanoate	141	—	
142	methyl 5-acetoxy hexanoate	676	—	
143	methyl 5-acetoxy octanoate	129	—	
145	methyl 5-hydroxy hexanoate			≤50
146	methyl 5-hydroxy octanoate			≤50
150	methyl butanoate	26	—	
161	methyl octanoate	34	—	
162	methyl pentanoate	5	—	
165	propyl acetate	6	—	
167	δ-heptanoate		34	
201	(3-hydroxyphenyl) ethyl alcohol	—	11	
207	1-hexanol	—	12	
213	2,3-butanodiol			50–500
216	2-allyl-phenol			50–500
217	2-butoxyethanol	74	24	
225	2-pentanol	7	—	
226	2-phenyl ethanol (1)	—	19	
229	3-methyl butan-1-ol	23	—	
230	3-methyl pentan-2-ol	9	—	
238	4-allyl-2,6-dimethoxyphenol	—	31	
240	4-vinyl guaiacol			>1000
241	4-vinyl phenol			50–500
242	benzylic alcohol			50–500
243	coniferlic alcohol			>1000
247	eugenol	—	18	
258	phenol	54	—	
308	benzaldehyde	11	9	
312	hexanal	10	—	
316	p-hydroxybenzaldehyde	45	6	
318	syringaldehyde	80	27	
319	vanillin	23	—	
407	2-pentanone	12	—	
502	2,5-dimethyl-4-hydroxy3(2H)-furanone	700	491	>1000
514	γ-decalactone	—	6	
517	γ-hexalactone	—	45	
518	γ-nonalactone	—	6	
519	γ-octalactone	—	8	
525	δ-hexalactone	26	—	
527	δ-octalactone	99	226	
704	acetic acid	109	—	50–500
707	cinnamic acid	—	65	
711	hexadecanoic acid			>1000
712	hexanoic acid	23	11	

[a]Wu and others (1991) (from pineapple juice, cultivar not reported).
[b]Sinuco and others (2004) pineapple pulp (perolera cultivar).
Note: Volatile compounds numbers correspond to those included in Table 21.1.

In their work, Takeoka and others (1991) identified and studied sulfur-containing constituents from pineapple essence and their contribution to pineapple odor. Volatiles were extracted with pentane. They identified for the first time 26 pineapple constituents and reported methyl and ethyl 3-(methylthio)-(*Z*)-2-propenoate as the major volatile constituents found in pineapple with concentrations in the range of 1–6 μg/kg.

PINEAPPLE FLAVOR PROFILE CHANGES

Volatile compounds play an important role in flavor perception (Kays 1997); however, their content can be altered by cultural practices before harvest, postharvest handling practices, maturity stage, and processing procedures, which might include refrigeration, minimal processing, juice extraction, filtration, heat processing, and others.

Influence of Cultivars and Maturity Stages on the Flavor of Pineapple Fruit

Many volatile compounds of pineapple have been identified from fresh fruit. However, in many cases, their concentrations depend on the cultivar as well as the degree of ripeness.

Umano and others (1992) worked with green and ripened pineapples from the Philippines (cultivar was not reported) and found differences in volatile constituents composition. They identified 157 volatile compounds; 144 were found in green fruits and 127 in ripened fruit. Ethyl acetate (24.5%), ethyl 3-(methylthiopropanoate (10.4%), and ethyl 3-acetoxyhexanoate (8.7%) were the major volatile components in green pineapple, compared with ethyl acetate (33.5%), *threo*-butane-2,3-diol diacetate (13.0%), and 3-hydroxy-2-butanone (8.7%) in ripened pineapples.

Brat and others (2004) studied the volatile compounds for a new pineapple hybrid (Flhoran 41) for different stages of maturity and compared them with the Smooth Cayenne cultivar. These authors found that major components were aliphatic, hydroxyl, and acetoxy esters and terpenes. Figure 21.2 shows four graphs for the main pineapple volatile compounds for the Flhoran 41 cultivar. Limonene was the most abundant constituent, and it decreased significantly as the fruit ripened (1300, 810, 975, and 1410 μg/100 g, for very green, green, ripe, and very ripe fruit, respectively). Volatile compound composition changed throughout the different stages of maturity; ripe pineapple had larger contents of most of the volatile compounds as compared with green and very green fruits.

Additionally, they found some differences in the volatile component profile and concentrations for ripe pineapple of Flhoran 41 and Smooth Cayenne cultivars (Fig. 21.3). Ripe pineapple from both varieties showed similar volatile composition but some components were only found in Flhoran 41 fruits (*n*-butyl acetate, ethyl 2-hydroxypropanoate and (*E*)-β-caryophyllene); some had higher concentrations in Flhoran 41 pineapples ((*E*)-β-ocimene, γ-butyrolactone, 2,5-dimethyl-4-methoxy-3(2H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and some esters [methyl 2-methylbutanoate, methyl 2-hydroxy-2-methylbutanoate, methyl 2-methyl 3-oxobutanoate, and ethyl 3-(methylthio)propanoate]). Other volatile constituents

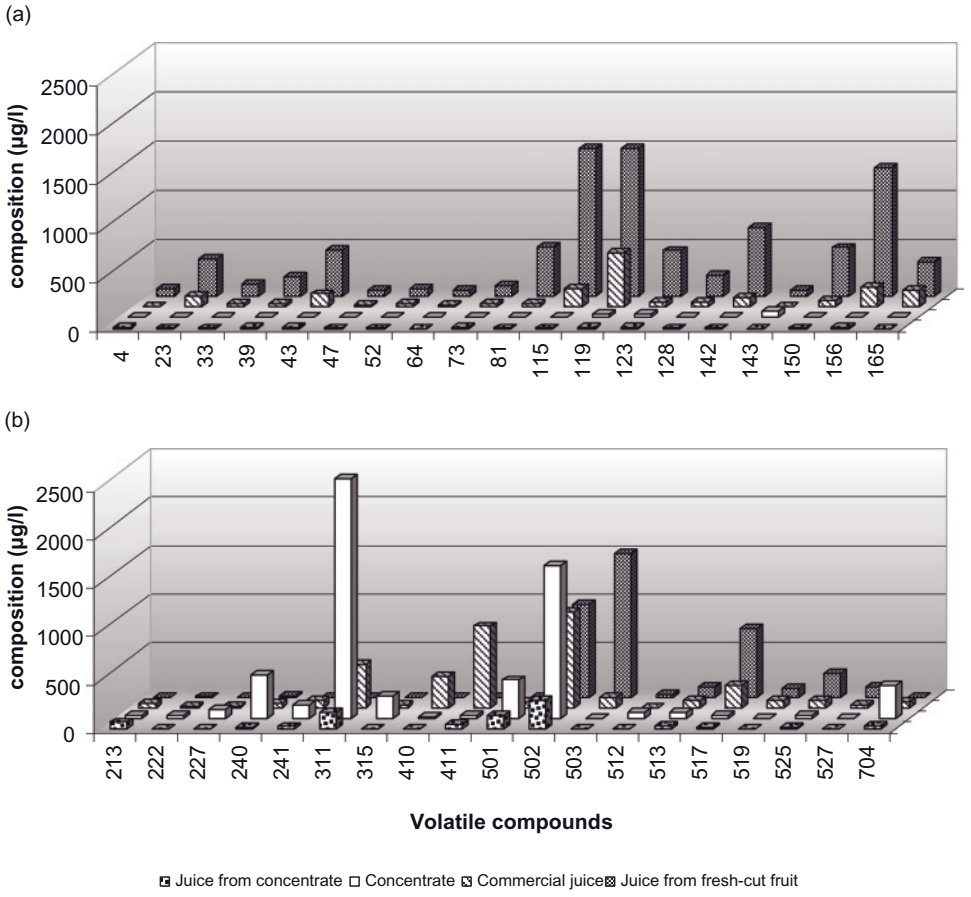


Figure 21.2. Esters (a) and alcohols, aldehydes, ketones, lactones, and other compounds (b) average composition (µg/l) in pineapple products. Volatile compound numbers correspond to those in Table 21.1. Adapted from Elss and others (2005).

were present in higher concentrations in Smooth Cayenne cultivar, such us methyl 5-acetoxyhexanoate, methyl 3-acetoxyhexanoate, dimethyl malonate, and methyl hexanoate.

Effects of Processing on the Flavor of Pineapple Fruit

The pineapple fruit flavor can be easily modified during fruit processing. A representative example is the pineapple juice, which is usually a by-product (outflowing juice, the juice from the peel, and the pineapple core) obtained during the production process of canned pineapples, or from concentrated pulp. In both cases, thermal treatments are employed resulting in the loss or transformation of some volatile compounds.

Elss and others (2005) studied the flavor profile of juice made from fresh-cut pineapple fruits, juice concentrates, commercial juices, and juice made from the

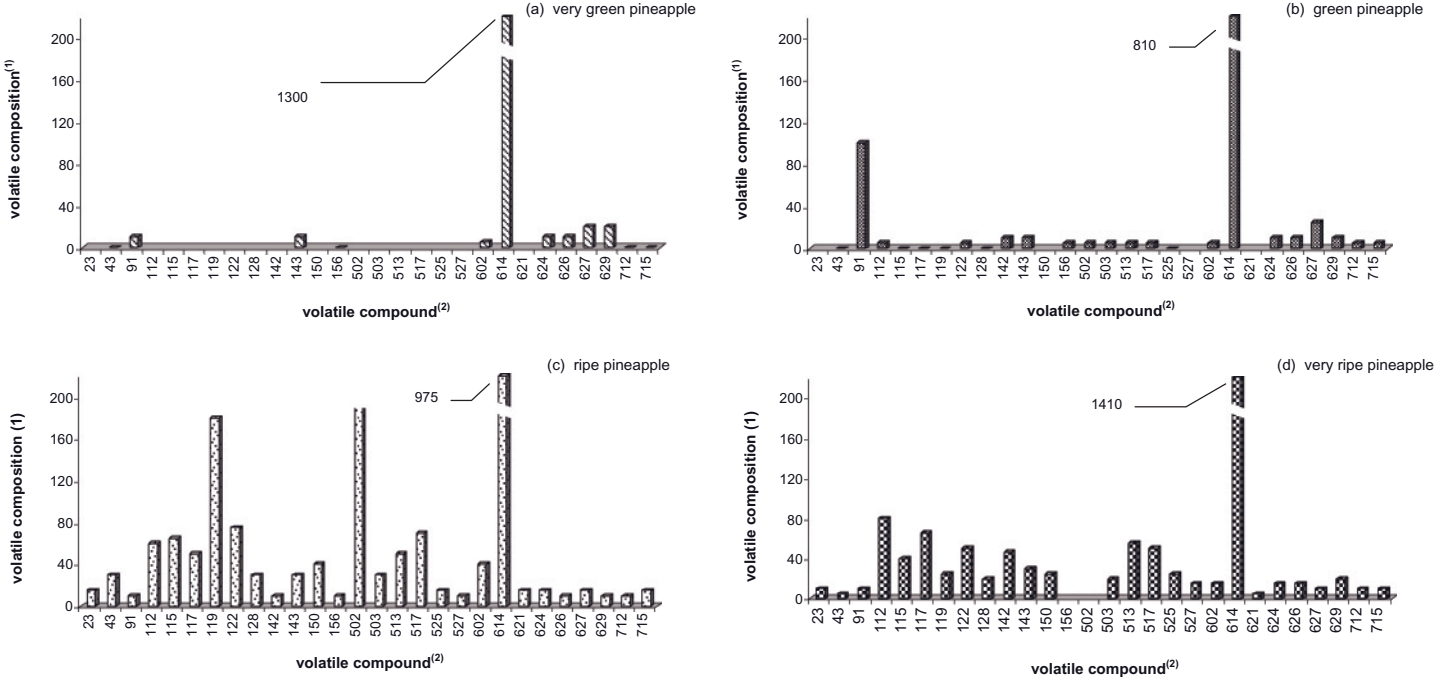
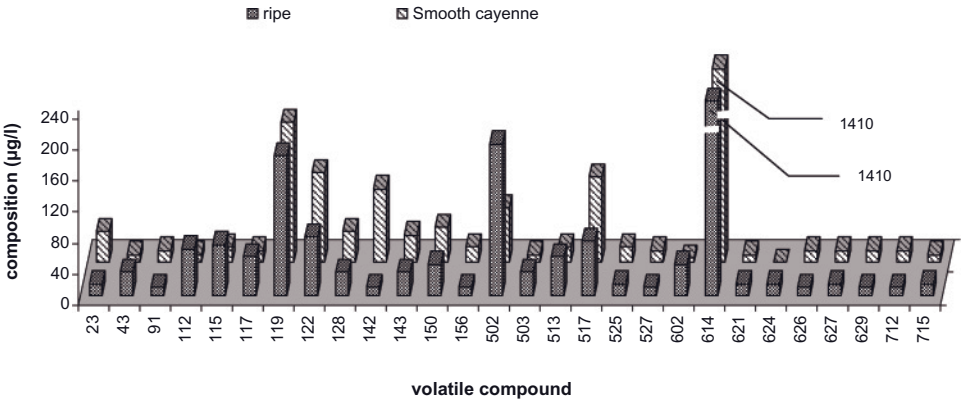


Figure 21.3. Changes in major pineapple volatile compounds composition during maturation of Flhoran41 cultivar pineapple from French West Indies (a–d show results for very green, green, ripe, and very ripe pineapple, respectively). Adapted from Brat and others (2004). [1]Micrograms of z-heptanol equivalent per 100 g fresh weight, µg/100 g; [2]volatile compound numbers correspond to those in Table 21.1.



[1:] volatile compound numbers correspond to those included in Table 1

Figure 21.4. Volatile compounds composition (µg/l) of ripe Flhoran41 and ripe Smooth cayenne pineapples. Volatile compound numbers correspond to those included in Table 21.1. Adapted from Brat and others (2004).

concentrate (Fig. 21.4). Their results showed considerable differences among flavor profile of fresh pineapple juice and other processed samples, since in most cases, the characteristic methyl esters and hydroxy or acetoxy esters were lacking completely or had only minor amounts in processed products. Ester content of juice prepared from fresh-cut pineapple was much larger than that of concentrate and juices (Fig. 21.4A). Differences can be due to losses during processing once the fruit is peeled, cut, and processed. Thermal processes used to prepare pineapple concentrates facilitate volatile compound losses and might also contribute to the production of new chemical compounds. They observed that methyl 2-methylbutanoate, methyl 3-(methylthio)propanoate, and methyl hexanoate were the most abundant esters in pineapple juice prepared from fresh-cut fruit, followed by methyl 5-acetoxyhexanoate, ethyl hexanoate, methylbutanoate, ethyl 3-(methylthio) propanoate, and methyl 3-acetoxyhexanoate. Commercial juices had similar aroma constituents but in much lower concentrations, while concentrates and juices prepared from concentrates had even lower concentrations.

In addition, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2,5-dimethyl-4-methoxy-3(2H)-furanone, and γ-hexalactone were the most important non-ester volatile components in juice prepared from fresh fruit. The first of them was important for all pineapple products studied, while the second and third were present in much lower concentrations in commercial juices, concentrates, and juices from concentrates. Furfural, 4-vinylguaiacol, 4-vinylphenol, 2,5-dimethyl-3(2H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and acetic acid were present in higher concentrations in pineapple concentrates, than the juice from fresh fruits and other pineapple products. Differences confirm changes in volatile composition occurring during processing.

Some other volatile compounds found in juices from concentrates include small amounts of terpenes associated with contamination during processing since they were not found in juices made from fresh-cut fruit (*p*-cymene, β-myrcene, α-terpineol, or linalool).

Because of the convenience offered to consumers, consumption of fresh-cut fruit including pineapples has increased considerably in the last years. However, the shelf life of cut fruits is considerably lower than that of the intact fruit. Once a fruit is cut, it becomes a different product from what it was in its entire form. Therefore, producer must ensure the fruit's original flavor characteristics, quality, and safety.

Spanier and others (1998) studied the effect of storage (4 °C for 3, 7, and 10 days) on the flavor volatile profile of fresh-cut pineapples. They observed that pineapple-like flavors increased very slightly during storage (acetic acid 1-methylethyl ester, acetic acid propyl ester, and 1-butanol 3-methyl acetate). While unpleasant odors and volatiles such as fermented, cheesy, sourdough, alcohol, and oily showed dramatic increases and masked the more desirable pineapple flavor leading to a diminution of the overall flavor quality of the product. The large increase in the level of low boiling alcohols in stored pineapple suggested that fermentative events occurred during storage. They confirmed that yeast was the source of the fermentation-derived alcohols.

More recently, Lamikanra and Richard (2004) indicated that the stress adaptation process of fruit to exposure of tissue resulting from fresh-cut processing involves the reduction of volatile aroma compounds, particularly esters, and synthesis of sesquiterpene compounds with phytoalexin properties. In fact, they evaluated the effect of storage and UV-induced stress on the volatile aroma compounds of fresh-cut pineapple. According to their results, storage at 4°C for 24 h, and exposure of cut fruit to UV radiation for 15 min caused a considerable decrease in the concentration of esters and an increase in the relative amount of copaene, sesquiterpene which inhibit microbial growth in fruits when it is added to fresh-cut fruit (Lamikanra et al. 2002; Lamikanra and Richard 2004). Furthermore, they identified other sesquiterpene considered as a potent antimicrobial agent, ocimene, which was present in the fruit but their production was not photo-induced by UV irradiation. The loss of esters and changes in volatile aroma composition during storage, including production of terpene phytoalexins, will potentially affect the fruit flavor during storage. However, sesquiterpene phytoalexins could contribute to the defense mechanism in wounded pineapple tissue.

SENSORY CHARACTERIZATION OF PINEAPPLE FLAVOR

Total aroma of the fruit is a result of a specific blend of individual component aromas with specific quantity of each of them. For this reason, it is necessary to achieve proper separation and identification of odor-contributing constituents in combination with sensory evaluation of the fruit and its individual components. Most of pineapple aroma studies have been done on identification and quantification of volatile constituents, and only a few have been done with sensory analysis (Flath 1980; Tokitomo et al. 2005).

Several researchers have studied the contribution of different volatile compounds to overall aroma of pineapple fruit (Flath 1980; Takeoka et al. 1991; Tokitomo et al. 2005; Wu et al. 1991). Relative composition and proper characteristics of each volatile constituent are two factors involved in their contribution to fruit aroma; however, it is not necessarily related to the component concentration. Therefore, the determination of the contribution of different volatile compounds to overall aroma

perception of fresh and processed pineapple is very important. For this reason, the use of sensory analysis is essential, in combination with separation techniques and proper analytic analysis to measure volatile component threshold and composition. Table 21.3 summarizes some sensory data and odor description for pineapple volatile compounds reported in the last years.

Flath (1980) cited studies by Pittet and others (1970) and Rodin and others (1965) who reported odor and taste thresholds of furanone in water as 0.1–0.2 ppm and 0.3 ppm, respectively, and while furanone concentration in pineapple flesh reported as 1.2 ppm on winter Smooth Cayenne fruit by Silverstein and others (1965). In this work, furanone odor was described as caramel, sweet, and fruity. A coconut note has also been reported in the aroma of fully ripe pineapple, probably caused by odorous lactones like γ-octalactones and δ-octalactones (Flath 1980).

Contribution of volatile constituents to pineapple aroma have been studied by comparing their concentrations and odor detection thresholds (Berger et al. 1985; Takeoka et al. 1991); as the ratio of average concentration to odor detection threshold increases, the contribution of the volatile compound becomes larger. Berger and others (1983) considered that α-patchoulene contributed to the strong fruit-spice odor of pineapple. In 1985, the same authors used a capillary gas chromatographic sniffing technique to compare analytic data with sensory judgments. They were able to identify several volatile constituents not reported before from whole ripe pineapple fruits from the Ivory Coast. They reported that even though 1-(*E*,*Z*)-3,5-undecatriene and 1-(*E*,*Z*,*Z*)-3,5,8-undecatraene had low average concentrations, their odor detection thresholds was also low, and thus they concluded that these compounds probably have an important contribution to the overall impression of pineapple flavor. Their isomers 1-(*E*,*E*)-3,5-undecatriene and 1-(*E*,*E*,*Z*)-3,5,8-undecatraene were found to have much less odor. Ethyl hexanoate and ethyl 3-(methylthio) propanoate were also reported by the same authors as important contributors to pineapple aroma; both of them were present in larger concentrations, and their odor detection threshold were also higher.

Takeoka and others (1991) reported ethyl-2-methyl-butanoate (S-(+) enantiomer) as a potent odorant with an odor threshold of 0.006 μg/kg, they considered it as the second largest odor contributor to pineapple aroma after pineapple furanone.

Sinuco and others (2004) used high-resolution gas chromatography with olfactometry (HRGC/O) to separate and describe the odor of each pineapple aroma components with the help of a group of aroma experts. They studied volatile constituents of fresh pineapple fruits (Perolera cultivar), using fruits grown in Colombia. They reported methyl esters of 2-methyl-butanoic and hexanoic acids as responsible for fresh pineapple odor, while reported esters such as ethyl butanoate, ethyl-2-methylbutanoate, butyl acetate, 2-methyl acetate, methyl-3-hexenoate, methyl 2-hydroxy-2-methybutanoate, methyl 3-hydroxybutanoate, and ethyl 3-acetoxy-2-methylbutanoate as low-impact volatile compounds for pineapple aroma. Furanone, γ-butyrolactone, γ-hexalactone, γ-octalactone, γ-decalactone, and δ-octalactone were reported as important for the Perolera cultivar pineapple aroma.

Tokitomo and others (2005) used the aroma extract dilution analysis (AEDA) approach to identify the most odor-active compounds. They studied Super Sweet (F-2000) pineapple cultivar purchased in Germany and Japan, and found 29 odor-active volatile components and estimated an odor activity value (OAV) to compare

TABLE 21.3. Sensory Characteristics of Pineapple Volatiles

	Volatile Compound	Average Concentration (μg/kg)	Odor Detection Threshold (μg/kg)	Odor Descripiton
3	2,3-butanediol diacetate			honey-like (7)
4	2-methyl butylacetate	≤50		pineapple (4)
8	2-propenyl n-hexanoate	<0.5	50–100	fruity, estery, near its detection threshold resembling 1-(E,Z)-3,5-undecatriene (2)
16	butyl acetate	≤50		fruity, sweet, pineapple (4)
23	dimethyl malonate	50–100		odor not detected by experts (4)
27	ethyl (E)-2-hexenoate	5	14–20	fruity, slightly pungent (2)
28	ethyl (E)-3-hexenoate	15	25–50	pungent, pineapple peel like (2)
31	ethyl (Z)-3-hexenoate		1–2	fruity, pineapple-like (2)
33	ethyl 2-(methylthio) acetate	<0.5	200–300	pungent, pineapple peel like (2)
35	ethyl 2-hydroxy hexanoate			ripened pineapple-llike (7)
37	ethyl 2-hydroxy-2-methyl butanoate	≤50		odor not detected by experts (4), grape-like (7)
38	ethyl 2-hydroxy-3-methyl butanoate			ripened pineapple-like (7)
39	ethyl 2-methyl butanoate	≤50[4], 157[6]	0.006[5], 0.15[6]	pineapple heart (4), fruity (6)
40	ethyl 2-methyl propanoate	48.0	0.02	fruity, sweet (6)
43	ethyl 3-(methylthio) propanoate	100[1], ≤50[4]	1–2[1]	fruity, pineapple-like (2)
44	ethyl 3-(methylthio)-(E)-2-propenoate		246	(5)
46	ethyl 3-acetoxy butanoate	≤50		metallic (4), mint-like (7)
49	ethyl 3-acetoxy pentanoate			ripened pineapple-llike (7)
50	ethyl 3-acetoxy-2-methyl butanoate	≤50		pineapple like (4), grape-like, powdery (7)
51	ethyl 3-hydroxy butanoate			grape-like (7)
52	ethyl 3-hydroxy hexanoate	≤50		odor not detected by experts (4)

TABLE 21.3. ***Continued***

	Volatile Compound	Average Concentration (μg/kg)	Odor Detection Threshold (μg/kg)	Odor Descripiton
53	ethyl 3-hydroxy octanoate			apple-like (7)
54	ethyl 3-hydroxy pentanoate	≤50		mushroom, soil (4), fruity, pineapple-like (7)
55	ethyl 3-hydroxy-2-methyl butanoate			grape-like, powdery (7)
57	ethyl 4-(methylthio) butanoate		19	(5)
58	ethyl 4-acetoxy butanoate			sour yogurt like (7)
59	ethyl 4-acetoxy hexanoate	≤50		coconut, sweet (4)
60	ethyl 4-acetoxy octanoate	≤50		odor not detected by experts (4)
61	ethyl 4-acetoxy pentanoate			pineapple like (7)
62	ethyl 4-hydroxy octanoate			milk-like (7)
64	ethyl 5-acetoxy hexanoate	100–150		honey (4)
65	ethyl 5-acetoxy octanoate	≤50		caramel, red (4)
70	ethyl acetate	≤50		odor not detected by experts (4), solvent-like, fruity (6)
72	ethyl butanoate	≤50[4], 75.2[6]	1[6]	pineapple (4), fruity (6)
81	ethyl hexanoate	500[2], ≤50[4, 6]	1–2[2]	fruity (2), fruity, pineapple, banana (4), fruity (6)
92	geranyl acetate		9	(5)
96	methyl (E)-3-hexenoate	≤50		pineapple, fruity, humid (4)
98	methyl (E)-4-hexenoate		147	(5)
101	methyl (Z)-3-hexenoate	≤50		odor not detected by experts (4)
104	methyl (Z)-4-hexenoate	≤50		green, fruity, rancid (4)
105	methyl (Z)-4-octenoate	≤50		raw potato (4)
109	methyl 2,4-hexadienate	≤50		row potato (4)
110	methyl 2-acetoxy butanoate	≤50		odor not detected by experts (4)

TABLE 21.3. ***Continued***

	Volatile Compound	Average Concentration (μg/kg)	Odor Detection Threshold (μg/kg)	Odor Descripiton
112	methyl 2-hydroxy-2-methyl butanoate	50–100		pineapple (4), woody (7)
114	methyl 2-hydroxy hexanoate	≤50		odor not detected by experts (4)
115	methyl 2-methyl butanoate	100–150[4], 1190[6]	2[6]	rancid, pineapple (4), fruit, apple-like (6)
116	methyl 2-methyl propanoate	154	6,3	fruity, sweet (6)
119	methyl 3-(methylthio) propanoate	100–150		penetrating, onion (4)
120	methyl 3-(methylthio)-(E)-2-propenoate		95	(5)
121	methyl 3-(methylthio)-(Z)-2-propenoate		25	(5)
122	methyl 3-acetoxy butanoate	50–100		odor not detected by experts (4)
123	methyl 3-acetoxy hexanoate	≤50		odor not detected by experts (4)
125	methyl 3-acetoxy-2-methyl butanoate	≤50		caramel (4), burnt (7)
127	methyl 3-hydroxy butanoate	≤50		pineapple, fermentation (4)
128	methyl 3-hydroxy hexanoate	≤50		unpleasant (4)
130	methyl 3-hydroxy pentanoate			honey-like (7)
131	methyl 3-hydroxy-2-methyl butanoate			woody (7)
132	methyl 3-hydroxy-3-methyl butanoate	≤50		coconut, sweet (4)
133	methyl 3-methyl butanoate	≤50		odor not detected by experts (4), fruit, apple-like (6)
134	methyl 4-(methylthio) butanoate		133	(5)
135	methyl 4-acetoxy hexanoate	100–150		odor not detected by experts (4)
139	methyl 4-acetoxy octanoate	≤50		pineapple (4)
141	methyl 5-acetoxy heptanoate	≤50		coconut (4)

TABLE 21.3. ***Continued***

	Volatile Compound	Average Concentration (μg/kg)	Odor Detection Threshold (μg/kg)	Odor Descripiton
142	methyl 5-acetoxy hexanoate	50–100		acid, metalic (4)
143	methyl 5-acetoxy octanoate	≤50		odor not detected by experts (4)
144	methyl 5-hexenoate		194	(5)
145	methyl 5-hydroxy hexanoate	≤50		green pineapple (4), smoky tobacco-like, woody (7)
147	methyl acetate	≤50		odor not detected by experts (4)
149	methyl benzoate	≤50		odor not detected by experts (4)
150	methyl butanoate	50–100		apple, sweet, toast (4)
156	methyl hexanoate	100–150		pineapple (4)
161	methyl octanoate	≤50		fruity, winy, orange (4)
162	methyl pentanoate	≤50		odor not detected by experts (4)
209	1-octen-3-ol		1,3	(5)
217	2-butoxy-ethanol	≤50		pineapple (4)
243	coniferilic alcohol	≤50		basamic, floral (4)
253	nonanol		50	(5)
311	furfural	≤50		carmel, toasted (4)
314	octanal	19,1	8	citrus, fatty (6)
319	vanillin	5,99	25	vanilla-like (6)
401	(Z)-1,5-octadien-3-one			geranium-like (6)
404	2,3-butanedione			buttery (6)
408	3-acetoxy-2-butanone			sour (7)
414	4-hydroxy-4-methyl-2-pentanone	≤50		apple, meaty, green (4)
415	acetone	≤50		odor not detected by experts (4)
418	methyl amyl ketone	≤50		odor not detected by experts (4)
419	β-damascenone	0,083	0,00075	fruity, sweet (6)
502	2,5 dimethyl-4-hydroxy-3-(2H)-furanone	1200[3], ≤50[4], 26800[6]	100–200[3], 10[6]	fruity, spicy (3), sweet, caramel, toast (4), sweet, pineapple-like, caramel-like (6)
503	2,5-dimethyl-4-methoxy-3(2H)-furanone	≤50		cherry (4), caramel-like (6)

TABLE 21.3. ***Continued***

	Volatile Compound	Average Concentration (μg/kg)	Odor Detection Threshold (μg/kg)	Odor Descripiton
508	3-hydroxy-4,5-dimethyyl-2(5H)-furanone			seasoning-like (6)
513	γ-butyrolactone	≤50		coconut, sweet (4)
514	γ-decalactone	≤50		fruity, sweet, peach (4), fruity, sweet, peach-like (6)
515	γ-dodecalactone			fruity, sweet (6)
516	γ-heptalactone	≤50		odor not detected by experts (4)
517	γ-hexalactone	≤50		sweet, caramel, toast (4)
518	γ-nonalactone			peach-like, fruity (6)
519	γ-octalactone	≤50		caramel, red (4), fruity, coconut-like (6)
521	δ-decalactone	≤50[4], 32.7[6]	160[6]	odor not detected by experts (4), sweet, coconut-like (6)
524	δ-heptalactone	≤50		odor not detected by experts (4)
525	δ-hexalactone	≤50		honey (4)
527	δ-octalactone	≤50[4], 78.2[6]	400[6]	sweet, caramel (4), coconut-like (6)
603	1-(E,E)-3,5-undecatriene	<0.5	750–1000	musty (2)
604	1-(E,E,Z)-3,5,8-undecatraene	<0.5	20–30	sweet, fruity (2)
605	1-(E,Z)-3,5-undecatriene	1[4], 8.89[6]	0.001–0.002[4], 0.02[6]	balsamic, spicy, pinewood (2), fresh, pineapple-like (6)
606	1-(E,Z,Z)-3,5,8-undecatraene	1	0.002–0.004	resembling 1-(E,Z)-3,5-undecatriene, more fruit (2), fresh, pineapple-like (6)
623	α-patchoulene (1)			strong fruity-spicy (1)
706	butanoic acid			sour (6)
710	dimethyl trisulfide		0,01	(5)
712	hexanoic acid	≤50		pineapple (4)
715	octanoic acid	≤50		odor not detected by experts (4)
716	phenylacetic acid			honey-like (6)

1-Berger and others (1983); 2-Berger and others (1985); 3-Flath (1980); 4-Sinuco and others (2004); 5-Takeoka and others (1991); 6-Tokitomo and others (2005); 7-Umano and others (1992).
Note: Volatile compounds numbers correspond to those included in Table 21.1.

among volatile constituents, taking into consideration the odor threshold and concentration. They reported furanone (2,5-dimethyl-4-hydroxy-3(2H)-furanone), ethyl 2-methylpropanoate and ethyl 2-methylbutanoate as the three most odor-active compounds, followed by methyl 2-methylbutanoate, 1-(*E*,*Z*)-3,5-undecatriene and β-damascenone. They corroborated that fresh pineapple-like aroma was due to 1-(*E*,*Z*)-3,5-undecatriene, as reported before. Sensory evaluations were performed by the authors to corroborate the above results. They used the main 12 odorants of pineapple and prepared models using the same compound concentrations as found in pineapple and seven odor descriptors: sweet, citrus like, fresh, fruity, green or grassy, woody, and pineapple like. When furanone or ethyl 2-methylbutanoate were excluded from the models, panelists noticed aroma changes. Absence of furanone resulted in lack of sweet, pineapple-like aroma, while absence of ethyl 2-methylbutanoate was reflected as a lack of fresh pineapple flavor.

Recently, Schulbach and others (2007) evaluated overall acceptability of fresh pineapple from five different countries and six different producers. They used a descriptive sensory analysis with eight descriptive terms: sweetness, sourness, pineapple flavor intensity, firmness, juiciness, off-flavor, banana character, and coconut character, along with a rating for overall acceptability. Their results showed that the attributes sweetness, pineapple flavor intensity, and off-flavor were the most important factors in determining acceptability. Pineapple flavor rating was more significant than sweetness in determining pineapple sensory quality as long as the sugar content of the fruit was adequate. In addition, this experiment provides strong evidence that increasing the aroma volatiles in pineapple will not only result in a pineapple with higher flavor intensity, but also with more apparent sweetness and better overall acceptability.

OTHER FLAVOR COMPONENTS

Many other constituents that stimulate the sense of taste have also been identified. The sugars, for instance, produce sensations of sweetness, while organic acids are responsible for sour tastes. Both, acidity and sweetness contributes with pineapple aroma. Acid content varies as the fruit develops and ripens. Citric and malic acids are the major nonvolatile acids in pineapple. Malic acid content can vary from 18% to 30% of total acids, while citric acid content is about 28–66% (Paull 1993).

Sugar content is an important characteristic that directly affects flavor. It is used as a quality parameter to indicate both maturity stage and quality. Major sugars in ripe fruit include sucrose, glucose, and fructose (Paull 1993). Content of inverted sugars is much larger during the early stages of the fruit development and decreases as the fruit ripens. Total sugars increase as the fruit ripens up to 12–18%, depending on the cultivar, weather conditions, and others (Flath 1980).

FINAL REMARKS

Nearly 370 volatile and nonvolatile constituents have been recognized up to 2005 in fresh and processed pineapple. However, factors such as cultivar, stage of maturity, and processing conditions, as well as pre- and postharvest practices, can directly

affect pineapple aroma profile. In fact, original flavor characteristics, quality, and safety of pineapple are also affected during their processing. In addition, even though MD2 cultivar has substituted a large portion of the pineapple world market, no information is available about its impact aroma compounds and how they change during processing. Studies in this subject are still very limited, and more efforts should be made not only to identify impact components but to study changes due to processing and storage, including sensory analyses of pineapple volatile compounds.

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REFERENCES

Badilla-Porras M. 2005. Caracterización de compuestos volátiles en jugos pulposos y clarificados de frutas de exportación. Trabajo final de graduación presentado en la Escuela de Tecnología de Alimentos, Facultad de Ciencias Agroalimentarias, Universidad de Costa Rica.

Berger RG, Drawert F, Kollmannsberger H, Nitz S, Schraufstetter B. 1985. Novel volatiles in pineapple fruit and their sensory properties. *J Agric Food Chem* 33:233–5.

Berger RG, Drawert F, Nitz S. 1983. Sesquiterpene hydrocarbons in pineapple fruit. *J Agric Food Chem* 31:1237–9.

Brat P, Thi Hoang LN, Soler A, Reynes M, Brillouet JM. 2004. Physicochemical characterization of a new pineapple hybrid (FLHORAN41 Cv.). *J Agric Food Chem* 52:6170–7.

Buttery RG, Takeoka GR, Ling LC. 1995. Furaneol: Odor threshold and importance to tomato aroma. *J Agric Food Chem* 43:1638–40.

Dahlen T, Hauck T, Wein M, Schwab W. 2001. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone as a secondary metabolite from D-fructose-1,6-diphosphate metabolism by *Zygosaccharomyces rouxii*. *J Biosci Bioeng* 91:352–8.

Elss S, Preston C, Hertzig F, Heckel F, Richling E, Schreier P. 2005. Aroma profiles of pineapple fruit (*Ananas comosus* [L.] Merr.) and pineapple products. *Lebensm Wiss Technol* 38:263–74.

Flath RA. 1980. Pineapple. In Nagi S, Shaw PE (eds.), *Tropical and Subtropical Fruits. Composition, Properties and Uses*. Westport, CT: AVI Publishing, pp. 157–83.

Flath RA, Forrey RR. 1970. Volatile components of Smooth Cayenne pineapple. *J Agric Food Chem* 18:306–9.

Haagen-Smit A, Kirchner JG, Prater AN, Deasy CL. 1945a. Chemical studies of pineapple (*Ananas sativus* Lindl). I. The volatile flavor and odor constituents of pineapple. *J Am Chem Soc* 67:1646–50.

Haagen-Smit A, Kirchner JG, Deasy CL, Prater AN. 1945b. Chemical studies of pineapple (*Ananas sativus* Lindl). II. Isolation and identification of a sulfur-containing ester in pineapple. *J Am Chem Soc* 67:1651–2.

Honkanen E, Pyysalo T, Hirvi T. 1980. The aroma of Finnish wild raspberries. *Z Lebensm Unters Forsch* 171:180–2.

Kays SJ. 1997. *Postharvest Physiology of Perishable Plant Products*. Athens, GA: Exon Press.

Lamikanra O, Richard OA. 2002. Effect of storage on some volatile aroma compounds in fresh-cut cantaloupe melon. *J Agric Food Chem* 50:4043–7.

Lamikanra O, Richard OA. 2004. Storage and ultraviolet-induced tissue stress effects on fresh-cut pineapple. *J Sci Food Agric* 84:1812–6.

Lamikanra O, Richard OA, Parker A. 2002. Ultraviolet induced stress response in fresh cut cantaloupe. *Phytochemistry* 60:27–32.

Miller PH, Libbey LM, Yang HY. 1973. Loganberry flavor components of commercial essence. *J Agric Food Chem* 21:508.

Paull RE. 1993. Pineapple and papaya. In Seymour GB, Taylor J, Tucker G (eds.), *Biochemistry of Fruit Ripening*. London: Chapman-Hall, pp. 291–323.

Perez AG, Olias R, Sanz C, Olias JM. 1996. Furanones in strawberries: Evolution during ripening and postharvest shelf life. *J Agric Food Chem* 44:3620–4.

Pickenhagen W. 1999. Flavor chemistry. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry. Thirty Years of Progress*. New York: Kluwer Academic, pp. 75–87.

Pickenhagen W, Velluz A, Passerat JP, Ohloff G. 1981. Estimation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) in cultivated and wild strawberries, pineapples and mangoes. *J Sci Food Agric* 32:1132–4.

Pittet AO, Rittersbacker P, Moralidhara R. 1970. Flavor properties of compounds related to maltol and isomaltol. *J Agric Food Chem* 18(5):929–33.

Re L, Maurer G, Ohloff GA. 1973. A simple synthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), an important aroma component of pineapple and strawberry. *Helv Chim Acta* 56:1882–94.

Rodin JO, Himel CM, Silverstein RM, Leeper RW. 1965. Volatile flavor and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. *J Food Sci* 30:280–5.

Schulbach KF, Portier KM, Sims CA. 2007. Evaluation of overall acceptability of fresh pineapple using the regression tree approach. *J Food Qual* 30:993–1008.

Silverstein RM, Rodin JO, Himel CM, Leeper W. 1965. Volatile flavor and aroma components of pineapple II. Isolation and identification of chavicol and γ-caprolactone. *J Food Sci* 30:668–72.

Sinuco DC, Morales AL, Duque C. 2004. Free and glycosidically bound volatile components from pineapple (*Ananas comosus* L.) var. Perolera. *Rev. Colomb. Quím.* 33:47–56.

Spanier AM, Flores M, James C, Lasater J, Lloyd S, Miller JA. 1998. Fresh-cut pineapple (*Ananas* sp.) flavor. Effect of storage. In Contis ET, Ho CT, Mussinan CJ, Parliament TH, Shahidi F, Spanier AM (eds.), *Food Flavors: Formation, Analysis and Packaging Influence and Developments in Food Science*. Amsterdam: Elsevier, pp. 331–43.

Takeoka GR, Buttery RG, Teranishi R, Flath RA, Gütert M. 1991. Identification of additional pineapple volatiles. *J Agric Food Chem* 39:1848–51.

Tokitomo Y, Steinhaus M, Büttner A, Schieberle P. 2005. Odor-active constituents in fresh pineapple (*Ananas comosus* [L.] Merr.) by quantitative and sensory evaluation. *Biosci Biotechnol Biochem* 69:1323–30.

Tonsbeek CHT, Plancken AJ, Weerdhof T. 1968. Components contributing to beef flavor. Isolation of 4-hydroxy-5-methyl-3(2h)-furanone and its 2,5-dimethyl homolog from beef broth. *J Agric Food Chem* 16:1016–21.

Umano K, Hagi Y, Nakahara K, Shoji A, Shibamoto T. 1992. Volatile constituents of green and ripened pineapple (*Ananas comosus* (L.) Merr.). *J Agric Food Chem* 40:599–603.

Wu P, Kuo MC, Hartman TG, Rosen RT, Ho CT. 1991. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *J Agric Food Chem* 39:170–2.

CHAPTER 22

The Flavor of Plums

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THE IMPORTANCE OF PLUM GROWING WORLDWIDE

For fresh and dry consumption, or for use as a flavoring, beverage, or condiment to accompany other foods, plums are widely grown throughout the temperate regions of the world. Of the pome and stone fruit crops, plums are second only to apples in terms of orchard area, with over 2.1 million hectares planted worldwide (Table 22.1). The diversity of habitats where plum species grow naturally have probably assisted in the breeding and development of new varieties adapted to specific localities and environmental conditions. Hence, plums are now cultivated throughout the temperate zones of both the northern and southern hemispheres.

Worldwide plum production has increased dramatically during the last decade, with a majority of the increase coming from new Asian orchards. Specifically, China is the current world's leader in plum production and is responsible for nearly half of the world's harvested tonnage. The total harvested area of Asian orchards has increased approximately 60% since 1996, while maintaining the same yield efficiency (Table 22.2). Harvested ton per hectare has similarly remained constant in African orchards during the same time period, while increasing harvested area by approximately 20%. European plum orchard area has decreased significantly since 1996; however, the level of plum production per hectare has risen sharply (5.51 MT/ha in 1996 vs. 7.85 MT/ha in 2006). Romania and Serbia rank second and third in world plum production with 2006 harvests of 598,753 and 556,227 MT, respectively. Throughout the world, there are 13 nations producing over 100,000 MT of plums annually. The highest overall plum yields per hectare are now achieved in North and South American orchards, but harvested orchard area in these regions have declined during the last decade.

Plums vary widely in their flavor and aroma characteristics, and many plums have been developed to be used for specific purposes. Large volumes of plums are used throughout Central and Eastern Europe in the production of the popular "slivovitz"

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

TABLE 22.1. Pome and Stone Fruit Crops, Worldwide Production, 2006 (FAOSTAT Data 2008)

Crop	Harvested Area (ha)
Apple	4,786,080
Plum	**2,154,196**
Pear	1,669,743
Peach	1,448,047
Apricot	474,942
Sweet cherry	341,436
Sour cherry	214,287
Quince	63,106

TABLE 22.2. Plum Production and Harvested Orchard Area in the World's Growing Regions, 1996 and 2006 (FAOSTAT Data 2008)

World Region	Production (MT × 1000)		Area Harvested (ha × 1000)		MT/ha	
	1996	2006	1996	2006	1996	2006
Africa	210	244	31.6	37.8	6.67	6.46
Americas	1201	920	108.0	95.0	11.13	9.70
Asia	3393	5505	1037.0	1670.0	3.27	3.29
Europe	3392	2727	615.0	347.0	5.51	7.85
Oceania	31	34	4.6	3.8	6.87	8.76

or plum brandy (Satora and Tuszyński 2008). Dried plums or "prunes" are produced from specific varieties with a high sugar content to ensure consumer palatability. Elevated sorbitol content is also of great importance in prune-type plums as a preventative of excessive caramelization (product darkening) during the drying process (Cinquanta et al. 2002). The fresh plum industry in California orchards has relied on "Japanese" type plums that ripen from May through the end of September, with diverse skin and flesh colors. Dozens of varieties are currently grown for both domestic and export marketing. Postharvest treatments have been developed to increase the length of the effective marketing periods by maintaining firmness, juiciness, and flavor (Mitchell 1987; Salvador et al. 2003; Valero et al. 2002). Both cultivated and native/wild plums are collected and preserved in a wide variety of forms (chutney, compote, glacé, jam, pickling, etc.) for direct consumption or served as an accompaniment to enhance the culinary experience.

THE IMPORTANT PLUM SPECIES AND THEIR CHARACTERISTICS

Plums are a large and botanically diverse group of stone fruits with over 2000 named varieties being selected from over a dozen different *Prunus* species. Currently, cultivated varieties are also diverse in their climactic preferences, as specific varieties have adapted to various regions throughout the entire temperate fruit-growing zones. Horticultural and botanical characteristics vary greatly between the various plum species. Numerous botanical characteristics are useful in distinguishing between various species, among them fruit color, size, and flavor of the fruit.

Based on original geographic distributions of plant habitats, plum germplasm can be divided into three broad groups of species: (1) European–Asian, (2) North American, and (3) Oriental. These groups can be further subdivided, based on various pomological characters. Morphological similarities of many plum species and interspecific hybridization between species in overlapping habitats have perplexed both traditional taxonomists and molecular biologists for over a century (Hedrick et al. 1911; Rohrer et al. 2004; Shaw and Small 2005; Waugh 1901). From the numerous plum species found throughout the world's temperate zones, Table 22.3 lists only those of economic importance through marketing or use of their fruit.

TABLE 22.3. Grouping of Cultivated Plum Species Based on Geographic Distribution

Geographic Grouping	*Prunus* Species	Pomological Grouping	Representative Varieties
European–Asian	*P. domestica* L.	Green Gages	Imperial Gage, Jefferson, Reine Claude
		Prunes	Agen, Hungarian, Italian, Sugar
		Yellow Eggs	Golden Drop, Monroe, Yellow Egg
		Imperatrices	Arch Duke, Englebert, Monarch
		Lombards	Bradshaw, Compote, Pond, Victoria
	P. domestica var. *insititia* Bailey	Damsons	Crittenden, Grand Duke, Shropshire
		Bullaces	Black Bullace, Royal Bullace, White Bullace
		Mirabelles	Drap d'Or, Late Mirabelle
		St. Julians	Saint Julian
	P. cerasifera Ehrh.	Myrobalans	Lindsayae, Marianna, Nigra
American		Common wild plums	
	P. americana Marsh.		Cherokee, DeSoto, Golden Queen
	P. nigra Ait.		Aitkin, Crimson, Oxford
		Wild Goose (Chickasaw) plums	
	Prunus hortulana Bailey		Cumberland, Golden Beauty, Wayland
	Prunus angustifolia Marsh.		Caddo Chief, Ogeeche
	Prunus munsoniana Wight and Hedr.		Jewell, Osage, Pottawattamie, Texas Belle
		Beach plums	
	P. maritime Marsh.		Bassett's American
		Pacific Coast plums	
	P. subcordata Benth.		Sisson
Oriental		Japanese plums	
	P. salicina Lindl.		Blackamber, Friar, Satsuma
		Apricot plums	
	P. simonii Carr.		Climax, Wickson (hybrids with *P. salicina*)

European–Asian Plums

The European or common garden plum, *Prunus domestica* L., is well-known for its diversity of fruit sizes and flavors and is by far the most desirable of the plum species in terms of fruit quality. The wide variety of fruit colors and flavors, as well as a lengthy fruit ripening season, yields abundant culinary uses. Prunes are produced exclusively from specific European–Asian plum varieties.

Many *P. domestica* fruits are somewhat oblong in shape, or with an elongated neck at the point of fruit attachment. Growth habit is usually an upright tree form, and new vegetative shoots are covered with a fine pubescence. A more dwarf and compact growth habit as well as a more ovate leaf shape distinguish *P. domestica* var. *insititia* from various forms of *P. domestica*.

Besides strict botanical distinctions between *P. domestica* and *P. domestica* var. *insititia*, each group has been further subdivided into various pomological classes. While some of the representative varieties listed in Table 22.1 are currently in commercial production, others have faded from popularity. The pomological classes used by growers and nurserymen were perhaps more important in a previous time as compared with plum growers today. Nonetheless, certain classes still have a special meaning for producers or in commerce. For canned plums, still a popular product in the United Kingdom, the Green Gage, Yellow Egg, and Lombard plum classes are most recognized and preferred by consumers for their high fruit quality (Luh et al. 1986). The prunes of *P. domestica* are another specific pomological class with widespread consumer recognition today. Their high sugar content and rich flavor profile ensures consumer acceptance after the drying process. Among the dried fruits available to consumers, prunes are second only to raisins in production volume (Anon. 2008).

Unlike the other European–Asian plums, *Prunus cerasifera* accessions are generally lacking in fruit quality. This species is more commonly known as the cherry or Myrobalan plum and is native to the Caucasus Mountains. The fruits are typically cherry like in form, of 1–2 cm in diameter, and with watery, soft flesh. The fruits are generally somewhat sweet and subacid but with a poor flavor. Fruits from most accessions of *cerasifera* are used for processing into juice, jams, or compotes.

North American Plums

Breeding of the North American plum species began in the mid-1800s in an effort to expand plum production into those areas where *P. domestica* was not well adapted. The wide diversity of native North American plums has led to hundreds of varieties and interspecies hybrids. Perhaps the most frequently used North American plum was *Prunus americana*, owing to its general cold hardiness and late bloom, as compared with domesticated plums (Hedrick et al. 1911). Ranging from Eastern Montana and Wyoming in the west to Rhode Island in the east, and from the Florida panhandle through the Dakotas to the Canadian border in the north, this species is certainly the most dominant in terms of its geographic range and density in North America. In its native state, *P. americana* produces somewhat low-topped thorny thickets that flourish along riverbanks. Prolific fruit set is very typical, and fruit thinning can provide a dramatic increase in fruit size. Fruits are predominantly yellow or reddish yellow, typically clingstone in nature and with a characteristically

astringent skin. Fruit quality is said to be generally good, when the astringent skin is removed from the flesh. Subjective evaluations of plum flavor and aroma early in the 20th century determined that *P. americana* plums generally surpassed both Japanese and European plums in flavor "bouquet" (Waugh 1901).

The Wild Goose plums are native to more southerly North America as compared with *P. americana* and *Prunus nigra*. Numerous varieties were created and named from this group, and its importance in North American plum breeding is second only to that of *P. americana*. Fruit skin is typically much lighter in color and much thinner as compared with *P. americana*.

P. nigra is of much lesser horticultural importance as compared with *P. americana*. Cold hardiness is the single most important character obtained from this species.

The Beach Plum, *Prunus maritima*, so named for its native seaside distribution on the upper east coast of the United States, has not been a major contributor to plum breeding in North America. Its specific geographic distribution and small fruit size (1.0–1.5 cm) have provided little reason for its pursuit as a progenitor in plum breeding programs. "Bassett's American" is said to be the single variety selected from this species.

North America's westernmost plum species, *subcordata*, was a very important food source for settlers of California and Oregon during western migrations in the mid-1800s. Typically a small tree or bush, the species can rise to erect specimens of 7–10 m in height. The fruits are comparatively large in size for native plums, and skin color is predominantly bright red. Of all the North American native plum species, *Prunus subcordata* most resembles European plums in fruit quality characteristics. Small-scale commercial entrepreneurs harvest this native plum in Northern California and Southern Oregon to produce wine, juice, and jam for boutique markets.

Oriental Plums

Through numerous hybridizations, breeding programs in the early 20th century combined the qualities of both Oriental plum species, *Prunus salicina* and *Prunus simonii*, with those of *P. subcordata* and *P. americana*. The diploid nature of the Oriental plums made interhybridization possible with the North American plums, whereas European–Asian plums could not be used due to their hexaploid nature. Burbank's variety "Santa Rosa" is still commercially popular today and combines the firmness, size, and color of Oriental plums with the aromatic notes from *P. americana* (Howard 1945). Since Luther Burbank's early efforts in developing plum culture in California, hundreds of varieties have been bred and grown successfully. The vast majority of currently grown Oriental varieties are undoubtedly multi-species hybrids that have stabilized genetically and can be further interhybridized in the development of newer varieties. Fruit size and diversity in fruit color are beneficial characteristics attributed to *P. salicina*. Fruit firmness and distinct flavor are characteristics associated with *P. simonii*'s contribution to currently grown Oriental plums.

The Oriental plum species generally exhibit a more spreading growth habit as compared with trees of European–Asian origin. Compared with the elongated fruit forms of *P. domestica*, Oriental plums have a more rounded fruit shape. *P. simonii* is characterized by a flattened pistil end of its fruit. Both *P. salicina* and *P. simonii*

have large and juicy fruits as compared with many of the European–Asian plums and the vast majority of North American plums.

THE FLAVOR OF PLUMS

Stone fruit quality is a complex combination of sensory attributes and proportions that provide value in terms of human consumption. While each consumer judges fruit quality to different standards, the general perception of quality relates to fruits that look good, are firm fleshed, and offer ample flavor and nutritive value (Crisosto 1994).

In contrast to apricots and other types of *Prunus*, less research has been accomplished on the aroma and flavor of plums. As previously stated, cultivars and plum species differ in appearance, aroma and flavor. The sensory properties are very important to consumers and can be a determining factor in the development and release of new fruit cultivars. The first reported study of plum volatiles was carried out by Forrey and Flath (1974) with *P. salicina*. After this first study, some of the most significant works on flavor and aroma of plums can be found in the studies of Ismail and others (1980, 1981) and Etievant and others (1986) in *P. domestica*, Gómez and Ledbetter (1993) and Gómez and others (1993) in *P. salicina*, Gómez and Ledbetter (1994) in *P. simonii*, and Horvat and others (1992) in *P. salicina* × *P. americana*.

MAIN COMPOUNDS IDENTIFIED IN THE DIFFERENT PLUM SPECIES

A list of the identified compounds in the different species is shown in Table 22.4. The volatile compounds identified in plums include carbonyl compounds, esters, hydrocarbons, lactones, and other miscellaneous compounds. When compared with the aromatic profile of other *Prunus* such as apricot, the results indicated that plums

TABLE 22.4. Compounds Identified in the Different Plum Species

Constituent	*P. salicina*	*P. simonii*	*P. domestica*	*P. salicina* × *P. americana*	Reference
Ketones					
Butanone			x		9
3-Octanone			x		9
Isophorone	x	x			1, 2
Acetophenone	x		x		1, 2, 8
α-Ionone		x			2
Geranylacetone	x	x			1, 2
β-Ionone	x	x		x	1, 2, 3
3-Hydroxy-2-butanone	x		x		4, 7, 8, 9
β-Damascenone				x	3
1-Phenyl acetone	x				6
Furfuryl methyl ketone			x		8

TABLE 22.4. ***Continued***

Constituent	*P. salicina*	*P. simonii*	*P. domestica*	*P. salicina* × *P. americana*	Reference
Alcohols					
Methanol	x				4
Ethanol	x				4
Propanol	x				4
2-Methyl-3-buten-1-ol	x				4
2-Methyl-propanol	x		x		4, 7
Butanol			x		7, 8, 9
3-Methyl-1-butanol	x		x		4, 9
2-Methyl-1-butanol	x		x		4
Butane-2,3-diol			x		8
Pentanol	x		x		4, 8
Penten-2-ol	x				4
4-Methylpentanol		x			2
(*Z*)-3-Hexen-1-ol	x	x		x	1, 2, 3, 4
(*E*)-2-Hexen-1-ol	x	x			1, 2, 4
Hexanol	x	x	x	x	1, 2, 3, 4, 7, 9, 10
2-Ethylhexanol	x				1, 2, 6
Heptanol	x		x		4, 9
Octanol	x	x	x		1, 2, 4, 9
Linalool	x	x	x	x	1, 2, 3, 4, 6, 7
Nonanol		x	x		2, 9
α-Terpineol	x				1, 2, 4
Geraniol		x			2
4-Terpineol			x		9
Nerol	x				1
Nerolidol	X				2
Benzyl alcohol	x		x		4, 8
2-Phenylethanol	x		x		4, 9
Eugenol				x	3
Methyl eugenol				x	3
Aldehydes					
Acetaldehyde	x				4
2-Methylpropanal			x		9
2-Methylbutanal			x		9
3-Methylbutanal			x		9
2-Methyl-2-pentenal	x				4
Hexanal	x	x	x	x	1, 2, 3, 7, 8, 9, 10
(*E*)-2-Hexenal	x	x		x	1, 2, 3, 9, 10
Heptanal	x	x	x		1, 2, 8
2-Heptenal	x	x			1, 2
(*E*,*E*)-2,4-Heptadienal	x				1, 2
Phenylacetaldehyde	x	x	x		1, 2, 9, 10
Octanal			x		8
Nonanal	x	x	x	x	1, 2, 3, 5, 6, 7, 8, 10

TABLE 22.4. ***Continued***

Constituent	*P. salicina*	*P. simonii*	*P. domestica*	*P. salicina* × *P. americana*	Reference
Decanal	x				6
β-Cyclocitral		x			2
(*E*,*Z*)-2,4-Decadienal		x			2
(*E*,*E*)-2,4-Decadienal		x			2
Benzaldehyde	x		x	x	3, 4, 7, 8, 10
Furfural			x		8, 10
Methylfurfural			x		8, 10
Esters					
Ethyl acetate	x		x		4, 9
Propyl acetate	x				4
2-Methyl-1-propyl acetate	x				4
Butyl acetate	x	x	x	x	1, 2, 3, 4, 9
3-Methylbutyl acetate	x	x	x	x	2, 3, 7
Pentyl acetate	x		x	x	3, 4, 9
(*Z*)-3-Hexenyl acetate	x		x		1, 2, 4, 9
Hexyl acetate	x	x	x	x	1, 2, 3, 4, 6, 9
(*E*)-2-Hexenyl acetate	x		x	x	1, 2, 3, 9
Heptyl acetate	x	x	x		2, 4, 7
Octyl acetate	x	x	x		2, 4, 8
Nonyl acetate		x			2
Decyl acetate				x	3
Dodecyl acetate		x			2
Benzyl acetate	x		x		4, 8
Ethyl phenyl acetate	x		x		4, 7
Geranyl acetate		x			2
Bornyl acetate	x	x			2
Butyl propanoate		x	x		2, 9
Pentyl propanoate			x		9
Hexyl propanoate			x		9
3-Hexenyl propanoate					
Pentyl 2-methylpropanoate			x		9
Methyl butanoate			x		9
Ethyl butanoate	x		x	x	3, 4, 7, 9
Propyl butanoate			x		9
Butyl butanoate	x		x	x	3, 4, 9
2-Methylpropyl butanoate		x	x		2, 9
Methylbutyl butanoate			x		7
Pentyl butanoate			x		9
Hexyl butanoate			x		7, 9
(*E*)-2-Hexenyl butanoate	x	x	x		2, 6, 9
Heptyl butanoate	x		x		6, 9
Octyl butanoate			x		9
Ethyl 2-methylbutanoate			x		9
Ethyl 3-methylbutanoate			x		9
Butyl 2-methylbutanoate			x		9
Hexyl 2-methylbutanoate			x		9
Butyl pentanoate			x		9

TABLE 22.4. ***Continued***

Constituent	*P. salicina*	*P. simonii*	*P. domestica*	*P. salicina* × *P. americana*	Reference
Hexyl pentanoate			x		7
Methyl hexanoate			x		9
Ethyl hexanoate	x		x		4, 7, 9
Propyl hexanoate			x		7
Methyl butyl hexanoate			x		7
Butyl hexanoate	x		x	x	6, 3, 7
Pentyl hexanoate		x			2
Hexyl hexanoate			x	x	3, 7, 9
(*Z*)-3-Hexenyl hexanoate	x	x			1, 2
Ethyl heptanoate			x		9
Methyl octanoate			x		9
Ethyl octanoate	x		x	x	1, 2, 3, 4, 7, 9
Propyl octanoate				x	3
Butyl octanoate			x	x	3, 7, 9
Hexyl octanoate			x	x	3, 9
Octyl octanoate				x	3
Ethyl nonanoate			x		9
Methyl decanoate			x		9
Ethyl decanoate	x		x		4, 7, 9
Ethyl anysate	x				4
Methyl salicylate			x		8
Methyl cinnamate			x		7
Ethyl cinnamate			x		9
Lactones					
γ-Hexalactone	x			x	3, 4
γ-Octalactone	x	x		x	1, 2, 3, 4
γ-Nonalactone			x	x	3, 7
δ-Decalactone				x	3, 6
γ-Decalactone	x	x	x	x	1, 2, 3, 4, 6, 7, 8
γ-Undecalactone		x			2
γ-Dodecalactone	x	x		x	1, 2, 3
Hydrocarbons					
1,4-Dimethyl benzene		x			2
1,2,3-Trimethylbenzene	x				2
Tridecane			x		9
Tetradecane			x		9
Pentadecane			x		9
Hexadecane			x		9
Heptadecane			x		9
2-Heptadecene			x		9
Limonene	x	x			2, 6
α-Pinene			x		9
β-Pinene			x		9
Carvone			x		8

1, Gómez and others (1993); 2, Gómez and Ledbetter (1994); 3, Horvat and others (1992); 4, Forrey and Flath (1974); 5, Ismail and others (1977); 6, Gómez and Ledbetter (1993); 7, Ismail and others (1981); 8, Ismail and others (1980); 9, Etievant and others (1986); 10, Sabarez and others (2000).

presented lower numbers of volatile compounds. In the extensive study of Etievant and others (1986) with *P. domestica*, 130 compounds were detected, including 62 esters, 14 hydrocarbons, 11 aldehyde, 10 alcohols, 8 lactones, and 8 ketones, and in one study with *P. salicina* and *P. simonii* (Gómez and Ledbetter 1994), a total of 12 alcohols, 6 hydrocarbons, 10 aldehydes, 7 ketones, 21 esters, and 4 lactones were identified in both species.

Aldehydes and alcohols of six-carbon atoms were identified in all species. These compounds were hexanal, 2-hexenal, hexanol, 2-hexenol, and 3-hexenol. The presence of these compounds is probably due to lipoxygenase activity, actions initialized by the disruption of the fruit tissues when it was blended (Frankel 1982), since most of the studies include a homogenization step, except when the headspace aroma is studied. Accordingly, most of these compounds were not identified in the study of plum headspace volatile compounds by Gómez and Ledbetter (1993). These C6-compounds seem to be important to plum aroma (Ismail et al. 1981) and contribute to the green note of the fruit (Guichard et al. 1990).

Other carbonyl compounds have been identified in plums. The presence of nonanal is significant as a characteristic constituent of skin waxes of plums having a fragrant, woody-like aroma (Ismail et al. 1981; Williams and Ismail 1981). The cuticular wax layer removed from whole plums had a creamy, fragrant wood-like odor. These characteristics were considered a significant contribution relative to the aroma of some plums. Nonanal has been identified in the volatiles isolated from many fruits and vegetables, but it has only been reported to be of significance in the aroma of lemon oil (Ikeda et al. 1962), cauliflower, and broccoli (Buttery et al. 1976), although it has also been isolated from peach leaves (Kemp et al. 1971).

Among the ketones, geranylacetone, detected in *P. salicina* and *P. simonii*, can be regarded as a norterpenoid arising from isoprenoid degradation (Takeoka et al. 1990). Another ketone found in some samples was isophorone, identified previously in kiwi flowers (Tatsuka et al. 1990).

Esters are the main compounds responsible for the fruity aroma. Among the esters identified in plums, butyl propanoate and 3-methyl-1-butyl acetate have a strong banana aroma, and 2-methylpropyl butanoate and octyl acetate have a pleasant fruity aroma. These esters, together with hexanal and some lactones, contribute to the plum-like aroma and the characteristic fruity flavor of some plum cultivars.

Among alcohols, some terpenols have been identified. The concentration of one of the most significant terpenols, linalool, was in general, present at lower concentration than in other *Prunus* such as apricots (Gómez et al. 1993). This was also true for the other terpenoids, as we could not detect geranial, or pseudoionone, compounds normally appearing in other *Prunus* species (Tang and Jennings 1968).

Hydrocarbons are more prevalent, quantitatively, than in other *Prunus* such as the apricot. This was probably related to the composition of the skin, which is very rich in waxes. One of the identified compounds, limonene, besides its sensory significance, has been described as an important odor attractant for some insects (Leskey et al. 2001).

A large number of lactones have been identified. γ-Octalactone, decalactone, and γ-dodecalactone were the most important quantitatively in plums.

MAJOR AROMA COMPOUNDS IN DIFFERENT PLUM SPECIES

P. domestica

On the basis of the studies of Williams and Ismail (1981) with *P. domestica*, linalool and ethyl butanoate are very important compounds in the aroma of European plums. These authors also determined that hexanal, when diluted, has a plum-like aroma. Ismail and others (1981) also considered γ-decalactone as well as ethyl nonanoate and benzaldehyde to be associated with the plum aroma of this species. When headspace analysis was employed, all extracts were dominated by hexanol and nonanol, together with 3-methylbutanol and linalool. These authors stated that the regions of the chromatogram containing benzaldehyde, linalool, methyl cinnamate, and γ-decalactone were associated with fresh plum aroma.

The same authors studied the aromatic profile of *P. domestica* canned fruits, showing that the headspace aroma of these plums was dominated by carbonyls, benzaldehyde, and linalool, whereas methyl cinnamate, γ-octalactone, and γ-decalactone were not detected. The absence of these compounds from headspace analyses may account for the differences in the aroma of canned plums as compared with freshly harvested fruit.

The studies of Etievant and others (1986) showed that hexyl, butyl, and ethyl esters were very abundant in the headspace of fresh mirabelle plums (*P. domestica ss. insitia*), whereas they could not detect linalool, α-terpineol, or geraniol. Esters accounted for 88% of total volatiles, the major being hexyl esters. They stated that the absence of linalool, damascenone, and methyl cinnamate (claimed to be main contributors to *P. domestica* aroma) might explain the differences in the aroma of mirabelle plums.

Dried European plums, known as prunes, are consumed in large amounts due, among other things, to their health benefits. During drying, there are a number of chemical reactions that may influence the aroma of the final product. Sabarez and others (2000) studied the volatile changes during dehydration of d'Agen prunes. As in previous studies with fresh European plums, the determined main volatiles were C6-compounds (especially when the fruit was blended) and nonanal and phenylacetaldehyde. After drying, C6-compounds disappeared, and the two aldehydes were retained. Three major new compounds were generated and indentified in the dry product: benzaldehyde (probably due to the degradation of its glucoside precursor, amygdalin, during heating as stated by Williams and Ismail 1981), 2-furancarboxyaldehyde (from degradation of sugars alone or in contribution with amino acids), and ethyl cinnamate.

P. salicina

The first study on *P. salicina* indicated that esters and lactones were the main volatile constituents in this fruit (Forrey and Flath 1974). In this primary study, it was demonstrated that acetate esters predominated in the Santa Rosa variety, but appreciable quantities of the higher γ-lactones appeared as well. Many carbonyl compounds and related esters were also identified by Gómez and others (1993), with γ-decalactone and γ-dodecalactone being the most important lactones found in the study.

The relative contribution of various constituents to the blended plum aroma was determined in the study of Gómez and others (1993) by calculating the number of odor units (Uo). The odor unit was defined by Guadagni and others (1966) as the concentration of the compound divided by its odor threshold. This value gives an indication of the significance each volatile contributes to the plum aroma. The compounds with the highest odor unit value were hexanal, described, when diluted as a plum-like aroma (Williams and Ismail 1981); nonanal, a characteristic constituent of plum skin waxes, with a fragrant woody-like aroma; (*E*,*E*)-2,4-decadienal; and the lactones γ-decalactone and γ-dodecalactone, which are described as being responsible for the fruity, peach, and coconut background aromas of the fruit (Takeoka et al. 1990).

As stated previously, hybridizations between Japanese and Native American plums at many U.S. locations have led to numerous cultivars being adapted to a wide range of environmental conditions. The studies of Horvat and others (1992) with hybrids of *P. salicina* and *P. americana* demonstrated that for most of the studied cultivars, (*E*)-2-hexenal, butyl acetate, butyl butanoate, and γ-dodecalactone were the major constituents. The major lactone reported in this study was γ-dodecalactone, whereas other lactones were found in trace amounts. The distribution of plum lactones differed from those of peaches and other plums where γ-decalactone and δ-decalactone in peaches had been found in higher concentrations than γ-dodecalactone (Horvat et al. 1990).

P. simonii

This plum presented a very interesting aromatic profile, with a high number of identified compounds. In a study where its profile was compared with *P. salicina* (Gómez and Ledbetter 1994), some compounds were only found in *P. simonii*. For example, among ketones, β-ionone appeared only in the profile of *P. simonii* as well as the alcohols, octanol, nonanol, and geraniol. The number of esters was both qualitatively and quantitatively higher in *P. simonii*. Twelve of the 21 identified esters appeared only in *P. simonii*. Also, the concentration of lactones, important for the fruity aroma (Guichard et al. 1990), was greater in *P. simonii*.

Some of the most characteristic components of plum aroma showed a significantly higher concentration in *P. simonii* samples. These compounds were hexenal, hexanal, and hexanol; the esters butyl acetate, hexylacetate, and (*Z*)-3-hexenyl acetate; as well as γ-decalactone and γ-dodecalactone. These esters and lactones, together with hexanal, probably contribute to the plum-like aroma and the characteristic fruity flavor of *P. simonii*.

In the study of Gómez and Ledbetter (1994), it can be seen that the most important compounds, as determined by the odor units, are β-ionone (despite its low concentration, this compound has a very low odor threshold) and nonanal, as well as hexyl acetate. This ester is also present at a high concentration in apples (Willaert et al. 1983) and is responsible for the characteristic apple-like aroma found in this species.

P. simonii has demonstrated itself to be a useful parent in plum breeding programs because of its unique aromatic components and quantitative presence. Of the 60 quantified compounds in this study, 23 are only present in *P. simonii*, showing significantly more esters than other plum species; taken collectively, they probably contribute to the intense fruity aroma of *P. simonii*.

THE FLAVOR OF PLUMCOTS

The Agricultural Research Service's Horticultural Crops Research Laboratory in Fresno, CA, began the development of plum–apricot hybrids in 1989. With these hybrids, it was expected to provide consumers with new fruit types. The hybrids are commonly known as "plumcots" and vary widely with regard to skin and flesh color, fruit size and shape, ripening period, and flesh texture and flavor. The main breeding interest was focused on those types having flesh texture characteristics similar to that of Japanese plum and aroma/flavor characteristics resembling those of apricot.

The studies of Gómez and Ledbetter (1993, 1997) and Gómez and others (1993) demonstrated that almost all of the compounds identified in apricots and plums were also found in plumcots (Table 22.5). There were also some unique compounds only found in one or more of the hybrids, and not in apricot or plum: ethyl benzoate; ethyl salicylate, and β-phellandrene. Ethyl salicylate has been described as having a pleasant odor, whereas ethyl benzoate has been identified also in peaches (Horvat et al. 1990). Those esters are described as having a sweet and fruity aroma.

Among plumcots, the compounds having the higher odor units were those characteristic of apricot such as lactones, linalool, and (*E*,*E*)-2,4-decadienal and those characteristic of plums such as hexyl acetate and nonanal. Besides these compounds, the contribution of the C6-compounds to the plumcot aroma was higher than in either apricot or plum.

From the results obtained in this study, it appears that the ability to produce aromatic volatiles may be paternally transmitted as discrete characteristics. A second point with regard to the structural genes responsible for the transmission of specific aromatic constituents is that compounds that are quantitatively important compounds in the parents of plum × apricot hybridizations may also be produced in quantitatively high levels in the progeny. Geranylacetone was identified in all of the parents of this study but was quantitatively much higher in one of the hybrids. Similarly, geranylacetone was also identified in all plumcot progeny, but at higher levels in progeny for which the elevated geranylacetone apricot parent had been used. A similar case can be made with γ-decalactone and γ-dodecalactone. Another compound, nonanal, contributed significantly to Blackamber (*P. salicina*) aroma. Those plumcots having Blackamber as a parent had higher odor unit values for nonanal than those plumcots for which another plum (*P. salicina* cv. Friar) was used.

In the last decade, the world has seen dramatic increases in plum production, with many breeding programs developing new plum varieties adapted to wide-ranging environmental conditions. Varietal development for the last several decades has focused primarily on the producer, with very little attention being paid to specific organoleptic characteristics. Currently, there is a renewed focus on consumer acceptance in plum improvement, with flavor and fruit aroma being important criteria in new variety development. Plums exhibit a great deal of genetic diversity relative to their natural habitats, as well as both in the kinds and amounts of compounds responsible for characteristic plum flavor. However, specific research on plum flavor and the responsible aromatic compounds must progress to the level of flavor research achieved in other fruits and vegetables. Collaborations between flavor chemists and plum breeders will certainly assist in the development of new high flavor plum varieties for the future. With the wealth of plum germplasm diversity available to the breeder and modern analytic tools available to the

TABLE 22.5. Aroma Compounds Detected in Plumcots (Gómez et al. 1993)

Ketones	Alcohols	Aldehydes	Esters	Lactones	Hydrocarbons
3-Hexanone	1-Methylcyclopentanol	Hexanal	Butyl acetate	γ-Octalactone	2,3-Dimethyl-2-pentene
2-Hexanone	(*Z*)-3-hexen-l-o1	(*E*)-2-hexenal	3-Methylbutyl acetate	γ-Nonalactone	1,4-Dimethylbenzene
2,2,6-Trimethylcyclohexanone	(*E*)-2-hexen-l-o1	Heptanal	(*Z*)-3-hexenyl acetate	γ-Decalactone	1,3,5-Cyclooctatriene
Isophorone	Hexanol	(*E*)-2-heptenal	Hexyl acetate	δ-Decalactone	2,4-Dimethyl-2-decene
Acetophenone	2-Ethylhexanol	(*E*,*E*)-2,4-heptadienal	(*E*)-2-hexenyl acetate	γ-Undecalactone	α-Phellandrene
2-Ethylcyclohexanone	Linalool	Phenylacetaldehyde	Ethyl benzoate	γ-Dodecalactone	α-Pinene
Dihydro-β-ionone	2,6-Dimethylcyclohexanol	Citral methyl acetal	(*Z*)-3-hexenyl butanoate		1,2,3-Trimethylbenzene
α-Ionone	4-Terpinenol	Nonanal	(*E*)-2-hexenyl butanoate		1,3,5-Trimethylbenzene
Geranylacetone	α-Terpineol	2-Undecenal	Methyl salicylate		2,2,8-Trimethyldecane
2,6-Bis(1,1-dimethylethyl-2,5-cyclohexadiene-l,4-dione	Geraniol	β-Cyclocitral	Ethyl octanoate		*m*-Cymene
β-Ionone	Nerol	(*E*,*E*)-2,4-decadienal	Octyl acetate		Limonene
Pseudoionone	Nerolidol	(*E*,*Z*)-2,4-decadienal	Ethyl phenylacetate		2,5-Dimethyl-2-undecene
(*E*,*E*)-Pseudoionone	Farnesol		Ethyl salicylate		2,2,5,5-Tetramethylhexane
6,10,14-Trimethyl-2-pentadecanone	2,6-Bis(1,1-dimethylethyl)-4-ethylphenol		Bornyl acetate		3,8-Dimethylundecane
6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one			2-Ethyl-3-hydroxyhexyl-2-methylpropanoate		Naphthalene
6-Methyl-5-hepten-2-one			Geranyl acetate		2-Oxo-l-methyl-3-isopropylpyrazine
			(*Z*)-3-hexenyl hexanoate		1-Methyl-4-(methylthio) benzene
			(*E*)-2-hexenyl hexanoate		Megastigma-4,6(*Z*),8(*Z*)-triene
			Ethyl decanoate		Megastigma-4,6(*E*),8(*E*)-triene
			Methyl-l0-methyldodecanoate		1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene
			Ethyl pentadecanoate		Megastigma-4,6(*E*),8(*Z*)-triene
					Megastigma-4,6(*Z*),8(*E*)-triene
					2-Ethyl-l,4-dimethylbenzene
					Dihydroactinidiolide

flavor chemist, future plum varieties might well possess both the horticultural traits important to the producer as well as a complex and pleasing flavor bouquet desired by consumers.

REFERENCES

Anon. 2008. Noncitrus Fruits and Nuts 2007 Preliminary Summary. USDA National Agricultural Statistics Service. January 2008. http://usda.mannlib.cornell.edu/usda/nass/NoncFruiNu/2000s/2008/NoncFruiNu-01-23-2008.pdf.

Buttery R, Gaudagni D, Ling D, Seifert R, Lipton W. 1976. Additional volatile components of cabbage, broccoli, and cauliflower. *J Agric Food Chem* 14:829–32.

Cinquanta L, Di Matteo M, Esti M. 2002. Physical pre-treatment of plums (*Prunus domestica*). Part 2. Effect on the quality characteristics of different prune cultivars. *Food Chem* 79:233–8.

Crisosto C. 1994. Stone fruit maturity indices: A descriptive review. *Postharvest News Inf* 5:65–8.

Etievant P, Guichard E, Issanchou S. 1986. The flavour components of Mirabelle plums. Examination of the aroma constituents of fresh fruits: Variation of the headspace composition induced by deep-freezing and thawing. *Sci Aliments* 6:417–32.

FAOSTAT Data. 2008. Agriculture Production. http://faostat.fao.org/faostat/ (accessed April 2008).

Forrey R, Flath R. 1974. Volatile components of *Prunus salicina* var. Santa Rosa. *J Agric Food Chem* 22:496–8.

Frankel E. 1982. Volatile lipid oxidation products. *Progress Lipid Res* 22:1–33.

Gómez E, Ledbetter C. 1993. Transmission of biochemical flavor constituents from apricot and plum to their interspecific hybrids. *Plant Breed* 111:236–41.

Gómez E, Ledbetter C. 1994. Comparative study of the aromatic profiles of two different plum species: *Prunus salicina* and *Prunus simonii* L. *J Sci Food Agric* 65:111–5.

Gómez E, Ledbetter C. 1997. Development of volatile compounds during fruit maturation: Characterization of apricot and plum x apricot hybrids. *J Sci Food Agric* 74:541–6.

Gómez E, Ledbetter C, Hartsell P. 1993. Volatile compounds in apricot, plum and their interspecific hybrids. *J Agric Food Chem* 41:1669–76.

Guadagni D, Buttery R, Harris J. 1966. Odour intensity of hop oil constituents. *J Sci Food Agric* 17:142–4.

Guichard E, Schlick P, Issanchou S. 1990. Composition of apricot aroma: Correlations between sensory and instrumental data. *J Food Sci* 55:735–8.

Hedrick UP, Alderman WH, Dorsey MJ, Taylor OM, Wellington R. 1911. The plums of New York. State of New York, Department of Agriculture, Eighteenth Annual Report, Vol. 3, Part II, J.B. Lyon Company, Albany, NY.

Horvat R, Chapman G, Robertson J, Meredith F, Scorza R, Callahan A, Morgens A. 1990. Comparison of the volatile compounds from several commercial peach cultivars. *J Agric Food Chem* 28:234–7.

Horvat R, Chapman G, Senter S, Robertson J. 1992. Comparison of the volatile compounds from several commercial plum cultivars. *J Sci Food Agric* 60:21–3.

Howard WL. 1945. Luther Burbank's plant contributions. California Agricultural Experiment Station Bulletin No. 691, University Press of the Pacific, Honolulu, Hawaii.

Ikeda R, Rolle L, Nannier S, Stanley W. 1962. Isolation and identification of aldehydes in cold-pressed lemon oil. *J Agric Food Chem* 10:98–102.

Ismail H, Brown G, Tuknott O, Holloway P, Williams A. 1977. Nonanal in epicuticular wax of Golden Egg plums (*Prunus domestica*). *Phytochemistry* 16:769–70.

Ismail H, Williams A, Tucknott O. 1980. The flavor components of canned plums. An investigation into the volatile components of canned plums. *Z Lebensm Unters Forsch* 171:265–8.

Ismail H, Williams A, Tucknott O. 1981. The flavour components of plums: An examination of the aroma components present in the headspace above four cultivars of intact plums, Marjorie's seedling, Merton Gem, NA 10 and Victoria. *J Sci Food Agric* 32:498–502.

Kemp T, Stolz L, Packett L. 1971. Aromatic hydrocarbons: Examination of peach fruit and foliage volatiles. *Phytochemistry* 10:478–9.

Leskey T, Prokopy R, Wright S, Phelan P, Haynes L. 2001. Evaluation of individual components of plum odor as potential attractants for adult plum curculios. *J Chem Ecol* 27:1–17.

Luh BS, Kean CE, Woodroof JG. 1986. Canning of fruits. In Woodroof JG, Luh BS (eds.), *Commercial Fruit Processing*, 2nd Ed. Westport, CT: AVI Publishing, pp. 163–262.

Mitchell FG. 1987. Influence of cooling and temperature maintenance on the quality of California grown stone fruit. *Int J Refrig* 10:77–81.

Rohrer JR, Ahmad R, Southwick SM, Potter D. 2004. Microsatellite analysis of relationships among North American plums (*Prunus* sect. *Prunocerasus*, Rosaceae). *Plant Syst Evol* 244:69–75.

Sabarez H, Price W, Korth J. 2000. Volatile changes during dehydration of d'Agen prunes. *J Agric Food Chem* 48:1838–42.

Salvador A, Cuquerella J, Martínez-Jávega JM. 2003. 1-MCP treatment prolongs postharvest life of "Santa Rosa" plums. *J Food Sci* 68(4):1504–10.

Satora C, Tuszyński T. 2008. Chemical characteristics of Śliwowica Łącka and other plum brandies. *J Sci Food Agric* 88:167–74.

Shaw J, Small RL. 2005. Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus* subgenus *Prunus* section *Prunocerasus*, Rosaceae). *Am J Bot* 92(12):2011–30.

Takeoka G, Flath R, Mon T, Teranishi R, Guentert M. 1990. Volatile constituents of apricot (*Prunus armeniaca* L.). *J Agric Food Chem* 38:471–7.

Tang C, Jennings W. 1968. Lactonic compounds of apricots. *J Agric Food Chem* 16:252–4.

Tatsuka K, Suekane S, Sakai J, Sumitani H. 1990. Volatile constituents of kiwi flowers: Simultaneous distillation and extraction versus headspace sampling. *J Agric Food Chem* 38:2176–80.

Valero D, Pérez-Vicente A, Martínez-Romero D, Castillo S, Guillén F, Serrano M. 2002. Plum storability improved after calcium and heat postharvest treatments: Role of polyamines. *J Food Sci* 67(7):2571–5.

Waugh FA. 1901. *Plums and Plum Culture*. New York: Orange Judd.

Willaert G, Dirinck P, De Pooter H, Schamp N. 1983. Objective measurements of aroma quality of Golden Delicious apples as a function of controlled-atmosphere storage time. *J Agric Food Chem* 31:809–13.

Williams A, Ismail H. 1981. The volatile flavor components of plums and their sensory evaluation. In Solms J, Hall R (eds.), *Criteria of Food Acceptance*. Zurich, Switzerland: Forster Publishing, pp. 333–45.

CHAPTER 23

Strawberry Flavor

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INTRODUCTION

Strawberry fruit is consumed for its pleasant flavor as well as its nutrient content. The modern cultivated strawberry (*Fragaria* × *ananassa* Duch.) is the most widely distributed strawberry crop due to its genotypic diversity and broad range of environmental adaptation. Two other species, *Fragaria vesca*, the most widely distributed of wild species, and *Fragaria moschata* Duch., are also grown commercially but on a much smaller scale (Hancock and Bringhurst 1979). The octoploid cultivated strawberry *Fragaria* × *ananassa* was derived from hybridization between two wild American native octoploid species, *Fragaria virginiana* Duch. and *Fragaria chiloensis* Duch., in 18th-century European gardens. Since that time, extensive hybridization between the parent species and their descendents has occurred, making *Fragaria* × *ananassa* a highly variable and adaptive species with a wide range of morphological and physiological characteristics (Larson 1994). Due to their hybrid origins, strawberries are adapted to many different climates: temperate, moderate, Mediterranean, subtropical, and even tropical if grown at high altitudes. This high adaptability and consumer-positive attitudes about strawberry have contributed to the rise of production worldwide.

The modern cultivated strawberry, *Fragaria* × *ananassa* Duch., produces an aggregate fruit that comprises a number of one-seeded fruits, or achenes, arranged in a spiral fashion on an enlarged receptacle (Winston 1902). The achenes are the true fruits of the strawberry, each containing a single embryo. However, the fleshy receptacle constitutes the edible part of the fruit. Arrangement of the achenes in the receptacle affects distribution of growth and, therefore, berry size and shape. Growth of the receptacle is principally a function of cell enlargement in its cortex and pith (Cheng and Breen 1992). The presence of auxin produced by the achenes is essential for the expansion of the receptacle during strawberry fruit development (Nitsch 1950), and the decline in the concentration of this hormone in the achenes as strawberry fruit matures triggers fruit ripening and the development of its characteristic

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flavor (Given et al. 1988; Manning 1994). The biosynthesis of strawberry flavor depends on two main factors: the availability of substrates and the inherent properties of the involved enzymes (Pérez et al. 1992; Zabetakis and Holden 1997). Some of these enzymes have been identified and purified. Most of them show to be developmentally regulated and associated with ripening (Mitchell and Jelenkovic 1995; Moyano et al. 2004; Pérez et al. 1996b, 1999b; Raab et al. 2006).

Since Manning (1994) first extracted strawberry mRNA and studied changes in gene expression during ripening, several molecular studies on strawberry have been initiated with the aim of providing more precise information on the regulation of the main ripening-related enzymes. The advent of molecular tools, such a cDNA microarray analysis, now adds a new dimension to gene expression studies. This type of analysis provides a powerful means for systematically studying the expression profiles of genes in a given tissue under specific physiological and environmental conditions. Combining the appropriate biochemical knowledge with gene expression data can provide indirect evidence for the elucidation of gene function. In order to make this review simpler, mention of enzymes and genes participating in the synthesis of volatile compounds will be restricted to those reported for strawberry.

STRAWBERRY FLAVOR

Fruit flavor is determined by a large number of volatile compounds whose distribution and biosynthesis is dependent on many factors, such as cultivar, maturity, and postharvest conditions (Forney et al. 2000; Larsen and Poll 1992). Although exhaustive information regarding strawberry volatile composition is available, few detailed biochemical studies have been done in relation to aroma biosynthesis. Reasons for this limited number of biochemical studies on strawberry are the high content of polyphenols and pectins in unripe and ripe fruits and the low protein content of strawberry fruit, hindering any process of enzyme isolation. Understanding the properties of enzymes involved in the production of aroma volatiles could improve strawberry flavor following shipping and marketing (Forney et al. 2000).

Volatile Composition of Strawberry Flavor

Strawberry flavor consists of a huge variety of volatile compounds that has been intensively studied. These compounds represent less than 0.01% of the fruit fresh weight but have a major impact on its quality (Buttery 1981). To date, more than 300 substances have been identified (Honkanen and Hirvi 1990; Latrasse 1991; Nijssen et al. 1996; Zabetakis and Holden 1997) including esters, furans, terpenoids, aldehydes, alcohols, ketones, acids, lactones, aromatic compounds, sulfur compounds, and acetals (Table 23.1). The relative abundance of individual volatiles is a fingerprint of a particular cultivar and species (Ulrich et al. 1997). The flavor characteristics of cultivated and wild strawberries differ significantly, the latter displaying higher aroma intensities compared with the former. The diversity and intensity of wild strawberry aromas cause the interest of plant breeders to use these species as donors of volatiles in breeding programs (Olbricht et al. 2008).

Esters are quantitatively and qualitatively the most abundant class of the flavor compounds in cultivated strawberries, comprising 25–90% of the total volatiles in

TABLE 23.1. Volatile Compounds Identified in Cultivated (C) and Wild Strawberries (W)

Compound	Source	Reference[a]	Compound	Source	Reference[a]
Esters					
(*E*)-Hex-2-enyl acetate	C, W	1	Ethyl pentanoate	C, W	1, 9
(*E*)-Hex-2-enyl butanoate	C	1	Ethyl propanoate	C, W	1, 9
(*E*)-Hex-3-enyl hexanoate	C	1	Hept-1-en-3-yl acetate	C	1
(Z)-Hex-3-enyl acetate	C, W	1	Hex-1-en-3-yl acetate	C	1
(*Z*)-Hex-3-enyl butanoate	C	1	Hex-2-enyl hexanoate	C	1
(Z)-Hex-3-enyl octanoate	C	1	Hex-4-enyl 2-methylpropanoate	C	3
(Z)-Hex-3-enyl propanoate	C	1	Hexyl 2-methylbutanoate	C	1
1-Methylbutyl 2-methylpropanoate	C	3	Hexyl 2-methylpropanoate	C	3
1-Methylbutyl acetate	C	1	Hexyl acetate	C, W	1
1-Methylbutyl butanoate	C	1	Hexyl butanoate	C, W	1
1-Methylbutyl hexanoate	C	1	Hexyl decanoate	C	1
1-Methylhexyl acetate	C	1	Hexyl formate	C, W	1
1-Methylhexyl butanoate	C	1	Hexyl hexanoate	C	1
1-Methylhexyl hexanoate	C	1	Hexyl octanoate	C	1
1-Methyloctyl butanoate	C	1	Isoproppyl hexanoate	C	3
1-Methylpentyl acetate	C	1	Isopropyl 2-methylbutanoate	C	1
2-Methylbutyl 2-methylbutanoate	C	1	Isopropyl 2-methylpropanoate	C	3
2-Methylbutyl acetate	C, W	1, 9	Isopropyl acetate	C	1
2-Methylpropyl 2-methylbutanoate	C	1	Isopropyl butanoate	C	1
2-Methylpropyl acetate	C	1	Isopropyl decanoate	C	1
2-Methylpropyl butanoate	C	1	Isopropyl hexanoate	C	5
2-Methylpropyl nonanoate	C	1	Isopropyl octanoate	C	1
3-Methylbut-2-enyl acetate	C, W	1	Methyl (*E*)-hex-2-enoate	C	3
3-Methylbutyl 2-methylbutanoate	C	1	Methyl 2-methylbutanoate	C, W	1, 9
3-Methylbutyl acetate	C, W	1, 9	Methyl 2-methylpropanoate	C	1
3-Methylbutyl acetate	C	1	Methyl 3-hydroxybutanoate	C	1
3-Methylbutyl butanoate	C	5	Methyl 3-hydroxyhexanoate	C	1
3-Methylbutyl formate	C	1	Methyl 3-methyl-(*E*)-but-2-enoate	C	3
3-Methylbutyl hexanoate	C	1	Methyl 3-methylbutanoate	C	2
3-Methylbutyl nonanoate	C	1	Methyl 4-methylpentanoate	C	1

TABLE 23.1. ***Continued***

Compound	Source	Reference[a]	Compound	Source	Reference[a]
3-Methylbutyl octanoate	C	1	Methyl acetate	C	1
Butyl 2-methylbutanoate	C	1	Methyl but-2-enoate	C	3
Butyl 3-methylbutanoate	C	1	Methyl butanoate	C, W	1
Butyl acetate	C, W	1	Methyl decanoate	C, W	1
Butyl butanoate	C	1	Methyl dodecanoate	C, W	1
Butyl formate	C, W	1	Methyl formate	C	1
Butyl hexanoate	C	1	Methyl heptanoate	C	1
Butyl octanoate	C	1	Methyl hexadecanoate	C	1
Butyl propanoate	C	3	Methyl hexanoate	C, W	1
Decyl acetate	C, W	1	Methyl nonanoate	C	1
Decyl butanoate	C, W	1	Methyl octadec-9-enoate	C	1
Decyl hexanoate	C	1	Methyl octadeca-9,12,15-trienoate	C	1
Ethyl (*E*)-hex-2-enoate	C	1	Methyl octadecanoate	C	1
Ethyl (*Z*)-hex-3-enoate	C, W	9	Methyl octanoate	C, W	1
Ethyl 2-methylbutanoate	C, W	1, 9	Methyl pentanoate	C	4
Ethyl 2-methylpropanoate	C	1	Methyl propanoate	C	1
Ethyl 3-hydroxyhexanoate	C	1	Nonyl acetate	C	3
Ethyl 3-methylbutanoate	C	1	Octyl 2-methylbutanoate	C	1
Ethyl 3-oxobutanoate	C	1	Octyl acetate	C, W	1
Ethyl acetate	C, W	1	Octyl butanoate	C	1
Ethyl acetoacetate	W	1	Octyl hexanoate	C	1
Ethyl but-2-enoate	C, W	1	Pent-3-enyl butanoate	C	1
Ethyl butanoate	C, W	1	Pentyl acetate	C, W	1, 7
Ethyl decanoate	C, W	1	Pentyl butanoate	C	1
Ethyl dodecanoate	C, W	1	Pentyl hexanoate	C	1
Ethyl formate	C	1	Propyl 3-methylbutanoate	C	3
Ethyl heptanoate	C	1	Propyl acetate	C	1
Ethyl hexanoate	C, W	1	Propyl butanoate	C	1
Ethyl octanoate	C, W	1			

Furans					
2,5-Dimethyl-3-hydroxy-4-methoxy-2,3-dihydrofurane	C	3	2-Furancarboxylic acid	C	1
2,5-Dimethyl-4-hydroxy-2H-furan-3-one	C, W	1	2-Furfural	C	1
2,5-Dimethyl-4-methoxy-2H-furan-3-one	C, W	1	2-Pentylfurane	C	3
Terpenoids					
Borneol	C	1	Nerol	W	9
Bornyl acetate		5	Nerolidol	C, W	1, 9
Citronellol	W	1	*p*-Menth-1-en-8-ol	C, W	9
Damascenone	C	1, 10	Pulegone	C	3
Isofenchyl alcohol	C	1	Terpinene	C, W	9
Limonene	C	1	α-Ionone	C	1
Linalool	C, W	1	α-Pinene	C, W	1, 6
Linalool oxides	C	1	α-Terpineol	C, W	1
Myrtenal	C, W	9	β-Ionone	C	1
Myrtenol	W	1	β-Phellandrene	W	6
Myrtenyl acetate	W	6	β-Pinene	C, W	1, 6
Aldehydes					
(*E*)-Hex-2-enal	C	1	But-2-enal	C	1
(*E*,*E*)-Deca-2,4-dienal	C	1	Butanal	C	1
(*E*,*E*)-Nona-2,4-dienal	C	1	Decanal	C	1
(*E*,*E*)-Nona-2,6-dienal	C	3	Hept-2-enal	C	1
Heptanal	C	1	Nonanal	C	1
Hex-2-enal	W	1	Oct-2-enal	C	1
Hexa-2,4-dienal	C	1	Octanal	C	4
Hexanal	C, W	1	Pent-2-enal	C	1
(*Z*)-Hex-3-enal	C	1	Pentanal	C	1
(*Z*)-Non-2-enal	C	8	Propanal	C	1
2-Methyl-pent-4-enal	C	4	Propenal	C	1
Acetaldehyde	C	1			
Alcohols					
(*E*)-Hex-2-en-1-ol	C, W	1	Hexan-1-ol	C, W	1
(*Z*)-Hex-2-en-1-ol	W	1	Hexan-2-ol	C	1
(*Z*)-Hex-3-en-1-ol	C	1	Hexan-3-ol	C	1
2-Ethylhexan-1-ol	W	1	Methanol	C	1
2-Methylbutan-1-ol	C	1	Non-1-en-3-ol	C	1

TABLE 23.1. ***Continued***

Compound	Source	Reference[a]	Compound	Source	Reference[a]
2-Methyl-butan-2-ol	C	1	Non-2-en-1-ol	C	3
2-Methylpropan-1-ol	C	1	Nonan-1-ol	C, W	1
3-Methyl-2-heptanol	C	3	Nonan-2-ol	C	1
3-Methylbutan-1-ol	C, W	1	Oct-1-en-3-ol	C	1
3-Methylbutan-2-ol	W	1	Oct-3-en-1-ol	C	1
6-Methylhept-5-en-2-ol	W	1	Octan-1-ol	C, W	1
Butan-1-ol	C	1	Octan-2-ol	C	1
Butan-2-ol	C	1	Octan-3-ol	C	1
Butane-1,2-diol	C	3	Pent-1-en-3-ol	C	1
Dec-2-en-1-ol	C	3	Pent-3-en-2-ol	C	1
Decan-1-ol	C, W	1	Pentadecan-2-ol	C, W	1
Decan-2-ol	C	1	Pentan-1-ol	C, W	1
Dodecan-1-ol	C	1	Pentan-2-ol	C, W	1,9
Dodecan-2-ol	C	1	Pentan-3-ol	C	1
Ethanol	C, W	1	Propan-1-ol	C	1
Heptan-1-ol	C	1	Propan-2-ol	C	1
Heptan-2-ol	C, W	1	Tridecan-2-ol	C, W	1
Heptan-3-ol	C	1	Undecan-1-ol	W	1
Hex-1-en-3-ol	C	1	Undecan-2-ol	C	1
Ketones					
3-Hydroxy-butanone	W	1	Hexan-2-one	C	1
3-Methylpentan-2-one	C	4	Methylbutanone	C	1
4-Hydroxy-4-methyl-pentan-2-one	C	1	Nonan-2-one	C, W	1
4-Methylpentan-2-one	C	3	Octan-2-one	C	1
5-Methyl-2-hexanone	C	4	Pent-3-en-2-one	C	1
6-Methyl-hept-5-en-2-one	C	4	Pentadecan-2-one	W	1
Butan-2-one	C	1	Pentan-2-one	C	1
Butane-2,3-dione	C, W	2, 9	Pentan-3-one	C	1
Butane-2,4-dione	C	1	Propanone	C	1
Decan-2-one	C	1	Tridecan-2-one	W	1

Heptadecan-2-one	W	1	Undecan-2-one	C, W	1
Heptan-2-one	C, W	1			
Acids					
2-Methylbut-2-enoic acid	C	1	Hex-2-enoic acid	C	1
2-Methylbutanoic acid	C, W	1, 9	Hexadec-9-enoic acid	C	1
2-Methylpent-2-enoic acid	C	1	Hexadecanoic acid	C	1
2-Methylpent-3-enoic acid	C	1	Hexanoic acid	C, W	1
2-Methylpropanoic acid	C, W	1	Non-3-enoic acid	C	1
3-Hydroxyhexanoic acid	C	1	Nonadecanoic acid	C	1
3-Hydroxyoctanoic acid	C	1	Nonanoic acid	C	1
3-Methylbutanoic acid	C	1	Oct-2-enoic acid	C	1
4-Methylpentanoic acid	C	1	Octadec-9-enoic acid	C	1
5-Methylhexanoic acid	C	1	Octadeca-9,12,15-trienoic acid	C	1
Acetic acid	C, W	1	Octadeca-9,12-dienoic acid	C	1
Butanoic acid	C, W	1	Octanoic acid	C, W	1
Dec-2-enoic acid	C	1	Pentadecanoic acid	C	1
Decanoic acid	C, W	1	Pentanoic acid	C	1
Dodecanoic acid	C, W	1	Propanoic acid	C, W	1
Eicosanoic acid	C	1	Tetradec-2-enoic acid	C	1
Formic acid	C	1	Tetradecanoic acid	C, W	1
Heptadecanoic acid	C	1	Tridecanoic acid	C	1
Heptanoic acid	C	1	Undecanoic acid	C	1
Lactones					
Undecalactone	C	10	γ-Octalactone	C, W	1
γ-Decalactone	C, W	1	δ-Decalactone	C	1
γ-Dodecalactone	C, W	1	δ-Heptalactone	C	1
γ-Heptalactone	W	1	δ-Hexalactone	C, W	1
γ-Hexalactone	C, W	1	δ-Octalactone	C, W	1
Aromatic compounds					
(*E*)-Cinnamic acid	C, W	1	Cinnamyl acetate	C	3
(*E*)-Cinnamyl alcohol	C	1	Cinnamyl alcohol	W	1
1-Methylnaphthalene	C	1	Cyclohexyl acetate	C	3
2-(4-Hydroxyphenyl)ethanol	C	1	Ethyl benzoate	C	3
2-Hydroxybenzoic acid	C	1	Ethyl cinnamate	C, W	1

TABLE 23.1. ***Continued***

Compound	Source	Reference[a]	Compound	Source	Reference[a]
2-Methoxy-4-vinylphenol	C, W	1	Ethyl salicylate	C	1
2-Methylnaphthalene	C	1	Ethylbenzene	C	3
2-Phenethyl acetate	C, W	1, 9	Eugenol	C, W	1
2-Phenylethanol	C, W	1	Isopropyl nicotinate	W	1
3-Phenyl propanol	C, W	1, 5	Methyl anthranilate	C, W	1, 10
3-Phenylpropanoic acid	C	1	Methyl benzoate	W	1
4-Methylbenzoic acid	C	1	Methyl cinnamate	C, W	1
4-Vinylphenol	C, W	1	Methyl *N*-formyl-anthranilate	W	1
Acetophenone	C	1	Methyl nicotinate	C, W	1, 9
Benzaldehyde	C, W	1	Methyl salicylate	C	1
Benzoic acid	C	1	Phenylacetic acid	C	1
Benzyl acetate	C, W	1	Toluene	C	3
Benzyl alcohol	C, W	1	Vanillin	W	1
Carvyl acetate	W	1	Verbenone	W	1
Sulfur compounds					
Dimethyl disulfide	C	1	Methanethiol	C	1
Ethyldithioethane	C	1	Methylthiol acetate	C	1
Ethylthioethane	C	1	Methylthiol butanoate	C	1
Acetals					
1,1-Diethoxyethane	C	1	1-Ethoxy-1-hexoxyethane	C	1
1,1-Diethoxyoctane	C	1	1-Ethoxy-1-methoxyethane	C	1
1,1-Diethoxypentane	C	1	1-Ethoxy-1-pentoxyethane	C	1
1,1-Dihexoxyethane	C	1	1-Ethoxy-1-propoxyethane	C	1
1,1-Dimethoxyethane	C	1	1-Methoxy-1-pentoxyethane	C	1
1-Butoxy-1-ethoxyethane	C	1	Diethoxymethane	C	1
1-Butoxy-1-methoxyethane	C	1	Dimethoxymethane	C	1
1-Ethoxy-1-hex-3-enoxyethane	C	1			

[a]Reviewed by (1) Zabetakis and Holden (1997); (2) Schieberle and Hofmann (1997); (3) Gomes da Silva and Chaves das Neves (1999); (4) Hakala and others (2002); (5) Azodanlou and others (2003); (6) Aharoni and others (2004); (7) Williams and others (2005); (8) Fukuhara and others (2006); (9) Ulrich and others (2007); (10) Olbricht and others (2008).

fresh ripe fruit (Douillard and Guichard 1990; Pyysalo et al. 1979; Schreier 1980). Other classes of compounds, which may represent up to 50% of strawberry volatiles, include aldehydes (Schreier 1980) and furanones (Larsen and Poll 1992). Alcohols may account for as much as 35% of the volatiles, but normally contribute little to strawberry flavor (Larsen and Watkins 1995). Terpenoids normally comprise <10% of strawberry volatiles and sulfur compounds <2%; both of them may contribute to strawberry flavor (Dirinck et al. 1981; Schreier 1980). Different studies based on odor threshold and concentration in the fruit have been performed to determine which volatiles contributed the most to the cultivated strawberry flavor (Larsen and Poll 1992; Larsen et al. 1992; Schieberle and Hofmann 1997). Data showed that among the hundreds of volatile compounds produced by fresh strawberries, in general, the most important volatile compounds seem to be methyl butanoate, ethyl butanoate, 2-methyl butanoate, ethyl hexanoate, methyl hexanoate, and methyl 2-methylpropanoate (fruity odor notes); 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 4-methoxy-2,5-dimethyl-3(2H)-furanone (caramel-like notes); (*Z*)-3-hexenal (green notes); butane-2,3-dione (buttery); and linalool (floral).

Terpenoid Biosynthesis in Strawberry Fruit

Terpenoids constitute just a small fraction of strawberry aroma, but they seem to play an important qualitative role either in cultivated and wild strawberries. The monoterpene linalool and sesquiterpene nerolidol seem to be the major constituents among the terpenoids of cultivated strawberry. Linalool imparts a sweet, floral, citrus-like note, and nerolidol, provides a rose, apple, green note. The presence of large amounts of linalool in the strawberry cultivar Senga Sengana contributes to the intense and pleasant character of this cultivar (Larsen and Poll 1992; Latrasse 1991). On the contrary, wild strawberry emitted mainly the olefinic monoterpenes α-pinene, β-myrcene, α-terpineol, and β-phellandrene, as well as myrtenol and myrtenyl acetate, having turpentine-like and woody odor notes.

Little is known about the synthesis of terpenoids in strawberry, although it is assumed that they are produced through the terpenoid synthetic pathways described in plants. Thus, terpenoids are derived either from the mevalonate pathway active in the cytosol or from the plastidial methylerythritol-4-phosphate pathway. Both pathways lead to the formation of isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMADP). IPP and DMADP are condensed by prenyl transferases to produce the monoterpene precursor geranyl diphosphate (GDP), the sesquiterpene precursor farnesyl diphosphate (FDP), and the diterpene precursor geranylgeranyl diphosphate (GGDP). Following the formation of these acyclic precursors, terpenoids are generated through the action of terpene synthases (TPSs) directly or after further modifications by hydroxylation, oxidation, reduction, acylation, or methylation (see Chapter 5 for more details).

Aharoni and others (2004) used cDNA microarray analysis to identify the *F. ananassa nerolidol synthase1* (*FaNES1*) gene in cultivated strawberry and showed that the recombinant FaNES1 enzyme produced in *Escherichia coli* generated both linalool and nerolidol when supplied with GPP or FPP, respectively. Characterization of additional genes very similar to *FaNES1* from both the wild and cultivated strawberry species (*FaNES2* and *F. vesca NES1*) showed that only *FaNES1* is exclusively present and highly expressed in the fruit of cultivated varieties. Protein localization

experiments suggest that a change in subcellular localization allowed the FaNES1 enzyme to produce linalool and nerolidol. The biosynthesis of linalool was suggested to occur in the cytosol rather than in the plastids, as would be expected for monoterpenes (Aharoni et al. 2004), based on the absence of a plastid-targeting sequence in *FaNES1* and on feeding experiments of labeled mevalonic acid to strawberries resulting in incorporation of the label into linalool, whereas feeding of the plastidic precursor 1-deoxy-D-xylulose did not result in labeled linalool (Aharoni et al. 2005). On the other hand, it seems that an insertional mutation might affect the expression of a TPS (*F. ananassa pinene synthase*) gene causing the loss of the biosynthesis of the typical wild species monoterpenes such as β-myrcene, α-pinene, and derived myrtenol and myrtenyl acetate. This phenomenon was demonstrated by cloning and characterizing a cytochrome P450 gene (*pinene hydroxylase*) that encodes the enzyme catalyzing the C10 hydroxylation of α-pinene to myrtenol (Aharoni et al. 2004).

The strawberry *FaNES1* gene has been used for performing metabolic engineering experiments in several plant species, focusing on the monoterpenoids and sesquiterpenoids (Aharoni et al. 2006). Results demonstrated that engineering of these compounds and their derivatives in plant cells is feasible, although it is necessary to take into account some important factors such as the subcellular localization of both the precursor pool and the introduced enzymes, the activity of endogenous plant enzymes modifying the introduced terpenoid skeleton, the effects on other pathways sharing the same precursor pool, and the phytotoxicity of the introduced terpenoids.

Furanone Biosynthesis in Strawberry Fruit

Strawberry fruits contain an uncommon group of key aroma compounds with a 2,5-dimethyl-3(2H)-furanone structure considered by most authors as the most important aroma constituents of cultivated strawberries (Douillard and Guichard 1989, 1990; Herrmann 1991; Hirvi 1983). Main compounds are 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF or furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF or mesifurane). Both compounds have strong, sweet, and pleasant odors. Furaneol imparts caramel burnt sugar notes at high concentrations and becomes fruity at lower concentrations (Re et al. 1973). Mesifurane is described as having a more sherry-like aroma (Hunter et al. 1974). Among these compounds, furaneol is the most important because of its high concentration (up to 55 μg/g strawberry fruit fresh weight) (Larsen et al. 1992; Sanz et al. 1995) and low odor threshold (10 ppb) (Schieberle and Hofmann 1997). Different studies have identified the presence of furaneol, mesifurane (Douillard and Guichard 1990; Hirvi and Honkanen 1982), and furaneol glucoside (Mayerl et al. 1989) in strawberries and have studied the evolution of these compounds along ripening and shelf life (Pérez et al. 1996a; Sanz et al. 1995), but these furanones have not been found in all cultivars. Factors such as furaneol's water-soluble nature and thermal instability could well account for the failure of some authors to detect these compounds (Sanz et al. 1994). Enantiomeric analyses showed that furaneol and mesifurane occur as racemates in different fruits (Bruche et al. 1995). Several efforts have been made to clarify the biosynthesis of furaneol and its derivatives.

Even though the biogenesis in fruits is quite unknown, all studies indicate that furaneol is derived from sugar metabolism (Schwab and Roscher 1997). Various

sugars have been suggested as precursors for furaneol synthesis, being fructose the most likely candidate (Pérez et al. 1999a; Roscher et al. 1998; Sanz et al. 1997; Zabetakis and Holden 1996). The first indications for the enzymatic formation of furaneol in strawberry fruit were provided by studies demonstrating the correlation of fruit ripening stage and furaneol concentration (Sanz et al. 1995). Incorporation experiments of radioactively labeled compounds in strawberry showed that D-[U-14C]fructose-1,6-diphosphate had the highest incorporation rate into the furanone structures among the tested compounds (Roscher et al. 1998). Further incorporation experiments with D-[U-13C]fructose proved the transformation of the complete carbon chain of D-fructose into furaneol (Schwab 1998), and studies with D-[2-2H] glucose demonstrated the involvement of phosphohexose isomerase in the conversion of D-glucose into the furanones (Wein et al. 2001). Pérez and others (1999) incubated *in vitro* grown strawberries with different sugars and sugar phosphates and demonstrate that D-fructose-6-phosphate is the likely precursor of furaneol in this fruit, contrary to what was found by Hecquet and others (1996a) in yeast. The slight increase in furanones showed by D-fructose incubations and similar to the control furanone production when D-fructose-1,6-diphosphate was used could indicate that the enzymatic system forming furaneol would need as a requirement a phosphorylated fructose molecule at carbon 6 and proposed the pentose phosphate cycle as the physiological source of D-fructose-6-phosphate in strawberry. Intermediates of this cycle have already been proposed as substrates for the biosynthesis of the furaneol homologue 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone in yeast (Sasaki 1996; Sasaki et al. 1991).

More recently, Raab and others (2006) reported the isolation and characterization of an enzyme involved in the transformation of D-fructose-1,6-diphosphate to furaneol in strawberry fruit. Sequence homology of the isolated enzyme with the protein sequence of an already sequenced cDNA led to the cloning and characterization of *Fragaria* × *ananassa* quinone oxidoreductase gene (*FaQR*). This gene is auxin dependent, and it is strongly induced during fruit ripening. Gene sequence analyses and determination of expression patterns suggested that FaQR was the last enzyme in the biosynthetic pathway leading to furaneol. On the basis of the observed reaction catalyzed by the heterologously expressed protein, FaQR would act as an enone oxidoreductase and identified 4-hydroxy-5-methyl-2-mehtylene-3(2H)-furanone as the natural substrate for this enzyme (Klein et al. 2007; Raab et al. 2006).

Furaneol is rapidly converted *in vivo* into mesifurane (Pérez et al. 1996; Roscher et al. 1997). Roscher and others (1998) demonstrated by *in vivo* feeding experiments the incorporation of the 14C-label into mesifurane after the application of *S*-[methyl-14C]-adenosyl-L-methionine (14C-SAM). Data supported the hypothesis that SAM is the natural source of the methyl in the 4-methoxy group of mesifurane. More recently, Lavid and others (2002) partially purified an O-methyltransferase in strawberry whose activity increases during fruit ripening and was able to transfer the SAM methyl group to furaneol, and Wein and others (2002) reported the cloning and characterization of an *S*-adenosyl-L-methionine-dependent O-methyltransferase gene (*FaOMT*), whose encoded protein was capable to carry out the methylation of furaneol to mesifurane. Northern hybridization indicated that the *FaOMT*-specific transcripts accumulated during ripening of strawberry fruits and were absent in the root, petiole, leaf, and flower. The protein was functionally expressed in *E.*

coli and exhibited substrate specificity for compounds having an *o*-diphenolic structure, such as furaneol in its dienolic tautomer. Lunkenbein and others (2006) assessed *in planta* the function of the FaOMT protein by up- and downregulating *FaOMT* in strawberry fruits through overexpression and antisense technology, respectively. Results from this study suggested that *FaOMT* is responsible for the last step in the biosynthesis of mesifurane.

Ester Formation in Strawberry Fruit

Volatile esters resulting from the esterification of an acyl moiety from acyl-coenzyme A (CoA) onto an alcohol are qualitatively and quantitatively the main components of strawberry aroma. The influence of esters on strawberry aroma makes alcohol acyltransferase (AAT), the enzyme catalyzing the esterification reaction, a key enzyme in strawberry aroma biochemistry. Strawberry AAT has been partially purified, and the specificity of this enzyme was correlated with the volatile composition of ripe fruit, suggesting that ester composition is dependent on the properties of the enzyme (Pérez et al. 1993). A dramatic increase in AAT activity has been observed in strawberry at the onset of ripening when the fruit acquires the ability to synthesize the characteristic aroma compounds. Data on AAT activity during strawberry postharvest shelf life point out the enzyme involvement not only in flavor but also in off-flavor generation (Pérez et al. 1996b). Purified proteins with AAT activity from different strawberry cultivars have been used for kinetic studies displaying a higher affinity for alcohols and acyl-CoAs with increasing carbon chain length (Olías et al. 2002). Comparative catalytic studies carried out with AAT from the wild *F. vesca* and different cultivated strawberries reflected interesting cultivar and species differences.

In recent years, genes encoding AATs have been isolated and characterized from different fruits (Souleyre et al. 2005; Wang and De Luca 2005; Yahyaoui et al. 2002), including cultivated and wild strawberries (Aharoni et al. 2000; Beekwilder et al. 2004). The proteins encoded by *AAT* genes show low sequence identity to other genes, but conserved motifs in their sequence could associate them to a superfamily of multifunctional acyltransferases, commonly referred as BAHD (St-Pierre and De Luca 2000). Genes isolated from strawberry encode ripening-enhanced and fruit-specific AATs. Biochemical evidences for the involvement of the *AAT* genes in the formation of fruity esters was provided by characterizing the recombinant protein expressed in *E. coli*. However, differences in the specificity of the recombinant enzymes for different substrates cannot fully account for the changes in ester composition during fruit development or the differences found among cultivars, suggesting that more than one form of AAT is present in strawberry fruit and that the availability of ester precursors seems to be an important factor determining the ester composition in strawberry.

Ester moieties may arise from more complex precursors including lipids and amino acids. Lipid metabolism is the source of a range of aldehydes and alcohols that, besides their direct contribution to fruit aroma, have an important role as precursors in the biosynthesis of esters. Enzymes such as hydrolase, lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) involved in the synthesis of lactones, aldehydes, and alcohols have also been described in strawberry (Leone et al. 2006; Mitchell and Jelenkovic 1995; Pérez et al. 1999b;

Schottler and Boland 1995). LOX is the first step in a biochemical route (LOX pathway) oxidizing linoleic (18:2) and linolenic (18:3) acids to the corresponding 9- or 13-hydroperoxide derivatives that, in turn, are cleaved by the enzyme HPL to form six-carbon aldehydes. These aldehydes have a very low odor threshold and are important contributors to the green odor notes of the fruit. The HPL activity from strawberry, which utilized the 13-hydroperoxide of linolenic acid as the preferred substrate to produce (*Z*)-hex-3-enal, increased markedly during the white stage of fruit development. In conjunction with a sharp increase in hexanal, this was taken to be evidence of a sequential pathway for the formation of green odor compounds in strawberry (Pérez et al. 1999b). More recently, Leone and others (2006) reported the presence of different LOX forms in strawberry, some of them associated to lipid–protein aggregates in specific locations within the cell and whose enzymatic activities are temporally differentiated. Based on their results, these authors suggested that the LOX pathway plays a role in converting lipids to six-carbon volatiles during strawberry ripening. Aldehydes produced through the LOX pathway can undergo reduction by ADH activity to form alcohols that are substrates for AAT activity to give rise to esters.

The metabolism of amino acids is also one of the main sources of substrates for ester biosynthesis in strawberry generating alcohols, aldehydes, and acids, either aliphatic, branched, or aromatic. These compounds contribute, and in some cases are determining, to the primary aroma of many fruits. Free amino acid content changes have been demonstrated to occur during ripening, when characteristic aroma is produced in most fruits, accounting for the different aroma profile patterns. Thus, Pérez and others (1992) reported a fourfold increase in ethyl esters concomitant to a 10-fold decrease in alanine content during strawberry ripening. Apart from the nitrogen reservoirs asparagine and glutamine, alanine was the major free amino acid found in this fruit. Metabolism of amino acids toward aroma biogeneration seems to occur via two consecutive enzymatic steps: deamination by aminotransferases and decarboxylation (see Chapter 5 for more details). Strawberry fruit displays differences in the level of metabolization for different amino acids, suggesting that the aminotransferase and/or specially the decarboxylating steps are critical for the release of precursors for the biosynthesis of branched esters, and probably ethyl esters, as suggested in banana (Wyllie and Fellman 2000; Wyllie et al. 1996). These relative activities could be affected by cultivar, maturity stage, and even environmental conditions, either on or off the plant.

Drawert (1975) postulated the oxidative decarboxylation of the 2-oxoacid resulting from deamination of amino acids to occur by an enzymatic complex similar to pyruvate dehydrogenase (PDH), involving as cofactors thiamine pyrophosphate, lipoic acid, FAD, NAD, and CoA. But the actuation of an enzyme similar to pyruvate decarboxylase (PDC), from the fermentative pathway, producing the nonoxidative decarboxylation of 2-oxoacids to form aldehydes is possible as well. In this sense, two strawberry cDNAs showing sequence similarity to *PDC* genes (*Fapdc1* and *Fapdc3*) from higher plants have been isolated by a differential display-PCR approach (Moyano et al. 2004). Sequence comparisons, Northern, and RT-PCR analysis showed that these strawberry genes are different and are expressed in a different expression pattern in vegetative tissues and in fruits during fruit development and ripening. RT-PCR studies indicated that only *Fapdc1* is induced during fruit ripening and its expression regulated by ripening-related hormones. Data

suggest that the strawberry *Fapdc1* gene could play an important role in fruit ripening and aroma biogenesis under normal and stress conditions. On the contrary, *Fapdc3* gene, constitutively present in strawberry fruit, would be involved in general metabolism to support energy production and biosynthesis of higher-molecular-weight compounds.

Aldehydes coming from the nonoxidative decarboxylation of amino acids or from the oxidative LOX pathway mentioned above are quickly reduced to the corresponding alcohols by ADH activity. ADH activity is strongly upregulated during the ripening of strawberry fruit (Mitchell and Jelenkovic 1995; Moyano et al. 2004), suggesting that this enzyme has an important role in flavor development, although it seems not to be a limiting factor. The metabolism of natural volatiles by intact strawberry fruit supports such a role for ADH (Hamilton-Kemp et al. 1996). Several isoforms of ADH are present in strawberry, and they exhibit broad substrate specificities implying they are involved in the interconversion of a number of flavor aldehydes and alcohols. Mitchell and Jelenkovic (1995) observed the presence of two NADPH- and NADH-dependent ADHs in strawberry receptacle with broad substrate specificities. NADH-dependent ADH showed higher activity against branched alcohols and nonaromatic aldehydes and an increase in activity during ripening, when characteristic aroma is produced. The presence of ADH is often associated with anaerobic metabolism, and carbon dioxide can cause some strawberry cultivars to accumulate acetaldehyde and ethanol (Fernández-Trujillo et al. 1999) that may contribute to the production of off-flavors (Ke et al. 1991; Moyano et al. 2004). Recent studies have demonstrated that cultivar variations in two aroma key enzymes, ADH and PDC, could explain the different susceptibility of strawberry varieties to postharvest disorders such as off-flavor development during modified atmosphere storage (Fernández-Trujillo et al. 1999; Watkins et al. 1999). Interestingly, strawberry cultivars tolerant to high CO_2 produced less ethanol and acetaldehyde than non-tolerant cultivars despite the fact ADH and PDC activity levels were higher.

CONCLUSION AND PERSPECTIVES

The cultivated strawberry *Fragaria* × *ananassa* Duch. is the most important berry fruit in the world, consumed for its nutrient content as well as pleasant flavor. Strawberry flavor consists of a huge variety of volatile compounds. Among them, a series of esters, furanones, and terpenes seem to be decisive for the characteristic strawberry flavor, though significant differences can be found between cultivated and wild strawberries and even between the different modern cultivars. Despite the huge information regarding strawberry volatile composition, few detailed biochemical and genetic studies have been done in relation to flavor biosynthesis. As we have seen throughout this chapter, strawberry flavor formation is a complex process in which quite different pathways are involved. These biochemical routes are interconnected, and most of them are not exclusively devoted to flavor formation but also give rise to some other important plant metabolites that have many different biological functions.

Flavor has been a secondary goal of the strawberry breeding programs, dominated by properties like appearance, firmness, color, harvest time, harvest behavior,

and shelf life. This has resulted in modern cultivars with high performance in yield, fruit size, and firmness but characterized by poor flavor. Thus, molecular genetic manipulation of specific genes or groups of genes to increase or modify flavor generation in strawberries might be an area of future research. However, social resistance to genetically engineered foodstuffs may hinder this research area. Instead, genetic knowledge may assist the development of marker-selected plant breeding using more conventional techniques. The combination of molecular techniques and metabolite profiling with traditional plant breeding methods promises to deliver in the short- to medium-term new strawberry cultivars with improved flavor quality.

REFERENCES

Aharoni A, Giri AP, Verstappen FW, Bertea CM, Sevenier R, Sun Z, Jongsma MA, Schwab W, Bouwmeester HJ. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–31.

Aharoni A, Jongsma MA, Bouwmeester HJ. 2005. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci* 10:594–602.

Aharoni A, Jongsma MA, Kim TY, Ri MB, Giri AP, Verstappen FWA, Schwab W, Bouwmeester HJ. 2006. Metabolic engineering of terpenoid biosynthesis in plants. *Phytochemistry Rev* 5:49–58.

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blass J, van Houwelingen AMML, De Vos RCH, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, Van Tunen AJ, O'Connell AP. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *Plant Cell* 12:647–61.

Azodanlou R, Darbellay C, Luisier JL, Villettaz JC, Amado R. 2003. Quality assessment of strawberries (*Fragaria* species). *J Agric Food Chem* 51:715–21.

Beekwilder J, Alavarez-Huerta M, Neef E, Verstappen FWA, Bouwmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135:1865–78.

Bruche G, Dietrich A, Mosandl A. 1995. Stereoisomeric flavour compounds LXXI: Determination of the origin of aroma active dihydrofuranones. *Z Lebensm Unters Forsch* 201:249–52.

Buttery RG. 1981. Vegetable and fruit flavours. In Teranishi R, Flath RA, Sugisawa H (eds.), *Flavor Research: Recent Advances*. New York: Marcel Dekker, pp. 175–216.

Cheng G, Breen PJ. 1992. Cell count and size in relation to fruit size among strawberry cultivars. *J Am Soc Hortic Sci* 117:946–95.

Dirinck PJ, De Pooter HL, Willaert GA, Schamp NM. 1981. Flavor quality of cultivated strawberries: The role of sulfur compounds. *J Agric Food Chem* 29:316–21.

Douillard C, Guichard E. 1989. Comparison by multidimensional analysis of concentrations of volatile compounds in fourteen frozen strawberry varieties. *Sci Aliments* 9:53–75.

Douillard C, Guichard E. 1990. The aroma of strawberry (*Fragaria ananassa*): Characterisation of some cultivars and influence of freezing. *J Sci Food Agric* 50:517–31.

Drawert F. 1975. Biochemical formation of aroma components. In Maarse H, Groenen PJ (eds.), *Aroma Research*. Wageningen, The Netherlands, pp. 13–39.

Fernández-Trujillo JP, Nock JF, Watkins B. 1999. Fermentative metabolism and organic acid concentrations in fruit of selected strawberry cultivars with different tolerances to carbon dioxide. *J Am Soc Hortic Sci* 124:696–701.

Forney CF, Kalt W, Jordan MA. 2000. The composition of strawberry aroma is influenced by cultivar, maturity and storage. *HortScience* 35:1022–6.

Fukuhara K, Li XX, Yamashita S, Hayata Y, Osajima Y. 2006. Analysis of volatile aromatics in "Toyonoka" strawberries (*Fragraria x ananassa*) using Porapak Q column extracts. *Acta Hortic* 708:343–8.

Given NK, Venis MA, Griergson D. 1988. Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta* 174:402–6.

Gomes da Silva M, Chaves das Neves H. 1999. Complementary use of hyphenated purge-and-trap gas chromatography techniques and sensory analysis in the aroma profiling of strawberries (*Fragaria ananassa*). *J Agric Food Chem* 47:4568–73.

Hakala MA, Lapveteläinen AT, Kallio HP. 2002. Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC-MS. *J Agric Food Chem* 50:1133–42.

Hamilton-Kemp TR, Archbold DD, Loughrin JH, Collins RW, Byers ME. 1996. Metabolism of natural volatile compounds in strawberry fruit. *J Agric Food Chem* 44:2802–5.

Hancock JF, Bringhurst RS. 1979. Ecological differentiation in perennial, octaploid species of *Fragaria*. *Am J Bot* 66:367–70.

Hecquet L, Sancelme M, Bolte J, Demuynck C. 1996. Biosynthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone by *Zygosaccharomyces rouxii*. *J Agric Food Chem* 44:1357–60.

Herrmann K. 1991. The aroma of fruits. Part III: Strawberry. *Erwerbs-Obstbau* 33:111–6.

Hirvi T. 1983. Mass fragmentographic and sensory analyses in the evaluation of the aroma of some strawberry varieties. *Lebensm Wiss Technol* 16:157–61.

Hirvi T, Honkanen E. 1982. The volatiles of two new strawberry cultivars "Annelie" and "Alaska Pioneer", obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca* Rügen and *Fragaria virginiana*. *Z Lebensm Unters Forsch* 175:113–6.

Honkanen E, Hirvi T. 1990. The flavour of berries. In Morton ID, MacLeod AJ (eds.), *Food Flavours*. Amsterdam: Elsevier Scientific Publications, pp. 125–93.

Hunter GLK, Bucek WA, Radford T. 1974. Volatile components of canned Alphonso mango. *J Food Sci* 39:900–3.

Ke D, Goldstein L, O'Mahony M, Kader AA. 1991. Effects of short-term exposure to low O_2 and high CO_2 atmospheres on quality attributes of strawberries. *J Food Sci* 56:50–4.

Klein D, Fink B, Arold B, Eisenreich W, Schwab W. 2007. Functional characterization of enone oxidoreductases from strawberry and tomato fruit. *J Agric Food Chem* 55:6705–11.

Larsen M, Poll L. 1992. Odor thresholds of some important aroma compounds in strawberries. *Z Lebensm Unters Forsch* 195:120–3.

Larsen M, Poll L, Olsen CE. 1992. Evaluation of the aroma composition of some strawberry (*Fragaria ananassa* Duch) cultivars by use of odour threshold values. *Z Lebensm Unters Forsch* 195:536–9.

Larsen M, Watkins CB. 1995. Firmness and concentration of acetaldehyde, ethyl acetate and ethanol in strawberries stored in controlled and modified atmospheres. *Postharvest Biol Technol* 5:39–50.

Larson KD. 1994. Strawberry. In Schaffer B, Andersen PC (eds.), *Handbook of Environmental Physiology of Fruit Crops. Volume I: Temperate Crops*. Boca Raton, FL: CRC Press, pp. 271–97.

Latrasse A. 1991. Fruits III. In Maarse H (ed.), *Volatile Compounds in Foods and Beverages*. New York: Dekker, pp. 329–87.

Lavid N, Schwab W, Kafkas E, Koch-Dean M, Bar E, Larkov O, Ravid U, Lewinsohn E. 2002. Aroma biosynthesis in strawberry: S-adenosylmethionine:furaneol O-methyltransferase activity in ripening fruits. *J Agric Food Chem* 50:4025–30.

Leone A, Bleve-Zacheo T, Gerrardi C, Melillo MT, Leo L, Zacheo G. 2006. Lipoxygenase involvement in ripening strawberry. *J Agric Food Chem* 54:6835–44.

Lunkenbein S, Salentijn EMJ, Coiner HA, Boone MJ, Krens FA, Schwab W. 2006. Up- and down-regulation of *Fragaria x ananassa* O-methyltransferase: Impacts on furanone and phenylpropanoid metabolism. *J Exp Bot* 57:2445–53.

Manning K. 1994. Changes in gene expression during strawberry fruit ripening and their regulation by auxin. *Planta* 194:62–8.

Mayerl F, Näf R, Thomas AF. 1989. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone glucoside: Isolation from strawberries and synthesis. *Phytochemistry* 28:631–3.

Mitchell WC, Jelenkovic G. 1995. Characterising NAD- and NADP-dependent alcohol dehydrogenase enzymes of strawberries. *J Am Soc Hortic Sci* 120:798–801.

Moyano E, Encinas-Villarejo S, López-Ráez JA, Redondo-Nevado J, Blanco-Portales R, Bellido ML, Sanz C, Caballero JL, Muñoz-Blanco J. 2004. Comparative study between two strawberry pyruvate decarboxylase genes along fruit development and ripening, postharvest and stress conditions. *Plant Sci* 166:835–45.

Nijssen LM, Visscher CA, Maarse H, Willemsens LC (eds.). 1996. *Volatile Compounds in Food, Qualitative and Quantitative Data*, 7th Ed. Zeist, The Netherlands: TNO Nutrition and Food Research Institute.

Nitsch JP. 1950. Growth and morphogenesis of the strawberry as related to auxin. *Am J Bot* 37:211–5.

Olbricht K, Grafe C, Weiss K, Ulrich D. 2008. Inheritance of aroma compounds in a model population of *Fragaria x ananassa* Duch. *Plant Breed* 127:87–93.

Olías R, Pérez AG, Sanz C. 2002. Catalytic properties of alcohol acyltransferase in different strawberry species and cultivars. *J Agric Food Chem* 50:4031–6.

Pérez AG, Olías R, Sanz C, Olías JM. 1996a. Furanones in strawberries: Evolution during ripening and postharvest shelf life. *J Agric Food Chem* 44:3620–4.

Pérez AG, Sanz C, Olías R, Ríos JJ, Olías JM. 1996b. Evolution of alcohol acyltransferase activity during fruit development and storage. *J Agric Food Chem* 44:3286–90.

Pérez AG, Olías R, Olías JM, Sanz C. 1999a. Biosynthesis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and derivatives in *in vitro* grown strawberries. *J Agric Food Chem* 47:655–8.

Pérez AG, Sanz C, Olías R, Olías JM. 1999b. Lipoxygenase and hydroperoxide lyase activities in ripening strawberry fruits. *J Agric Food Chem* 47:249–53.

Pérez AG, Ríos JJ, Sanz C, Olías JM. 1992. Aroma components and free amino acids in strawberry var. Chandler during ripening. *J Agric Food Chem* 40:2232–5.

Pérez AG, Sanz C, Olías JM. 1993. Partial purification and some properties of alcohol acyltransferase from strawberry fruits. *J Agric Food Chem* 41:1462–6.

Pyysalo T, Honkanen E, Hirvi T. 1979. Volatiles of wild strawberries, *Fragaria vesca* L. compared to those of cultivated berries, *Fragaria x ananassa* cv. Senga Sengana. *J Agric Food Chem* 27:19–22.

Raab T, López-Ráez JA, Klein D, Caballero JL, Moyano E, Schwab W, Muñoz-Blanco J. 2006. FaQR, required for the biosynthesis of the strawberry flavor compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone, encodes an enone oxidoreductase. *Plant Cell* 18:1023–37.

Re L, Maurer B, Ohloff G. 1973. An easy procedure for the synthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol), a flavor component of pineapple and strawberry. *Helv Chim Acta* 56:1882–94.

Roscher R, Bringmann G, Schreier P, Schwab W. 1998. Radiotracer studies on the formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in detached ripening strawberry fruits. *J Agric Food Chem* 46:1488–93.

Roscher R, Schreier P, Schwab W. 1997. Metabolism of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in detached ripening strawberry fruits. *J Agric Food Chem* 45:3202–5.

Sanz C, Olías JM, Pérez AG. 1997. Aroma biochemistry of fruits and vegetables. In Tomás-Barberán FA, Robins RJ (eds.), *Phytochemistry of Fruit and Vegetables*. Oxford: Clarendon Press, pp. 125–55.

Sanz C, Pérez AG, Richardson DG. 1994. Simultaneous HPLC determination of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and related flavor compounds in strawberries. *J Food Sci* 59:139–41.

Sanz C, Richardson DG, Pérez AG. 1995. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone and derivatives in strawberries during ripening. In Rouseff RL, Leahy MM (eds.), *Fruit Flavour*. Washington, DC: American Chemical Society, pp. 269–75.

Sasaki M. 1996. Isolation and identification of precursor of 4-hydroxy-2(or 5)-ethyl-5 (or 2)-methyl-3(2H)-furanone from isolated soybean protein and shoyu. *J Agric Food Chem* 44:230–5.

Sasaki M, Nunomura N, Matsudo T. 1991. Biosynthesis of 4-hydroxy-2(or 5)-ethyl-5 (or 2)-methyl-3(2H)-furanone by yeast. *J Agric Food Chem* 39:934–8.

Schieberle P, Hofmann T. 1997. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J Agric Food Chem* 45:227–32.

Schottler M, Boland W. 1995. Biosynthesis of dodecano-4-lactone in ripening fruit: Crucial role of an epoxide-hydrolase in enantioselective generation of aroma components of the nectarine (*Prunus persica* var. nucipersica) and the strawberry (*Fragaria x ananassa*). *Helv Chim Acta* 78:847–56.

Schreier P. 1980. Quantitative composition of volatile constituents in cultivated strawberries, *Fragaria ananassa* cv. Senga Sengana, Senga Litessa and Senga Gourmella. *J Sci Food Agric* 31:487–94.

Schwab W. 1998. Application of stable isotope ratio analysis explaining the bioformation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in plants by a biological Maillard reaction. *J Agric Food Chem* 46:2266–9.

Schwab W, Roscher R. 1997. 4-Hydroxy-3(2H)-furanones: Natural and Maillard products. *Recent Res Dev Phytochem* 1:643–73.

Souleyre EJF, Greenwood DR, Friel EN, Karunairetnam S, Newcomb RD. 2005. An alcohol acyl transferase from apple (cv Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. *FEBS J* 272:3132–44.

St-Pierre B, De Luca V. 2000. Evolution of acyltranferase genes: Origin and diversification of the BAHD superfamily of acyltransferases involved in secondary metabolism. In John RI, Romeo R, Varin L, De Luca V (eds.), *Recent Advances in Phytochemistry Evolution of Metabolic Pathways*, Vol. 34. Oxford: Elsevier Science Publishing, pp. 285–315.

Ulrich D, Hoberg E, Rapp A, Kecke S. 1997. Analysis of strawberry flavour—Discrimination of aroma types by quantification of volatile compounds. *Z Lebensm Unters Forsch* 205:218–23.

Ulrich D, Komes D, Olbricht K, Hoberg E. 2007. Diversity of aroma patterns in wild and cultivated *Fragaria* accessions. *Genet Resour Crop Evol* 54:1185–96.

Wang J, De Luca VD. 2005. The biosynthesis and regulation of biosynthesis of Concord grape fruit esters, including "foxy" methylanthranilate. *Plant J* 44:606–19.

Watkins B, Manzano-Méndez JE, Nock JF, Zhang JJ, Maloney KE. 1999. Cultivar variation in response of strawberry fruit to high carbon dioxide treatments. *J Sci Food Agric* 79:886–90.

Wein M, Lavid N, Lunkenbein S, Lewinsohn E, Schwab W, Kaldenhoff R. 2002. Isolation, cloning and expression of a multifunctional O-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2H)-furanone, one of the key aroma compounds in strawberry fruits. *Plant J* 6:755–65.

Wein M, Lewinson E, Schwab W. 2001. Metabolic fate of isotopes during the biological transformation of carbohydrates to 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry fruits. *J Agric Food Chem* 49:2427–32.

Williams A, Ryan D, Olarte-Guasca A, Marriott P, Pang E. 2005. Analysis of strawberry volatiles using comprehensive two-dimensional gas chromatography with headspace solid-phase microextraction. *J Chromatogr B* 817:97–107.

Winston AL. 1902. The anatomy of edible berries. *Connecticut Agr Exp Stn Rep* 26:288–325.

Wyllie SG, Fellman JK. 2000. Formation of volatile branched chain esters in bananas (*Musa sapientum* L.). *J Agric Food Chem* 48:3493–6.

Wyllie SG, Leach DN, Nonhebel H, Lusunzi I. 1996. Biochemical pathways for the formation of esters in ripening fruit. In Taylor A, Mottram DS (eds.), *Flavour Science, Recent Developments*. Cambridge, UK: Royal Society of Chemistry, pp. 52–7.

Yahyaoui F, Wongs-Aree C, Latché A, Hackett R, Grierson D, Pech JC. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyltransferase involved in the generation of aroma volatile esters during melon ripening. *Eur J Biochem* 269:2359–66.

Zabetakis I, Holden MA. 1996. The effect of 6-desoxy-D-fructose on flavour bioformation from strawberry (*Fragaria x ananassa*, cv Elsanta) callus cultures. *Plant Cell Tissue Organ Cult* 45:25–9.

Zabetakis I, Holden MA. 1997. Strawberry flavour: Analysis and biosynthesis. *J Sci Food Agric* 74:421–34.

CHAPTER 24

Temperate Fruit Juice Flavors

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The growth in fruit and vegetable consumption in the United States during the past two decades is attributed to the availability of new fruit and vegetable products, increased availability and convenience of these products, and increased interest in healthier lifestyles. Consumption of fruit juices accounts for 43% of fruit and vegetable consumption, primarily due to the convenience of these processed fruit products. Although apple juice and orange juice still account for a significant proportion of the market, juices from grapes, berries, and other fruits are also increasing in popularity (Pollack 2001). Consumer demand for greater variety and improved flavor in fruit juices has resulted in an increase in the production of blended juices. Berry juices are commonly used in blended fruit juices with the more traditional juices, including apple, orange, and cranberry (Roberts et al. 2004).

The health benefits associated with fruits and fruit juices have had a significant impact on the diversity of juices from different fruits available to consumers (Pollack 2001). The health-protective phytochemicals present in fruits and vegetables include vitamin E, vitamin C, β-carotene, and phenolic antioxidants (Gardner et al. 2000; Kaur and Kapoor 2001; Netzel et al. 2002). The juices from many of the fruits not only provide these health benefits but also have desirable flavor characteristics, which contribute to their high consumer acceptability.

Table 24.1 illustrates the similarities in the predominant volatile flavor compounds and their aroma description in the most common fruit juices from temperate growing regions. Each of the fruit juices contains key flavor compounds that characterize the unique flavor characteristics of these juices. However, for each fruit, there is also diversity in the content and the composition of the volatile flavor compounds, due to the effects of cultivar, growing conditions, maturity, and postharvest storage and handling. These factors may contribute to noticeable differences in the volatile flavor compounds identified in different studies. For each of these fruits and their respective fruit juices, differences in the minor volatile flavor compounds can contribute significant differences in the overall aroma characteristics of the juices (Pérez et al. 1992).

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TABLE 24.1. Aroma Descriptions of Volatile Flavor Compounds Frequently Identified in Fruits and Fruit Juices

Volatile Flavor Compound	Aroma Description	Apple	Grape	Strawberry	Raspberry	Blueberry
Esters						
Ethyl acetate	Chemical, fruity, orange		A	H		
Butyl acetate	Apple like	D, E, R, S				
2-Methylbutyl acetate	Apple like	B, C, E, I, S				
Pentyl acetate	Banana like, apple, fruity	M, R				
Hexyl acetate	Apple	B, C, E, I, M, R, S				
Benzyl acetate	Fresh			O		
Phenylethyl acetate	Sweet, pipe tobacco		A			
Ethyl propionate	Fruity	B, C, I, S				
Methyl butanoate	Floral/pineapple			H		
Ethyl butanoate	Apple, green, fruity/vanilla, floral	D, M	A	H, K	O	
Ethyl 3-hydroxybutanoate	Hay like, burnt marshmallow, muscadine		A			
Hexyl butanoate	Apple peel			O		
Ethyl 2-methylpropanoate	Fruity			H		
Methyl 2-methyl butanoate	Sweet, fruity					O
Ethyl 2-methyl butanoate	Cooked apple, fruity, green apple	E, F, M, Q	A	H		
Ethyl 3-methyl butanoate	Cooked pineapple			H		
Methyl hexanoate	Pineapple/fruity			H, O		
Ethyl hexanoate	Fruity/acid, green apple		A	H, O		
Ethyl octanoate	Fruity			O		
Ethyl decanoate	Grape			O		
Aldehydes						
Hexanal	Cut grass, green, green apple	D, G, J, M, Q	A	H, O	O	
trans-2-Hexenal	Sweet, green, sharp	D, G, J, M, Q		H		
cis-3-Hexenal	Grassy				N	
Octanal	Green, citrusy			O	O	
trans, *cis*-2,6-Nonadienal	Cucumber		A			
Phenylacetaldehyde	Caramel syrup, honey like, floral		A			
3-(Methylthio) propanal	Boiled potatoes, baked potatoes, nutty, stale		A			
Alcohols						
2- and 3-Methylbutanol	Malty, bitter, chocolate		A			
Hexanol	Green, earthy	M		O		
cis-3-Hexenol	Green grass					O

trans-2-Hexenol	Green, fruity	D				O
Heptanol	Chemical			O		
2-Octanol	Mushroom				O	
trans, *cis*-2,6-Nonadienol	Cucumber, melon		A			
Acids						
Acetic acid	Sour, pungent, vinegar		A			
2- and 3- Methylbutanoic acid	Sweaty, dried fruit		A			
Hexanoic acid	Fatty			O		
Ketones						
1-Hexen-3-one	Leaves, metallic				N	
1-Octen-3-one	Mushroom, woody, earthy		A		N	
1-Nonen-3-one	Pungent, mushroom				O	
2-Undecanone	Orange			O		
Terpenoid compounds						
Limonene	Lemon			O	O	O
Sabinene	Woody				O	
Linalool	Lemon				O	
Geraniol	Rose		A		O	
Citronellyl acetate	Rose			O		
β-Damascenone	Floral		A, P		N	
Furan compounds						
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	Caramel, cotton candy, burnt sugar		A	L, O	N	
2,5-Dimethyl-4-methoxy-3(2H)-furanone (mesifurane)	Sherry like			L		
3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	Maple, cotton candy				N, O	
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel like		A			
Phenolic compounds						
2-Phenylethanol	Honey, rosy		A			O
Methyl cinnamate	Strawberry			O		
o-Aminoacetophenone	Foxy, cat urine		A, P			

References: A, Baek and others (1997); B, Cheetham (2002); C, Dimick and Hoskin (1983); D, Duerr (1979); E, Echeverria and others (2004); F, Flath and others (1967); G, Flath and others (1969); H, Gomes da Silva and Chaves das Neves (1999); I, Lopez and others (1998); J, Panasiuk and others (1980); K, Pérez and others (1992); L, Pérez and others (1996a); M, Rizzolo and others (1989); N, Roberts and Acree (1996); O, Roberts and others (2004); P, Shure and Acree (1994); Q, Su and Wiley (1998); R, Williams and Knee (1977); S, Young and others (1996).

FLAVOR CHARACTERISTICS OF FRUIT JUICES

Esters, alcohols, aldehydes, terpenoids, ketones, ethers, and other volatile flavor compounds contribute to the unique flavor characteristics of fruits and fruit juices. Esters contribute floral and fruity aroma attributes and represent the major class of volatile flavor compounds in fruit juices. Alcohols and aldehydes contribute green and pungent aroma attributes. Terpenoid compounds are described as having piney, floral characteristics. Sugars, acids, and bitter compounds are important contributors to the taste of the juices and the overall flavor quality. Consumer acceptability of these fruit juices is a function of the presence of the key character-impact compounds and the balance of volatile and nonvolatile flavor compounds (Jella et al. 1998).

The key contributors to the fruity, apple-like aromas of apple juices are ethyl 2-methylbutanoate, hexyl acetate, ethyl propionate, 2-methylbutyl acetate, and butyl acetate (Cheetham 2002; Dimick and Hoskin 1983; Lopez et al. 1998; Young et al. 1996). Each of the apple cultivars has unique aroma characteristics shown by significant variability in the content and composition of the volatile flavor compounds (Lopez et al. 1998; Poll 1981; Williams et al. 1981; Young et al. 1996). Ethyl *trans*-2-*cis*-4-decadienoate is the major volatile flavor compound present in pears. Other esters, which are also found in apple juice, include ethyl-2-octenoate, hexyl acetate, ethyl-3-decenoate, butyl acetate, and ethyl butanoate (Cheetham 2002).

Ideally, apples should be processed into juice shortly after harvesting for the best aroma characteristics. For extended storage, controlled atmosphere storage is frequently used to reduce fruit respiration and delay ripening and spoilage. However, the production of the characteristic volatile flavor compounds typical of apple is altered in the reduced oxygen atmosphere. This environment results in a decrease in the content of the desirable apple aroma compounds, such as butyl and hexyl esters, aldehydes, and ketones and increases in ethanol, acetaldehyde, and ethyl esters (Boylston et al. 1994; Mattheis et al. 1991; Plotto et al. 1999).

In grape juice, β-damascenone is a key flavor component for most grape cultivars (Shure and Acree 1994). As with apples, different grape cultivars differ in their profile of volatile flavor compounds. *o*-Aminoacetophenone, methylfuraneol, and methyl anthranilate have been identified as important odor-active compounds in Concord grapes (Shure and Acree 1994). The characteristic candy and foxy-like aroma characteristics of muscadine grape juice is attributed to furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) and *o*-aminoacetophenone (Baek et al. 1997). In addition to these unique volatile flavor compounds, aldehydes and alcohols, with green and grass-like aromas, esters with fruity aromas, and aldehydes and alcohols with green and grass-like aromas contribute to the characteristic flavor of grape juice (Baek et al. 1997; Serot et al. 2001).

Esters, especially methyl and ethyl esters of butanoic and hexanoic acids, are major contributors to the volatile flavor compounds present in fresh strawberry (Gomes da Silva and Chaves das Neves 1999; Pérez et al. 1992). Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) and mesifurane (2,5-dimethyl-4-methoxy-3(2H)-furanone), characterized as having strong, sweet, and pleasant aromas, have also been shown to be important contributors to strawberry aroma (Pérez et al. 1996a). Strawberry aroma has been described as including caramel, jam, fruity, floral, and green flavor notes. The contents of these important compounds to strawberry

aroma and overall strawberry aroma intensity increase during ripening (Pérez et al. 1996a).

The descriptors piney, lemon like, floral, buttery, maple syrup, vanilla, raspberry like, and violet like have been used to describe raspberry aroma (Roberts and Acree 1996). β-Damascenone, sabinene, sotolon, 2-octanol, and limonene have been identified as important contributors to the aroma of raspberry.

The predominant volatile flavor compounds in blueberries are alcohols and esters. In general, blueberries are less aromatic and contain fewer volatile compounds than other small fruits (Roberts et al. 2004).

Sweetness and tartness are important contributors to the taste and flavor perception of fruit juices. Fructose, sucrose, and glucose are the most common sugars identified in fruit juices, contributing to the sweet taste of the juices. Organic acids, such as malic acid in apples and pears (Blanco et al. 1992) and tartaric and malic acids in grapes (Buglione and Lozano 2002; Montgomery et al. 1982), contribute tartness to the juices. The ratio of sugars to acids, rather than the absolute content of the sugars and organic acids, is most important in determining the relative sweetness or tartness of the juices. As with the volatile flavor compounds, there are significant differences in the sweetness and tartness of different cultivars within a given fruit. Strong correlations have been reported between titratable acidity and perceived tartness and soluble solids and perceived sweetness. However, most often, the sugar:acid ratio (soluble solids-to-titratable acidity ratio) makes a greater impact on the perceived sweetness and tartness of the fruit juices, as well as the flavor perception and balance, and overall consumer acceptability (Fellers et al. 1988).

FLAVOR COMPOUNDS GENERATED DURING RIPENING

The development of aroma during ripening contributes to the development of the characteristic flavors of the fruits and is critical to consumer acceptability of the fruits and fruit products (Pérez et al. 1996a). During the ripening and maturation process, significant changes occur through a series of biochemical reactions that contribute to the development of desirable flavor compounds from the carbohydrates, lipids, proteins, and other plant constituents (Dixon and Hewett 2000). Typically, the formation of volatile flavor compounds occurs during the latter stages of the ripening process when the enzymes, which catalyze the formation of the flavor compounds, become active (Pérez et al. 1992). Therefore, juice processed from immature fruit often lacks the esters and other volatile compounds that contribute desirable fruity flavor characteristics (Song and Bangerth 1994; Yahia et al. 1990).

The volatile esters are among the most important groups of compounds that contribute to desirable fruity aromas. β-Oxidation of fatty acids and oxidation of fatty acids by lipoxygenase form straight-chain alcohols and acyl-coenzyme A (CoA), which are the precursors needed for the formation of esters. Amino acids are precursors to aliphatic and branched-chain alcohols, acids, carbonyls, and esters, which are formed through a series of deamination, decarboxylation, reduction, and esterification reactions (Pérez et al. 1992). Aldehydes and alcohols are formed from acyl-CoA through the activity of acyl-CoA reductase and alcohol dehydrogenase, respectively (Bartley et al. 1985; Echeverria et al. 2004). However,

alcohol acyltransferase, which catalyzes the esterification of alcohols by carboxylic acids, is the rate-limiting step in the formation of these fruity esters (Defilippi et al. 2005; Pérez et al. 1996b). The activity of alcohol acyltransferase, as well as the content of the alcohol precursors, increases in ripening (Defilippi et al. 2005; Echeverria et al. 2004; Pérez et al. 1996b). A comparison of different cultivars of strawberry identified a strong relationship between alcohol acyltransferase activity, ester formation, and aroma quality and intensity (Pérez et al. 1996b; Shalit et al. 2001). The preferred substrate for alcohol acyltransferase is dependent on the fruit. Hexanol is the preferred substrate for alcohol acyltransferase in strawberry (Pérez et al. 1996b) and apple (Defilippi et al. 2005; Echeverria et al. 2004). In addition, 2-methylbutanol and butanol also function as important substrates for alcohol acyltransferase in apple (Defilippi et al. 2005; Echeverria et al. 2004). On the other hand, aliphatic, aromatic, and sulfur-containing alcohols function as precursors to form esters in melons (cv. Arava) (Shalit et al. 2001).

Glycosidically bound flavor precursors, in which the aglycones are derived from fatty acid, shikimate, and monoterpenoids and C13 norisoprenoid metabolism, have been identified in apples (Schwab and Schreier 1990), raspberry pulp (Pabst et al. 1991), and tomatoes (Buttery et al. 1990). Although these flavor precursors do not contribute significantly to the aroma of the raw fruits, enzymatic hydrolysis with pectinases catalyzes the release of these volatile flavor compounds resulting in a significant contribution to the flavor of the juices (Buttery et al. 1990; Pabst et al. 1991).

During the latter stages of ripening, degradation of starches to form sugars and the synthesis of organic acids contribute positively to the flavor characteristics of the fruits and fruit juices through the formation of sweet and tart tastes. Juices of fruits with higher sugar : acid ratios tend to have a greater fruity intensity and have a higher flavor quality than juices with a lower sugar : acid ratio (Roberts et al. 2004). Although the accumulation of sugars occurs at approximately the same time as aroma formation, it is believed that independent biochemical reactions control these different aspects of flavor development (Shalit et al. 2001).

FLAVOR COMPOUNDS GENERATED DURING JUICE PROCESSING

Juice is extracted from the raw fruits through a maceration and pressing process. The maceration process can increase the content of hexanal, *trans*-2-hexenal, and other lipid oxidation products through stimulation of lipoxygenase activity (Su and Wiley 1998). Treatment of the juice pulp with pectinases to increase the extraction of the juices and improve clarity tend to increase the content of the volatile flavor compounds due to the release of the volatile flavor compounds from the glycosidically bound flavor precursors (Buttery et al. 1990; Pabst et al. 1991; Su and Wiley 1998).

To further improve the extraction of the juice from the pulp, press aids are often used. Roberts and others (2004) found that the use of dried apple pomace as a press aid not only increased the yield of the juice from strawberries, raspberries, and blueberries, but also contributed increased contents of esters and other desirable fruity volatile flavor compounds to enhance the aroma of the juice. Conventional press aids, such as rice hulls, paper pulp, wood fibers, or diatomaceous earth, on the

other hand, can contribute undesirable volatile flavor compounds to the juice to result in soapy, resinous, and chemical flavor characteristics (Roberts et al. 2004).

Thermal processing of juices is the most common processing method to ensure the safety of fruit juices. The Food and Drug Administration (FDA) legislation that mandates the application of sterilization technologies to reduce the target microorganism by 5 logarithm cycles to ensure the safety of the juice is a result of numerous outbreaks of foodborne illnesses attributed to the consumption of freshly squeezed juices (CFR 2001). Pasteurization and irradiation are among the processing treatments that effectively achieve the required 5-$\log_{10}$ reduction. However, the effect of these processing treatments on the flavor quality of the juices is also of significant concern.

Many of the volatile flavor compounds present in fruit juices are subject to degradation during thermal processing treatments, resulting in significant losses in the natural fruity aromas and the development of cooked aromas due to the thermal degradation of the aroma compounds (Poll 1985; Poll and Flink 1983; Su and Wiley 1998; Wang et al. 2003). In an artificial apple juice, contents of typical apple juice flavor compounds decreased significantly following heating due to thermal degradation (Su and Wiley 1998). Heat processing of strawberry juice results in a decrease in the content of the green aroma volatile compounds and fruity esters and an increase in caramel-like flavors formed through the Maillard reaction (Schieberle 1994).

Heat treatment of low pH fruit juices results in the hydrolysis of glycosides and the release of volatile flavor compounds (Buttery et al. 1990; Roberts and Acree 1996). During the heating process, the glycosidic precursors of several key aroma compounds, including raspberry ketone (Roberts and Acree 1996) and β-damascenone (Roberts and Acree 1996), phenylacetaldehyde (Buttery et al. 1990), and benzaldehyde (Buttery et al. 1990), are degraded to release these potent aroma compounds and increase the aroma intensity of the fruit.

Irradiation is an effective nonthermal method for the reduction of pathogenic microorganisms in juice (Wang et al. 2004, 2006), although the irradiation treatment also results in the formation of off-flavor compounds, which are specific to the juice and dependent on the irradiation dose. Irradiation of cantaloupe juices at greater than 3 kGy resulted in juices described as having "cheesy off-odor mixed with fruit fragrance" or "oxidation odor of butterfat." The off-odor produced through the irradiation of melon juice have been attributed to the formation of alcohols, aldehydes, and ketones with six to nine carbons, which are not typically present in the juice, from polyunsaturated fatty acids (Wang et al. 2006).

Potassium sorbate is frequently added to juices during processing to inhibit yeasts and molds, control fermentation, and extend the shelf life (Baroody and McLellan 1986; Luedtke and Powell 2002; Wright et al. 2000). The presence of sorbate in apple cider prior to irradiation had a protective effect on the content of the esters and other volatile flavor compounds, which are important contributors to apple flavor (Crook and Boylston 2004; Crook et al. 2004). The increased stability of these volatile flavor compounds is attributed to the ability of the sorbate to quench free radicals that initiate oxidation reactions that form lipid oxidation products and degrade esters and other flavor compounds (Boylston et al. 2003). However, descriptive sensory panelists have also detected a significantly higher intensity of musty off-flavors in irradiated apple cider containing sorbate. These off-flavors are

hypothesized to be formed through the degradation of sorbate during irradiation. These compounds are believed to be present at low concentrations, yet due to their low threshold, are more likely to be detected by sensory than by instrumental methods (Yulianti et al. 2005).

The production of juice concentrates is a common processing method for the manufacture of fruit juices. Thermal evaporation under a vacuum effectively reduces the water content of the juices, although some of the important volatile flavor compounds are also lost during the concentration of the juice. The volatile flavor compounds recovered during the thermal evaporation are added back to the juices to improve the flavor quality of the reconstituted juices. In single-strength apple juice, methyl 2-methylbutanoate and ethyl 2-methyl butanoate, two characteristic compounds responsible for apple flavor, are added to the juice. However, in the gas chromatographic analysis of commercial apple juices, other atypical volatile flavor compounds were identified, including limonene and α-terpineol, and γ-decalactone. These compounds are considered to be carryover contaminants from other production lines and can adversely affect the flavor characteristics of the juices. The concentrations of the characteristic apple aroma compounds added to the juices as recovery aromas are also important. These esters have typical apple aroma characteristics at low concentrations but can result in apple juices with pineapple-like aftertaste at high concentrations (Elss et al. 2007).

OFF-FLAVOR FORMATION IN FRUIT JUICES

Exposure of juices to fluorescent lights during commercial display contributes to off-flavor formation in fruit juices. In cloudy apple juices stored in glass bottles, sensory panelists noted the formation of metallic, plastic-like, oxidized, rotten, and waxy off-flavors following exposure to fluorescent light (3000 lx for 48 h). 1-Octen-3-one, along with several other unsaturated aldehydes and ketones were determined to be major contributors to the off-flavors, using gas chromatography–olfactometry. The off-flavor formation was greater with juices containing higher contents of suspended solids, indicating that the insoluble fraction contains the precursors of the off-flavor compounds. Oxidation of not only unsaturated fatty acids, but also terpenes and lycopene during exposure to light are hypothesized to contribute to the formation of these off-flavor compounds (Hashizume et al. 2007).

Off-flavor formation following juice processing is frequently caused by spoilage microorganisms, such as *Alicyclobacillus acidoterrestris* and *Streptomyces* spp. In apple juices in which distinct off-flavors have been detected, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 2-methylisoborneol, 1-octen-3-ol, fenchyl alcohol, geosmin, guaiacol, and 2,6-dibromophenol were present in concentrations greater than their detection threshold and contributed musty, moldy, earthy aroma characteristics to the juice (Siegmund and Pöllinger-Zierler 2006).

THE FUTURE OF THE FRUIT JUICE INDUSTRY

The positive health benefits and convenience associated with fruit juices is expected to contribute to further growth of the fruit juice market. Furthermore, the develop-

ment of juice blends with unique flavor characteristics is expected to be an area for further expansion. However, the successful growth of the fruit juice industry is dependent on the flavor quality of the juices and utilization of processing technologies to maximize the desirable flavor quality of these juices.

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REFERENCES

Baek HH, Cadwallader KR, Marroquin E, Silva JL. 1997. Identification of predominant aroma compounds in muscadine grape juice. *J Food Sci* 62:249–52.

Baroody T, McLellan MR. 1986. The effect of pH, acidity, and low level sorbate on the shelf life of inoculated apple juice. *J Food Qual* 9:415–23.

Bartley IM, Stoker PG, Martin ADE, Hatfield SGS, Knee M. 1985. Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. *J Sci Food Agric* 36:567–74.

Blanco D, Morán M, Gutiérrez M, Moreno J, Dapena E. 1992. Biochemical study of the ripening of cider apple varieties. *Z Lebensm Unters Forsch* 194:33–7.

Boylston TD, Kupferman EM, Foss JD, Buering C. 1994. Sensory quality of Gala apples as influenced by controlled and regular atmosphere storage. *J Food Qual* 17:477–94.

Boylston TD, Wang H, Reitmeier CA, Glatz BA. 2003. Effects of processing treatment and sorbate addition on the flavor characteristics of apple cider. *J Agric Food Chem* 51:1924–31.

Buglione M, Lozano J. 2002. Nonenzymatic browning and chemical changes during grape juice storage. *J Food Sci* 67:1538–43.

Buttery RG, Takeoka G, Teranishi R, Ling LC. 1990. Tomato aroma components: Identification of glycoside hydrolysis volatiles. *J Agric Food Chem* 38:2050–3.

CFR. 2001. January 19 Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice. Final Rule. CFR part 120. Available from: Federal Register online via GPO access. DOCID:fr19ja01-26. http://wais.access.gpo.gov

Cheetham PSJ. 2002. Plant-derived natural sources of flavor. In Taylor AJ (ed.), *Food Flavour Technology*. Sheffield, UK: Sheffield Academic Press, pp. 105–52.

Crook LR, Boylston TD. 2004. Flavor characteristics of irradiated apple cider during storage: Effect of packaging materials and sorbate addition. *J Food Sci* 69:C557–63.

Crook LR, Boylston TD, Glatz BA. 2004. Effect of gas environment and sorbate addition on flavor characteristics of irradiated apple cider during storage. *J Agric Food Chem* 52:6997–7004.

Defilippi BG, Kader AA, Dandekar AM. 2005. Apple aroma: Alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. *Plant Sci* 168:1199–210.

Dimick PS, Hoskin JC. 1983. Review of apple flavor—State of the art. *CRC Rev Food Sci Nutr* 18:387–409.

Dixon J, Hewett EW. 2000. Factors affecting apple aroma/flavour volatile concentration: A review. *N Z J Crop Hortic Sci* 28:155–73.

Duerr P. 1979. Development of an odour profile to describe apple juice essences. *Lebensm Wiss Technol* 12:23–6.

Echeverria G, Graell J, López ML, Lara I. 2004. Volatile production, quality and aroma-related enzyme activities during maturation of "Fuji" apples. *Postharvest Biol Technol* 31:217–27.

Elss SL, Kleinhenz S, Schreier P. 2007. Odor and taste thresholds of potential carry-over/off-flavor compounds in orange and apple juice. *Lebensm Wiss Technol* 40:1826–31.

Fellers PJ, Carter RD, de Jager G. 1988. Influence of the ratio of degrees Brix to percent acid on consumer acceptance of processed modified grapefruit juice. *J Food Sci* 53:513–5.

Flath RA, Black DR, Guadagni DG, McFadden WH, Schultz TH. 1967. Identification and organoleptic evaluation of compounds in Delicious apple essence. *J Agric Food Chem* 15:29–35.

Flath RA, Forrey RR, Teranishi R. 1969. High resolution vapor analysis for fruit variety and fruit product comparisons. *J Food Sci* 34:382–6.

Gardner PT, White TAC, McPhail DB, Duthie GG. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem* 68:471–4.

Gomes da Silva MDR, Chaves das Neves HJ. 1999. Complementary use of hyphenated purge-and-trap gas chromatography techniques and sensory analysis in the aroma profiling of strawberries (*Fragaria ananassa*). *J Agric Food Chem* 47:4568–73.

Hashizume M, Gordon MH, Mottram DS. 2007. Light-induced off-flavor development in cloudy apple juice. *J Agric Food Chem* 55:9177–82.

Jella P, Rouseff R, Goodner K, Widmer W. 1998. Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. *J Agric Food Chem* 46:242–7.

Kaur C, Kapoor HC. 2001. Antioxidants in fruits and vegetables—The millennium's health. *Int J Food Sci Technol* 36:703–25.

Lopez ML, Lavilla MT, Riba M, Vendrell M. 1998. Comparison of volatile compounds in two seasons in apples: Golden Delicious and Granny Smith. *J Food Qual* 21:155–66.

Luedtke AN, Powell DA. 2002. A review of North American apple cider-associated *E. coli* O157:H7 outbreaks, media coverage and a comparative analysis of Ontario apple cider producers' information and production practices. *Dairy Food Environ Sanit* 22:590–8.

Mattheis JP, Buchanan DA, Fellman JK. 1991. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. *J Agric Food Chem* 39:1602–5.

Montgomery MW, Reyes FG, Cornwell C, Beavers DV. 1982. Sugars and acid analysis and effect of heating on color stability of Northwest Concord grape juice. *J Food Sci* 47:1983–6.

Netzel M, Strass G, Kaul C, Bitsch I, Dietrich H, Bitsch R. 2002. In vivo antioxidative capacity of a composite berry juice. *Food Res Int* 35:213–6.

Pabst A, Barron D, Etiévant P, Schreier P. 1991. Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *J Agric Food Chem* 39:173–5.

Panasiuk O, Talley FB, Sapers GM. 1980. Correlation between aroma and volatile composition of McIntosh apples. *J Food Sci* 45:989–91.

Pérez AG, Olías R, Sanz C, Olías JM. 1996a. Furanones in strawberries: Evolution during ripening and postharvest shelf life. *J Agric Food Chem* 44:3620–4.

Pérez AG, Sanz C, Olías R, Ríos JJ, Olías JM. 1996b. Evolution of strawberry alcohol acyltransferase activity during fruit development and storage. *J Agric Food Chem* 44:3286–90.

Pérez AG, Rios JJ, Sanz C, Olías JM. 1992. Aroma components and free amino acids in strawberry variety Chandler during ripening. *J Agric Food Chem* 40:2232–5.

Plotto A, McDaniel MR, Mattheis JP. 1999. Characterization of "Gala" apple aroma and flavor: Differences between controlled atmosphere and air storage. *J Am Soc Hort Sci* 124:416–23.

Poll L. 1981. Evaluation of 18 apple varieties for their suitability for juice production. *J Sci Food Agric* 32:1081–90.

Poll L. 1985. The influence of apple ripeness and juice storage temperature on the sensory evaluation and composition (volatile and non-volatile components) of apple juice. *Lebensm Wiss Technol* 18:205–11.

Poll L, Flink JM. 1983. Influence of storage time and temperature on the sensory quality of single-variety apple juice. *Lebensm Wiss Technol* 16:215–9.

Pollack SL. 2001. Consumer demand for fruit and vegetables: The U.S. example. In Regmi A (ed.), Changing Structure of Global Food Consumption and Trade. Agriculture and Trade Report No. WRS-01-01, U.S. Department of Agriculture, Economic Research Service. May 2001. http://www.ers.usda.gov/publications/wrs011/wrs011h.pdf

Rizzolo A, Polesello A, Teleky-Vamossy GY. 1989. CGC/Sensory analysis of volatile compounds developed from ripening apple fruit. *J High Resolut Chromatogr* 12:824–7.

Roberts DD, Acree TE. 1996. Effects of heating and cream addition on fresh raspberry aroma using a retronasal aroma simulator and gas chromatography olfactometry. *J Agric Food Chem* 44:3919–25.

Roberts JS, Gentry TS, Bates AW. 2004. Utilization of dried apple pomace as a press aid to improve the quality of strawberry, raspberry, and blueberry juices. *J Food Sci* 69: SNQ181–90.

Schieberle P. 1994. Heat-induced changes in the most odour-active volatiles of strawberries. In Marrse H, van der Heij DG (eds.), *Trends in Flavour Research*. New York: Elsevier Sciences, pp. 345–51.

Schwab W, Schreier P. 1990. Glycosidic conjugates of aliphatic alcohols from apple fruit (*Malus sylvestris* Mill cult. Jonathan). *J Agric Food Chem* 38:757–63.

Serot T, Prost C, Visan L, Burcea M. 2001. Identification of the main odor-active compounds in musts from French and Romanian hybrids by three olfactometric methods. *J Agric Food Chem* 49:1909–14.

Shalit M, Katzir N, Tadmor Y, Larkov O, Burger Y, Shalekhet F, Lastochkin E, Ravid U, Amar O, Edelstein M, Karchi Z, Lewinsohn E. 2001. Acetyl-CoA: Alcohol acetyltransferase activity and aroma formation in ripening melon fruits. *J Agric Food Chem* 48:794–9.

Shure KB, Acree TE. 1994. Changes in the odor-active compounds in *Vitis labruscana* cv. Concord during growth and development. *J Agric Food Chem* 42:350–3.

Siegmund B, Pöllinger-Zierler B. 2006. Odor threshold of microbially induced off-flavor compounds in apple juice. *J Agric Food Chem* 54:5984–9.

Song J, Bangerth F. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. *Acta Hortic* 368:150–9.

Su SK, Wiley RC. 1998. Changes in apple juice flavor compounds during processing. *J Food Sci* 63:688–91.

Wang H, Reitmeier CA, Glatz BA. 2004. Inactivation of *Escherichia coli* O157:H7 and other naturally occurring microorganisms in apple cider by electron beam irradiation. *J Food Prot* 67:1574–7.

Wang H, Reitmeier CA, Glatz BA, Carriquiry AL. 2003. Mixed model analysis of sensory characteristics of irradiated apple cider. *J Food Sci* 68:1498–1503.

Wang Z, Ma Y, Zhao G, Liao X, Chen F, Wu J, Chen J, Hu X. 2006. Influence of gamma irradiation on enzyme, microorganism, and flavor of cantaloupe (*Cucumis melo* L.). *J Food Sci* 71:M215–20.

Williams AA, Knee M. 1977. The flavour of Cox's Orange Pippin apples and its variation with storage. *Ann Appl Biol* 87:127–31.

Williams AA, Lewis MJ, Tucknott OG. 1981. The neutral volatile components of cider apple juices. *Food Chem* 6:139–51.

Wright JR, Sumner SS, Hackney CR, Pierson MD, Zoecklein BW. 2000. A survey of Virginia apple cider producers' practices. *Dairy Food Environ Sanit* 20:190–5.

Yahia EM, Acree TE, Liu FW. 1990. The evolution of some odour-active volatiles during the maturation and ripening of apples on the tree. *Lebensm Wiss Technol* 23:488–93.

Young H, Gilbert JM, Murray SH, Ball RD. 1996. Causal effects of aroma compounds on Royal Gala apple flavours. *J Sci Food Agric* 71:329–36.

Yulianti F, Reitmeier CA, Glatz BA, Boylston TD. 2005. Sensory, flavor and microbial analyses of raw, pasteurized and irradiated apple ciders. *J Food Sci* 70:S153–8.

CHAPTER 25

Fruits from Central and South America

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INTRODUCTION

The flavor of exotic tropical fruits is of increasing interest for consumers throughout the world. Some tropical fruits, for example, passion fruit, already possess a great marketing potential, and, consequently, the demand for the respective flavorings increases. World production of some of the important tropical fruits of Central and South America is shown in Table 25.1. Lesser known fruit crops are either of regional commercial importance or are appealing to flavorists and analytic chemists because of their sensory properties.

The typical flavor of fruits is not present during early fruit formation but develops entirely during a rather brief ripening period. This flavor development period, or ripening, occurs during the climacteric rise in respiration. During this period, metabolism of the fruit changes to catabolism, and flavor formation begins. Minute quantities of lipids, carbohydrates, proteins, and amino acids are enzymatically converted to simple sugars or acids and volatile compounds. The rate of flavor formation reaches a maximum during the postclimacteric ripening phase (Reineccius 2006).

A typical fruit may have well over a hundred different volatile components, but in total, these compose only a few parts per million of the entire fruit (Fisher and Scott 1997). All fruits share a very high proportion of the same volatile compounds. For example, of the 17 esters identified in banana volatiles, only five are not found in apples. Most volatile constituents in fruits contain aliphatic hydrocarbon chains, or their derivatives (esters, alcohols, acids, aldehydes, ketones, lactones), with saturated ones predominating apples, unsaturated ones predominating in pears, and branched chains predominating in bananas (Fisher and Scott 1997). Esters are by far the largest chemical category of volatiles from fruits. The apple, which has a prominent odor, largely produces and emits esters of relatively low molecular weight. Pear odors are more subtle and contain esters of higher molecular weight.

Fruit aromas vary widely. Citrus, such as grapefruit, orange, lemon, and lime, are rich in terpenoids, whereas most non-citrus fruits, such as apple, raspberry, cranberry,

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TABLE 25.1. World Production of Avocado, Pineapple, and Papaya Fruits in 2000 (000 Metric Tons)

Name of Fruit	Total World Production	Total South American Production	Leading Producer Countries in South America
Avocado	2,406	429	Chile: 100 Columbia: 75
Pineapple	13,504	2,307	Brazil: 1353 Columbia: 408
Papaya	7,227	3,752	Brazil: 3300 Peru: 165

Source: FAO (2002).

and banana, are characterized by esters and aldehydes (Fisher and Scott 1997). In general, aldehydes are common to fruit flavors and are believed to play an important role in many fruits (Werkhoff et al. 1998). Literature data about the flavor compounds or the composition of volatiles of Central and South America fruits are scarce.

HISTORY AND BACKGROUND OF FLAVOR

Progress in flavor research has been an evolutionary process. From a historical view, flavor research was significantly driven by advances in instrumentation. Great strides were made when gas chromatography (GC) became generally available. Prior to GC, the isolation, separation, and identification of unknown volatile compounds was an extremely tedious task. The advent of fused silica capillary GC columns was particularly significant since fused silica column development did not limit high-resolution chromatography to a handful of experts but made it possible for all. The development of low-cost quadrupole mass spectrometers also has resulted in significant advances in flavor research. Low-cost instruments with excellent GC compatibility have also put this technique in the hands of many flavor researchers who otherwise could not afford the technique (Martin and Martin 1999).

Beyond instrumental developments, flavor chemistry has evolved in terms of understanding. Initially, researchers used GC–mass spectrometry (MS) to identify long list of aroma chemicals in foods. This has resulted in nearly 7000 aroma compounds identified in foods today (Maarse and Visscher 1994). Many of these aroma compounds are present naturally in foods, while others are the result of fermentation, thermal processing, or deteriorative reactions (e.g., lipid oxidation). Very soon it was established that food flavors could not be regenerated from these lists, and some logical approach had to be formulated to determine which aroma compounds made a significant contribution to food aroma and which were insignificant. Those aroma compounds that smelled like the food were considered most important. Unfortunately, many foods did not contain "character-impact compounds" but the aroma was the result of a combination of numerous noncharacteristic odorants. Historically, considerable effort has been devoted to identifying mechanisms of flavor formation in plants (biosynthesis), during heating (Maillard reaction), and fermentation (Reineccius 1999).

ANALYTIC METHODOLOGY OF FRUIT FLAVORS

The analysis of food has been dramatically improved over the past 30 years, mainly through the invention of new work-up procedures, modern separation techniques (high-resolution gas chromatography [HRGC] on fused silica capillaries, high-performance liquid chromatography [HPLC]), and more sensitive spectroscopic methods (nuclear magnetic resonance, MS, Fourier transform infrared spectroscopy [FTIR]), some of them being available as hyphenated techniques (HRGC-MS, HRGC-FTIR). This continuous development has, over the years, led to results and publications about virtually every food flavor. Thousands of flavor compounds have been identified in the different foods and are collected in libraries (Nijssen et al. 1996).

An important factor in properly interpreting any chromatogram is to understand how the flavor volatiles were concentrated and introduced on to the column. Today, steam distillation extraction is generally avoided as it is known to produce thermal decomposition artifacts. Current practices either extract the flavor components from the liquid or solid with a low boiling liquid, supercritical fluid or distill the volatile components under vacuum. Another possibility is to work with headspace gases and trap their volatiles either cryogenically or on a solid phase absorbent. Arthur and Pawliszyn (1990) used solid phase microextraction (SPME) with subsequent thermal desorption using fused silica fibers. This technique has been used to extract flavor volatiles in model flavor systems, as well as coffee, fruit juice and a butter-flavored vegetable oil (Yang and Peppard 1994). The most common methods to isolate unknown odorants from food products are distillation and solvent extraction combined with chromatography. However, introduction of artifacts, loss of highly volatile components during concentration, and coelution of odorants with the solvent can distort the results.

A method to isolate flavor compounds that is less prone to bias is headspace analysis. It is superior to extraction because in this the nonvolatile artifact-forming compounds are not extracted; therefore, the results are more representative of the system sampled. There are two types of headspace analysis: static and dynamic headspace (DHS) analysis. Static headspace analysis involves sampling air equilibrated above a food sample followed by direct injection into a GC-MS for identification and quantification. Compounds that would normally coelute with the solvent can be detected by static headspace analysis. To be detected by GC-MS analysis, volatiles must be present at levels equal to or greater that 10^{-5} g/L. The concentration of volatiles above a food product ranges from 10^{-11} to 10^{-4} g/L (Reineccius 1994), and humans can smell some aroma compounds in food that have concentrations less than 10^{-12} g/L (Fazzalari 1978); therefore, only the most abundant volatiles will be detected by this method.

DHS analysis uses a gas to purge the volatiles from a sample and is often more sensitive than static headspace methods (Reineccius 1994). Purge-and-trap analysis is a DHS method that traps the volatiles purged from a sample on an absorbent solid. The trap is designed to concentrate the volatiles, which are subsequently desorbed for GC-MS analysis. An advantage of this method is the ability to concentrate the product by a hundred- to a thousandfold (Taranishi and Kint 1993).

In the past decades, flavor volatiles have mainly been isolated by means of liquid–liquid extraction, simultaneous distillation and extraction, or DHS analysis. It is

TABLE 25.2. Major Volatile Compounds of Avocado Fruit

Group	Type of Compound(s)
Alcohols	Ethanol, pentanol, hexanol, (*Z*)-nerolidol
Esters	3-Methyl-butanol
Aldehydes	Tetradecanal, hexanal, deca-2(*E*), 4(*Z*)-dienal, deca-2(*E*), 4(*E*)-enal, hept-2(*E*)-enal, octanal, dec-4-enal, dec-2(*E*)-enal, deca-2(*E*), 4(*E*)-dienal
Hydrocarbons/ terpenes	α-Farnesene, myrcene, α-cubebene, α-copaene, β-caryophyllene, α-humulene, α-cadinene, oct-2(*E*)-ene, limonene, octane, δ-elemene, α-cubebene, elemene isomer, β-cubebene, bisabolene
Lactones/acids	3-Hydroxy-2-butanone, acetic acid
Others	2-Pentylfuran, 2-heptylfuran, caryophyllene oxide

Source: Lopez and others (2004); Sinyinda and Gramshaw (1998).

well-known from the literature that the composition of a flavor extract is dependent on the isolation procedure employed (Werkhoff et al. 1998). Large differences in the composition of volatiles have been observed when using different isolation techniques. For example, simultaneous distillation–extraction under atmospheric pressure (SDE) in a modified Likens–Nickerson apparatus (Schultz et al. 1977) led to an aroma spectrum of passion fruit aroma with much more variation than that obtained by liquid–liquid extraction (Engel and Tressl 1983a).

Microwave processing offers an alternative to blanch fruits, since the application of high temperature and short time often results in minimum damage. Lopez and others (2004) investigated the effect of microwave time, pH, and avocado leaves (independent variables) on avocado flavor (response) using SPME-GC-MS. Some of the important flavor compounds present in avocado fruit are listed in Table 25.2.

The development and use of SPME as an alternative to the traditional methods has grown enormously in recent years (Arthur and Pawliszyn 1990). This technique is highly sensitive, inexpensive, portable, reproducible, and rapid, and is a solvent-free method for extraction. The overall procedure involves the direct exposure of an SPME fiber to the headspace of or immersed in a sample in a closed vial. Once equilibrium has been reached, the fiber is introduced into a GC-MS for separation, identification, and quantification. Overall, if a qualitative approach to flavor analysis is desired, then the DHS and SPME sampling techniques have definite advantages related to their ability to concentrate the sample volatiles, thus facilitating structure identification. However, any quantitative aspects of both these methods are directly related to the sampling time and thus must be monitored closely (Coleman and Lawrence 1997).

Headspace analysis in the early stages was done simply by sampling vapors from various products with a syringe and injecting the vapors in a GC (Taranishi and Buttery 1962). Now, there are many sophisticated applications and variations of this method: purge-and-trap, cold trap, trap with polymeric solid phase, and so on. This method permits analyses with minimum delay from sampling to analysis, with the minimum of introduction of artifacts developed or introduced during sampling. Unless one resorts to vacuum distillation, distillation usually means that the sample has been exposed to temperatures above ambient. At high temperatures, some of

the thermally unstable compounds are destroyed, and some artifacts are produced by combination of fragments formed. Thus, headspace analyses permit analyses of volatiles emitted by plants or food products to provide data representing fresh flowers, fresh fruits, and vegetables, of products as they are, not data containing artifacts formed during distillation and/or extraction.

Significant differences for individual flavor compounds were observed in both the qualitative and quantitative compositions of extracts obtained by different isolation procedures (Werkhoff et al. 1998). The difference between the two headspace procedures, vacuum headspace (VHS) and DHS, is very obvious in that the DHS extract is mainly dominated by the more volatile components. A typical example is ethyl butyrate that comprised 23% of the DHS extract, while the VHS extract contained only 4.7%. These findings are typical for the DHS method and represent the physical principle of this procedure. The VHS method "extracts" much more of the higher boiling point components and delivers a much more balanced composition of the total flavor. Most of the higher boiling and more polar lactones, for example, that are very potent and important flavor compounds (e.g., γ-decalactone, *cis*-γ-jasmin lactone, *trans*-γ-jasmin lactone, γ-dodecalactone) could not be detected in the DHS experiment. Another example is the sulfur-containing flavor compounds, for example, 3-mercaptohexanol, 3-(methylthio) hexanol, 3-(methylthio) hexyl butyrate, 3-mercaptohexyl hexanoate, and 3-(methylthio) hexyl hexanoate, which are missing in the DHS extract. So, it can be easily explained why the DHS extract received a poor sensory judgment. On the other hand, the simultaneous distillation–extraction under vacuum (SDEV) extract was judged, by sensory evaluation, as being somewhat typical compared with the fresh fruit. The SDEV seems to be a valuable alternative to the SDE, which produces thermally induced artifacts and consequently quite atypical extracts. Typical examples for thermal degradation reactions are the monoterpenes 2,6,6-trimethyl-2-vinyltetrahydropyrane, the *trans*- and *cis*-anhydrolinalool oxides, nerol oxide, and *trans*-ocimenol, as well as some furans like 2-methyl-3(2*H*)-furanone, fufural, 5-methylfurfural, and furfuryl alcohol. All of the compounds mentioned were only identified in the SDE.

GC-MS is a powerful tool for the separation and characterization of chemicals whether they are odor active or not. In the analysis of flavor, GC-MS can selectively focus on the odor-active compounds once their spectral and chromatographic properties are known. However, the task of determining which compounds in a sample are odor active requires a bioassay. The type of constituents that may contribute to the characteristic sensory properties of the food product needs to be investigated (Taranishi 1998).

Since fruit flavors exist as complex chemical mixtures, analysis has primarily involved separation technologies. Advances in the analysis of flavors in general can be applied to fruit flavor research. Fruit flavors involve both volatile and nonvolatile flavor components. Sweet and sour are the major nonvolatile flavors in fruit, and HPLC has been the method of choice to determine the corresponding individual sugars and acids. Major fruit flavor-impact components are usually volatile and are typically determined using capillary GC, GC, GC-MS, or GC-FTIR including multidimensional GC, where the second column is a chiral column. Yellow passion fruit belongs to the best known tropical fruits in the world. It has its origin in South America and has a floral, estery aroma with a distinct tropical, sulfury note. The flavor of yellow passion fruit is quite complex in its composition. There are no real

character-impact compounds, but the flavor is a delicate balance of different chemical classes, for example, fruit esters, green compounds, monoterpenes, sulfur compounds, and lactones. The volatile composition of yellow passion fruits was reviewed by Whitfield and Last (1986) as well as by Shibamoto and Tang (1990).

Using passion fruit, Werkhoff and others (1998) compared four different flavor isolation techniques: VHS method, DHS method, SDE, and SDEV to obtain aroma concentrates that are truly representative of tropical passion fruit flavor. The most representative and typical extract was obtained by VHS sampling and subsequent liquid–liquid extraction of the aqueous phase. This VHS concentrate was prefractionated by medium-pressure adsorption chromatography on silica gel. For the first time, approximately 180 components were identified in the liquid chromatography fractions of passion fruit flavor. Of these compounds, 14 components have not been previously reported as naturally occurring flavor ingredients (Werkhoff et al. 1998). They clearly demonstrate that the composition of the extracts is dependent on the isolation procedures employed. There are numerous variations to sample concentration but their complete discussion is beyond the scope of this chapter.

Numerous reviews on sample preparation and isolation procedures have been published (Buttery and Ling 1996; Parliment 1997; Taranishi and Kint 1993; Wampler 1997), and the influence of these different techniques on the resultant flavor extracts has been discussed in detail by a number of workers (Blank 1997; Krumbein and Ulrich 1996; Misharina et al. 1994; Mistry et al. 1997).

FRUIT FLAVOR IN PROCESSED FOOD PRODUCTS

In commercial products, initial flavor has little meaning, if that same flavor is not present when the product is consumed. The flavor changes that occur between production and consumption are of enormous interest to the food and flavor industry. Many factors have to be considered. Because of their commercial importance, most of the work in this area has been done with apple or orange juice. Little work in this area has been done with tropical fruits.

Lopez and others (2004) characterized 23 flavor volatiles from avocado purees after microwave treatment at different pH values, and after the addition of avocado leaves by response surface analysis, and analysis by SPME-GC-MS, 19 compounds were derived from lipid oxidation and only 4 from the avocado leaves. In unprocessed avocado puree, six different volatile compounds were found: ethanol, 3-methylbutanol, acetic acid, 3-hydroxy-2-butanone, hexanol, and pentenol. In contrast, the compounds found in processed avocado were aldehydes, alcohols, and ketones, being 2-heptenal [*E*] the most abundant of these compounds, followed by octanal, 1-octen-3-one, and 2-octenal [*E*]. On avocado leaves, the main volatile compounds were identified as estragol, terpenoids, and 2-hexenal. In particular, terpenoids and volatile compounds derived from lipid degradation showed an interesting pattern depending on the variety, degree of ripeness, and extraction method. Previous studies (Sinyinda and Gramshaw 1998) reported 23 volatile compounds in fresh avocado; the larger differences compared with the results of Lopez and others (2004) may be due to the avocado variety as well as the extraction method used. When avocado leaves were added to avocado puree processed with microwaves, terpenoids (α-pinene, β-pinene, eucalyptol) and estragole were found in addition to

lipid volatile compounds. This enriches the aroma of microwaved puree. A decrease of hexanal content, together with an increase in the 2-hexanal [*E*] level was also found. This last compound imparts fresh and green flavor notes, which improve the sensorial quality of the puree. The levels of α-O-pinene, β-pinene, 2-hexenal [*E*], and estragole increased after microwave treatment of avocado leaves, whereas limonene, eucalyptol, and copaene showed a slight decrement. Estragole and 2-hexenal [*E*] showed increments of approximately 1.1- and 3-fold, respectively. From the statistical analysis of the experimental design, it was possible to determinate that the most important factors influencing the abundance of flavor compounds derived from lipids were microwaving time and pH. Surface response analysis showed that as the pH decreased and heating time increased, there was an increase in volatile compounds derived from lipids, and thus, lipids volatiles were strongly influenced by time and pH. The volatile content derived from avocado leaves increased when leaf content and heating time increased.

FLAVOR VARIETAL STUDIES

Because specific fruit cultivars can deliver very different flavor characters, research in the characterization of differences in flavor volatiles among cultivars has been conducted. Many of these investigations focus on correlating analytic and sensory data. Besides varietal differences, environmental factors, such as variation in growing temperatures, rainfall, irrigation, and soil nutrients, can change the amount and type of flavor compounds present in plants. It is generally accepted that stressed plants increase production of secondary metabolites and thus produce more flavorful fruits (Cook et al. 2003).

FLAVOR FORMATION IN FRUITS

Knowledge of precursors and pathways leading to the formation of flavor in fruits has progressed slowly over the years. Historically, emphasis has been placed on determining what constitutes flavor rather than on the mechanism of flavor formation. The continued demand for natural flavorings has renewed interest in this research area since a knowledge of biological pathways facilitates their production (or enhancement) under conditions that permit their labeling as natural (Reineccius 2006). This most often involves biotechnology (enzymology or fermentation).

Flavors are formed from major plant constituents under genetic control. Each metabolic pathway is connected to other metabolic pathways. As direct products of a metabolic pathway, or as a result of interactions between pathways or end products, a host of volatile compounds are produced, which contribute to the flavor (aroma) of ripe fruit (Reineccius 2006).

Two basic routes of flavor formation can be differentiated: biosynthesis of compounds via genetically determined pathways (Herderich 1999; Jones and Young 1996) and thermally induced reactions resulting in volatile compounds (Tressl and Rewicki 1999). In both areas, the investigation of model systems starting from potential precursors or intermediates plays an important role (Guntert et al. 1992; Williams et al. 1992).

BIOGENESIS OF FRUIT FLAVOR

It is generally recognized that fruit aroma varies qualitatively and quantitatively depending on the cultivar, maturity stage, climate and cultural conditions, and the production area for each cultivar. The most rapidly growing area in fruit flavor research is focused on understanding the biogenesis of flavor compounds and characterization of precursors. Hendrich and Winterhalter (1991) conducted significant research in the biogenesis of flavor volatile in passion fruit. Although esters are qualitatively and quantitatively one of the most important class of volatile compounds in fruit aroma, there are very few reports on the biochemical aspects of ester formation in fruits.

BIOSYNTHESIS OF FRUIT FLAVOR—LACTONES

Lactones are important flavor substances for pineapples, papayas, and passion fruits. Due to their low odor threshold, they have a high flavor value in the fruits. These lactones are made fairly expensively via chemical synthesis from keto acids. On the other hand, microbiologically produced lactones have the advantage of being pure optically and natural. There are numerous microorganisms that are known to synthesize lactones. Lactones can be formed by de novo synthesis; by β-oxidation from ricinoleic acid, free fatty acids, or hydroxyl acids; and by reduction from unsaturated lactones or from cheese (Leahy and Roderick 1999).

FACTORS AFFECTING THE DEVELOPMENT OF FLAVOR

There is little question that geographic location influences flavor of fruits. It is known that climate and soil conditions influence flavor, and that often the best fruit comes from handpicking of fresh and fully ripened fruit right from the tree. There is little disagreement that numerous factors influence the flavor in a plant. There is a great deal of literature available on the differences in flavor due to genetics, environment, harvesting time, and postharvest treatment (Baldwin et al. 2000; Fellman et al. 2000; Paillard 1981, 1990). Genetics determine the enzyme systems and precursors involved in flavor formation. Soil nutrition provides some of the essentials for flavor development. Growing conditions influence activity of different enzyme systems and can significantly alter flavor development. Certainly, the stage of maturity and storage conditions can further influence flavor.

AVOCADO

The avocado (*Persea americana* Mill.) belongs to the Lauraceae, a family of mainly (sub) tropical trees and shrubs. The English name derives from the Spanish word abogada, an adaptation of an Aztec word "ahuacatl," which became avocet in French (Samson 1986). Avocado has been originated in Central and South America, Mexico, and is now cultivated in all the tropical and subtropical regions.

The avocado has been popular for thousands of years, and still is, a popular food in Central America. It is a nutritious fruit but the sugar content is low; therefore, it

can be recommended as a high-energy food for the diabetic (Samson 1986; Swisher 1988). The avocado's status as a food varies with the region where it is consumed and the degree of familiarity with which it is regarded by the local populace. The fruit is a traditional staple in Guatemala and nearby countries. In some countries, the fruit is eaten with sugar, or in ice cream or milk shakes.

Volatile Components of Avocado Fruit

The chemical compositions of the edible portion of the avocado flesh are water 65%, protein 1–4%, sugar about 1%, and oil 3–30%. It is rich in B complex vitamins and moderately so in vitamins A and D. The oil, which is similar in composition to olive oil, is highly digestible. Because of the high oil content, avocados have the highest energy value of any fruit. The high oil content also contributes to the consistency and the special taste of the fruit (Purseglove 1968).

Sinyinda and Gramshaw (1998) reported that in the immediate extract of the avocado mesocarp, β-caryophyllene (60%) was the main sesquiterpene, followed by α-humulene (5.9%), caryophyllene oxide (4.8%), α-copaene (4.5%), and α-cubebene as the main hydrocarbons; alkanals were present but only in low concentrations. In the extract prepared following storage (2 h) of the mesocarp at room temperature, β-caryophyllene (28.8%) was the main sesquiterpene, followed by α-copaene (10.7%), a cadinene isomer (8.5%), α- and β-cubebene (7.7%), α-farnesene (5.3%), and octane (4.8%) as principal hydrocarbons; decenal (6.3%) and heptenal (3.2%) were the main aldehydes.

A greater number of aldehydes have been reported in the "stored" avocado mesocarp, 11 compounds compared with 7 in the "unstored" extract (Sinyinda and Gramshaw 1998). Yamaguchi and others (1983) found no sesquiterpenes in avocado mesocarp extracted under reduced pressure, but found 2-methyl-2-butanal, *N*,*N*-dimethyl formamide, and methanol, which were not found by Sinyinda and Gramshaw (1998).

PINEAPPLE

Pineapple (*Ananas comosus* L. Merr.), one of the most popular tropical fruits in the world, has been cultivated in South America since the 15th century. However, it was not until the beginning of the 20th century that advanced canning technology made it possible to deliver green pineapple to people all over the world. People now enjoy the pineapple's unique sweet and sour flavor either as a green fruit or in processed/canned form, such as in cakes or pies (Umano et al. 1992).

The pineapple plant (*A. comosus*, Bromeliaceae) originated from Brazil, and approximately 100 pineapple varieties are now known worldwide (Rohrbach et al. 2003). Native to Central and South America, pineapple grows in several tropical areas such as Hawaii, Australia, Mexico, India, Malaysia, the Philippines, and Thailand. Pineapple varieties are plentiful, but only a few leading types are commercially available. Thailand is the world's largest producer of fresh as well as canned pineapples. Thai pineapple accounts for nearly 18% of the global market, followed by the Philippines (14%) and Brazil (13%) (Ti 2000). The pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavor and refreshing sugar–acid balance.

Aroma Profiles of Pineapple and Pineapple Products

Pineapple juice is a by-product that originates during the production of canned pineapples. The outflowing juice, the pulp from the peel, and the pineapple core are the starting materials for the juice production. These pineapple parts are squeezed with the help of mills and screw presses resulting in the so-called single-strength juice after pasteurization (Askar and Treptow 2001). However, the majority of commercial pineapple juice is made from concentrate. Usually, thermal concentration is employed resulting in the flavorless concentrate and the flavor containing aqueous water phase. The volatiles from the latter can be further enriched by the techniques common in flavor technology leading to recovery of pineapple aroma. In order to reconstitute the juice, the legislation requires to combine concentrate and recovery aroma under dilution with water.

From previous studies carried out on pineapple flavor (Engel et al. 1990; Spanier et al. 1998; Takeoka et al. 1989, 1991; Teai et al. 2001; Umano et al. 1992; Wu et al. 1991), information about its composition and the contribution of several constituents to the overall flavor has been provided. In addition, the sensitivity of the genuine pineapple fruit flavor, which can be easily modified in the course of fruit processing, that is, from the postharvest storage until thermal procedures, is well-known.

Flavor Profile of Pineapple

The pineapple fruit aroma has already been studied extensively, leading to the identification of the classical "pineapple furanone" 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) (Rodin et al. 1965; Willhalm et al. 1965), esters such as methyl and ethyl 2-methylbutanaate, methyl hexanoate, and methyl and ethyl 3-(methylthio) propanoate, as well as several hydroxyl and acetoxy esters and γ-lactones as well as "key constituents" (Engel et al. 1990; Takeoka et al. 1989, 1991; Teai et al. 2001; Umano et al. 1992; Wu et al. 1991).

Elss and others (2005) identified more than 130 constituents in fresh pineapple fruit volatiles by HRGC-MS analysis. The qualitative pineapple fruit flavor profile reported by Elss and others (2005) agreed with the information provided earlier by Engel and others (1990), who have reported several methyl esters and some characteristic sulfur-containing esters, various hydroxyl esters and their corresponding acetoxy esters, as well as a number of lactones being responsible for the typical pineapple flavor profile. Quantitatively, however, the pineapple cultivars studied by Elss and others (2005) showed an extremely wide range of variations in the amount of volatiles.

Only a part of the water phases/recovery aromas (20%) under study contained a high number of the typical volatiles as found in the juices made from fresh-cut pineapple fruits (Elss et al. 2005). In comparison with the fruit flavor profile, however, significant differences have been found. In most cases, the characteristic methyl esters and hydroxyl or acetoxy esters were lacking completely or appeared only in minor amounts. There could be many reasons that might explain these findings. These can mainly be attributed to the selection of inappropriate fruit varieties and flavor losses or modifications during postharvest handling or in the course of distillation and flavor recovery (Elss et al. 2003). However, some of the processors are

now able to provide appropriate qualities; the problem, therefore, should rather be an economic than a technological one. As expected, the flavor profile of pineapple juice concentrates was determined by thermally formed compounds such as, for example, furfural and 4-vinylguaiacol and non-distillable substances, as typically furaneol (Elss et al. 2005).

The aroma profile of a number of commercial pineapple juices of both the single-strength juices as well as the products made from concentrates showed much less detectable compounds and quantitatively, also the amount of aroma constituents comprised only a small part of that found in juices made from fresh-cut fruits (Elss et al. 2005). Compared with the juices made from concentrates, the volatile profiles of the single-strength juices match better with the genuine fruit profiles. As expected, they also showed some components produced during thermal treatment (pasteurization), such as, for example, furfural, 3-hydroxy-[2H]-pyran-2-one, pantolactone, furaneol and 5-(hydroxymethyl) furfural (Elss et al. 2005). Hodgson and Hodgson (1993) reported a comparative data on the composition of pineapple varieties from America. A number of reviews on pineapple flavors have also been published during the last two decades (Berger and Kollmannsberger 1985; Engel et al. 1990; Karg 1983; Kishino and Kobayashi 1981).

Volatile Constituents of Green and Ripened Pineapple

Volatile constituents of green and ripened pineapples were isolated and identified by GC and GC-MS (Umano et al. 1992). Among a total of 157 volatile compounds identified in the samples, 144 were found in green fruit and 127 were found in ripened fruit. Fifty volatiles have been identified for the first time from pineapple. Esters, which constituted 84% of total volatiles, were the most abundant volatiles found in green pineapple, followed by ketones (5.9%) and lactones (4.6%). These results are consistent with those reported in a previous study (Takeoka et al. 1989).

In green pineapples, the major volatile constituents were found to be ethyl acetate (24.5%), ethyl 3-(methylthio) propionate (10.4%), and ethyl 3-acetoxyhexanoate (8.7%). In ripened pineapple, ethyl acetate (33.5%), *threo*-butane-2,3-diol diacetate (13.0%), and 3-hydroxy-2-butanone (8.7%) were the major constituents. Engel and others (1989) demonstrated that the concentrations of hydroxy and acetoxy acid esters increased during pineapple ripening. 3-Hydroxy-2-butanone has been reported as a main volatile constituent in a vacuum steam distillate from canned pineapple juice (Ohta et al. 1987). On the other hand, green pineapple contained it only in trace amounts (Takeoka et al. 1989). As the formation of ethanol was not observed in the ripening process, it was suggested that no fermentation has occurred (Umano et al. 1992).

Among many hydroxyl esters and acetoxy esters identified by Umano and others (1992), 20 hydroxy esters (such as methyl or ethyl 3-hydroxy-2-methylbutanoate, methyl or ethyl 2-hydroxy-2-methylbutanoate, methyl 3-hydroxy-3-methylbutanoate, and ethyl 2-hydroxy-3-methylbutanoate) and 8 acetoxy esters (such as methyl or ethyl 3-acetoxy-2-methylbutanoate) were found for the first time in pineapple fruits. Interestingly, these compounds apparently have never been reported in any other fruit so far.

Butane-2,3-diol diacetate, found in all samples, is a new constituent of pineapples, and its precursor, 3-acetoxy-2-butanol, was also detected in trace amounts (Umano

et al. 1992). Takeoka and others (1989) reported 2,3-butanediol from pineapple, but they did not find its acetate derivative. In addition to butane-2,3-diol diacetate, 3-hydroxy-2-butanone (acetoin) was found in ripened pineapple in large amounts.

Among sulfur-containing compounds identified, ethyl 3-(methylthio) propanoate and methyl 3-(methylthio) propanoate, which are known to have a characteristic pineapple flavor, were found as major constituents (Umano et al. 1992). Takeoka and others (1991) also reported several sulfur-containing esters that were similar to the major esters found in pineapple by Umano and others (1992). Takeoka and others (1991) identified a number of sulfur compounds in pineapple, such as, methyl 3-(methylthio)-(*E*)-2-popenoate, methyl 3-(methylthio)-(*Z*)-2-propenoate, ethyl 3-(methylthio)-(*E*)-2-propenoate, ethyl 3-(methylthio)-(*Z*)-2-propenoate, methyl 4-(methylthio) butanoate, ethyl 4-(methylthio) butanoate (tentative), and dimethyl trisulfide. Some of the important volatiles flavor compounds present in pineapple are listed in Table 25.3.

Free and Bound Aroma Compounds in Pineapple

The aromatic components of fruits are usually present either in a free form or bound to sugar in the form of glycoside. Wu and others (1991) studied free and glycosidically bound pineapple constituents and identified 2-pentanol, 2-butoxyethanol, hexanoic acid, phenol, 4-hydroxybenzaldehyde, vanillin, and syringaldehyde as aglycones. Previously, Wu and others (1990) also reported the presence of glycosidically bound 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) in pineapple. GC and GC-MS analyses of the glycosidically bound fraction showed DMHF as the most abundant compound.

The free volatile fraction had "fruity," "pineapple-like" aroma, while the glycosidically or phosphate bound fractions had no odor. Only after enzymatic hydrolysis did the glycosidically bound fraction have the characteristic fruity, pineapple-like aroma. In contrast, the β-glucosidase hydrolysis, the extract of phosphatase hydrolysate had no odor. No volatile compound was liberated by acid phosphatase as proven by GC and GC-MS (Wu et al. 1991).

TABLE 25.3. Most Odor-Active Volatiles from Fresh Pineapple

Group	Name of Compound(s)
Esters	Ethyl acetate, methyl 2-methylpropanoate, ethyl 2-methylpropanoate, methyl 2- and 3-methylbutanoates, ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate
Lactones	(*Z*)-1,5-octadien-3-one, β-damascenone, γ-octalactone, δ-octalactone, γ-nonalactone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, γ-decalactone, δ-decalactone, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, γ-dodecalactone, 4-methoxy-2,5-dimethyl-3(2H)-furanone
Hydrocarbons	1-(*E*,*Z*)-3,5-undecatriene, 1,3,5,8-undecatetraene
Aldehydes	Octanal
Acids	Butanoic acid, phenylacetic acid
Others	Vanillin

Source: Tokitomo and others (2005).

The glycosidically bound volatiles isolated from pineapple were mainly hydroxyl compounds and lactones. DMHF was the most abundant compound, followed by δ-octalactone and ethyl 3-hydroxhexanoate. Some glycosidic hydroxyl esters such as ethyl 3-hydroxyhexanoate and methyl 3-hydroxyoctanoate were reported for the first time in pineapple by Wu and others (1991). It is interesting to note that many lactones such as γ-hexalactone, δ-hexalactone, δ-heptalactone, γ-octalactone, δ-octalactone, γ-nonalactone, and γ-decalactone were found in the glycosidically bound fraction. These compounds may be present in the form of glycosidically bound hydroxyl acids in pineapple.

Many glycosidically bound phenolic compounds such as eugenol, 4-hydroxybenzaldehyde, (3-hydroxyphenyl)-ethyl alcohol, 4-allyl-2,6-dimethoxyphenol, and syringaldehyde and glycosidically bound alcohols, such as hexanol, 2-butoxyethanol, (3-hydroxyphenyl) ethyl alcohols, and 2-phenylethanol, were also reported in pineapple by Wu and others (1991). In addition, they have also reported the glycosidic acids such as hexanoic acid and cinnamic acid and glycosidically bound benzaldehyde in the pineapple.

PAPAYA

Papaya (*Carica papaya* L.) is a native of tropical America but is currently disseminated throughout the tropics. The Maradol Roja variety is well-known in Cuba, Mexico, Colombia, and neighboring countries. This variety has a high sugar content, and its pulp maintains a firm texture and high resistance to oxidation during ripening. Papaya, like many climacteric fruits, undergoes a variety of physical and chemical changes after harvest (Shiota 1991). The stage of ripeness determines the fruit's final quality.

Aroma Composition of Papaya

Almora and others (2004) evaluated the volatile components in four ripening stages as maturity indicators of papaya and reported that butanol, 3-methylbutanol, benzyl alcohol, and α-terpineol isolated by simultaneous distillation/solvent extraction method showed maximum concentrations in the third maturation stage, and can be used as ripeness indicators of Maradol Roja variety of papaya. On the other hand, Flath and others (1990) reported that linalool, benzyl isothiocyanate, and phenylacetonitrile were released in significant amounts at all four ripeness stages from papaya (*C. papaya* L., Solo variety), but linalool production increased dramatically as the fruit progressed from one-fourth to full ripeness. Free benzyl isothiocyanate levels also increased with fruit ripening, but phenylacetonitrile release fluctuated across the four fruit ripeness stages, showing no clear correlation with ripeness. Numerous esters and monoterpenes were only detected in volatile emissions from fully ripe fruit. Schwab and others (1989) reported the monoterpene alcohols linalool and 2,6-dimethyloct-7-ene-2,3,6-triol, were released by phosphatase activity in papaya fruit. They also found glycosidic hexanol and 2-phenylethanol and glycosidically bound 2-methylbutanoic acid/3-methylbutanoic acid, benzoic acid, and phenylacetic acid in papaya. Some of the important flavor components of papaya fruit are shown in Table 25.4.

TABLE 25.4. Major Volatile Compounds Typically Present in Papaya

Group	Type of Compound(s)
Esters	Methyl butanoate, 3-methylbutanol, ethyl hexanoate, ethyl dodecanoate, ethyl acetate, ethyl butyrate, prop-2-yl butyrate, methyl hexanoate, methyl octanoate, ethyl benzoate, butyl hexanoate, ethyl octanoate, butyl benzoate, 3-methylbutyl benzoate, ethyl butanoate
Alcohols	Butanol, benzyl alcohol, terpinen-4-ol, α-terpineol
Hydrocarbons	Myrcene, α-phellandrene, α-terpinene, β-phellandrene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, caryophyllene
Aldehydes	Hexanal, heptanal, benzaldehyde, octanal, nonanal, decanal
Lactones	γ-Hexalactone, γ-octalactone
Others	(*Z*)-linalool oxide, (*E*)-linalool oxide, linalool, benzyl isothiocyanate, dodecanoic acid, methyl salicylate, triacetin, methyl geranate, benzyl isothiocyanate, methyl thiocyanate, phenylacetonitrile, germacrene D, pentadecane, geranylacetone, pentane-2,4-dione, 6-methylhept-5-en-2-one, heptan-2-one, 4-hydroxy-4-methylpentan-2-one

Source: Almora and others (2004); Pino and others (2003); Flath and others (1990).

PASSION FRUIT

Yellow passion fruit (*Passiflora edulis* v. *flavicarpa*) is one of the most popular and best known tropical fruits having a floral, estery aroma with an exotic tropical sulfury note. The volatile composition of yellow passion fruit flavor is rather complex and was thoroughly reviewed by Whitfield and Last (1986) and Shibamoto and Tang (1990). To date, >200 components have been identified in yellow passion fruit flavor.

The attractive tropical flavor note of ripe passion fruits has been shown to be associated with trace levels of sulfur volatiles. Volatile sulfur components are important trace constituents of natural products and play an important role in the sensory properties of food flavors. To date, 12 volatile sulfur-containing trace constituents have been identified in different varieties of yellow passion fruits as well as in passion fruit juices (Engel and Tressl 1991; Winter et al. 1976).

Flavor Composition of Passion Fruit

The flavor composition of passion fruits has been investigated intensively in the past few years. Murray and others (1972), Parliment (1972), and Winter and Kloti (1972) gave a first insight into the complex mixture of aroma components of this tropical fruit. Degradation products of carotenoids (Demole et al. 1979; Naf et al. 1977; Whitfield et al. 1973; Winter et al. 1979a), sulfur-containing components (Winter et al. 1976), and unusual aliphatic esters (Winter et al. 1979b) were reported to play important roles in the unique and delicate flavor of passion fruit.

The chiral sulfur-containing flavor compounds 2-methyl-4-propyl-1,3-oxathiane, 3-mercaptohexanol, 3-(methylthio) hexanol and their acetates, butanoates, and hexanoates are among the most potent components responsible for the typical tropical-fruity notes of the yellow passion fruit (Engel and Tressl 1991; Weber et al. 1995; Winter et al. 1976).

To obtain aroma concentrates that are truly representative of the tropical passion fruit flavor, four different flavor isolation techniques, VHS method, DHS method, SDE, and SDEV, have been used by Werkhoff and others (1998). The most representative and typical extract was obtained by VHS sampling and subsequent liquid–liquid extraction of the aqueous phase. This VHS concentrate was prefractionated by medium-pressure adsorption chromatography on silica gel. For the first time, approximately 180 components were identified in the LC fractions of passion fruit flavor. Of these compounds, 14 components have not been previously reported as naturally occurring flavor ingredients (Werkhoff et al. 1998).

Casmir and others (1981) detected over 300 volatile flavorants in passion fruit and identified 22 components of this mixture as possessing passion fruit flavor. Esters (aliphatic, aromatic, terpenoidic) were the most abundant class of volatiles, followed by C_{13} norterpenoids and monoterpenoids. Winter and others (1976) and Engel and Tressl (1991) indicated that the sulfur-containing compounds, especially 3-methylthiohexanol and 2-methy-4-propyl-1,3-oxathianes, were considered key flavor components of yellow passion fruit. Engel and Tressl (1983b) reported that nerol, linalool, geraniol and α-terpineol were not present in yellow passion fruits in free form, but were present in bound, glycosidic forms. Thermal treatment of passion fruit pulp at pH 3.0 resulted in increased concentrations of a series of monoterpenes, alcohols, and oxides.

Engel and Tressl (1983a) reported that a pool of nonvolatile polar precursor compounds exist in passion fruit. This reservoir of nonvolatile compounds predominately consists of the glycosides of monoterpene alcohols as well as hydroxylated linalool derivatives. The presence of bound flavor compounds may explain the difficulty in comparing aroma profiles derived from the same cultivar, namely, yellow passion fruit. Thermal treatments, either during commercial processing or during laboratory preparation, strongly influence the type and amounts of aroma compounds detected. Yellow passion fruit essence is a mixture derived from (1) natural, unbound flavorants; (2) flavorants released from bound forms by juice acidity and thermal treatments; and (3) compounds derived from acid-catalyzed hydration–dehydration reactions and by other type of transformations.

Differences in free and bound compounds from passion fruits have been reported. Glycosidically bound volatile compounds have been detected in passion fruit (Engel and Tressl 1983b; Winterhalter 1990). Their results were generally obtained from different cultivars produced in different places, and in some cases, the exact identification of their cultivars was not known.

Preliminary results obtained for glycosidically bound volatile compounds in passion fruits reveal that about 22% glucosides, 12% arabinosylglucosides, 39% rutinosides, and 27% gentiobiosides are present (D. Chassagne, unpublished data). Among these compounds, 2-phenylethyl, linalyl, α-terpinyl, geranyl and neryl glucosides, and rutinosides and benzyl alcohol rutinoside have been identified by analytic optimum performance laminar chromatography (OPLC) and HPLC from the fractions isolated by preparative OPLC. The determination of the structure of isolated heterosidic fractions by MS, MS-MS, and HPLC shows that glucosides are only 22% of the bound compounds in passion fruit. Glucosides, arabinoglucosides, rutinosides, and gentiobiosides represent about 80% of bound compounds in passion fruit. Some of the important flavor components of passion fruit are shown in Table 25.5.

TABLE 25.5. Major Flavor Constituents of Yellow Passion Fruit

Group	Name of Compound(s)
Aldehydes	2- and 3-Methylbutanal, (*E*)-2-hexenal, benzaldehyde
Alcohols	1-Butanol, 2- and 3-methyl-1-butanol, 1-hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, 1-octanol, 4-terpineol, nerol, 3-mercaptohexanol, geraniol, benzyl alcohol
Esters	Ethyl butanoate, ethyl acetate, ethyl hexanoate, hexyl acetate, (*E*)-3-hexenyl acetate, hexyl butanoate, ethyl octanoate, (*E*)-3-hexenyl butanoate, ethyl 3-hydroxybutanoate, hexyl hexanoate, (*Z*)-3-hexenyl hexanoate, diethyl succinate, ethyl 3-hydroxyhexanoate, α-terpineol, benzyl acetate, hexyl octanoate, benzyl butanoate, benzyl hexanoate, ethyl cinnamate
Lactones	2-Butanone, cyclopentanone, 7,8-dihydro-β-ionone, β-ionone, γ-decalactone
Hydrocarbons/ terpenes	Myrcene, limonene, γ-terpinene, *trans*-β-ocimene, 3-hydroxy-2-butanone and terpinolene, (*E*)-4,8-dimethyl-1,3,7-nonatriene
Others	*trans*-Linalool oxide, linalool, hexadecanoic acid

Source: Werkhoff and others (1998).

In addition to the spectrum of biosynthesized volatiles in passion fruit, there exists a pool of nonvolatile polar precursor compounds, especially glycosides of monoterpene alcohols and hydroxylated linalool derivatives, which can be transformed into important aroma components by chemical or enzymatic reactions (Engel and Tressl 1983a). The degree of liberation and degradation decisively determines the spectrum of isolated volatiles. This may explain the difficulties while comparing the results of quantitative determinations of passion fruit aroma components in extracts obtained by different isolation techniques. The fact that monoterpene alcohols are present in passion fruits not in the free but in glycosidic form, may explain the decreasing concentrations of linalool and α-terpineol in the fruit during its development from the immature green to full ripe state (Casmir et al. 1977–1978). Possibly, the transformation of biosynthesized linalool and α-terpineol from the free into the glycosidic form takes place within this period of fruit maturation.

CONCLUSIONS

Nowadays, the consumers consider the characteristic exotic flavor of the tropical fruits as one of the most attractive attributes. Therefore, food industries are emphasizing more on these volatile flavor components to produce value-added newer products. In a fruit, flavor develops during a short ripening period either on the tree itself or after it has been picked from the tree at its fully ripe stage of maturity. Many factors are known to influence the flavor of fruits, such as plant genetics, soil nutrition, growing environment, stage of maturity, and conditions of storage from harvesting to consumption.

While a number of variables are outside of the control of the plant grower, some are within his or her control. The grower should consider the influence of plant

genetics, soil nutrition, and water regime in controlling the flavor of his or her fruit crop. To a grower, flavor should now become a more significant consideration affecting the value of his or her produce.

In spite of the limited information available on the flavor components of Central and South American fruits, the chapter has tried to emphasize the high variability that has been encountered in the amount of fruit volatiles depending on the cultivar (origin) and techniques used to determine the flavor components of these fruits.

REFERENCES

Almora K, Pino JA, Hernandez M, Duarte C, Gonzalez J, Roncal E. 2004. Evaluation of volatiles from ripening papaya (*Carica papaya* L., var. Maradol roja). *Food Chem* 86:127–30.

Arthur CL, Pawliszyn J. 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal Chem* 62:2145–8.

Askar A, Treptow H. 2001. Verarbeitung von tropischen Fruchten. In Echobinger U (ed.), *Frucht-und Gemusesafie* (3. Aufl.). Stuttgart, Germany: Ulmer, pp. 242–5.

Baldwin EA, Scott JW, Shewmaker CK, Schuch W. 2000. Flavor trivia and tomato aroma: Biochemistry and possible mechanisms for control of important aroma components. *HortScience* 35(6):1013–22.

Berger RG, Kollmannsberger H. 1985. Pineapple flavors, biosynthetic routes and sensorial properties. In Berge RG, Nitz S, Schreier P (eds.), *Topics in Flavour Research*. Marzling, Germany: Eichhorn, pp. 305–20.

Blank I. 1997. Gas chromatography-olfactometry in food aroma analysis. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 293–329.

Buttery RG, Ling LC. 1996. Methods for isolation of food and plant volatiles. In Takeoka GR, Teranishi R, Williams PJ, Kobayashi A (eds.), *Biotechnology for Improved Foods and Flavors*. Washington, DC: American Chemical Society, pp. 240–8.

Casmir DJ, Kefford JF, Whitfield FB. 1981. Technology and flavor chemistry of passion fruit juices and concentrates. In Chichester CO (ed.), *Advances in Food Research*, Vol. 27. New York: Academic Press, pp. 243–95.

Casmir DJ, Shaw KJ, Whitfield FB. 1977–1978. Volatile flavor constituents—passion fruit. *Div Food Res Rep Res (Aust CSIRO)*, pp. 17–18.

Coleman WM, Lawrence BM. 1997. A comparison of selected analytical approaches to the analysis of an essential oil. *Flav Frag J* 12:1–8.

Cook DJ, Davidson JM, Linforth RST. 2003. Measuring the sensory impact of flavor mixtures using controlled deliveries. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization: Sensory Analysis, Chemistry and Physiology*. New York: Marcel Dekker, pp. 135–50.

Demole E, Enggist P, Winter M, Furrer A, Schulte-Elte KH, Egger B, Ohloff G. 1979. Megastigma-5,8-dien-4-one, an aroma constituent of the yellow passion fruit and Virginia tobacco. *Helv Chim Acta* 62:67–75.

Elss S, Preston C, Hertzig C, Heckel F, Richling E, Schreier P. 2005. Aroma profiles of pineapple fruit (*Ananas comosus* [L.] Merr.) and pineapple products. *LWT* 38:263–74.

Elss S, Preston C, Hertzig C, Richling E, Schreier P. 2003. Uber das Aromaprofil der Ananas (*Ananas comosus* [L.] Merr.) Eine Nachernte-Studie. *Flussiges Obst* 70:734–7.

Engel KH, Heidlas J, Albrecht W, Tressl R. 1989. *Biosynthesis of Chiral Flavor and aroma Compounds in Plants and Microorganisms. ACS Symposium Series 388*. Washington, DC: American Chemical Society, pp. 8–22.

Engel KH, Heidlas J, Tressl R. 1990. The flavor of topical fruits (banana, melon, pineapple). In McLeod AJ (ed.), *Food Flavours Part C: The Flavour of Fruit*. Amsterdam: Elsevier, pp. 195–219.

Engel KH, Tressl R. 1983a. Formation of aroma components from nonvolatile precursors in passion fruit. *J Agric Food Chem* 31(5):998–1002.

Engel KH, Tressl R. 1983b. Differentiation of yellow and purple passion fruits by investigation of their ester composition. *Chem Mikrobiol Technol Lebensm* 8:33–9.

Engel KH, Tressl R. 1991. Identification of new sulfur-containing volatiles in yellow passion fruits (*Passiflora edulis* f. *flavicarpa*). *J Agric Food Chem* 39:2249–52.

FAO (Food and Agriculture Organization of the United Nations), Statistics Division. 2002. Production of fruits and vegetables during 2000. In *FAO Production Yearbook*, Vol. 54. FAO Statistics Series No. 163. Rome, Italy: Food and Agriculture Organization of the United Nations, pp. 182–5.

Fazzalari FA. 1978. *Compilation of Odor and Taste Threshold Values Data*. Philadelphia: American Society of Testing and Materials.

Fellman JK, Miller TE, Mattinson DS, Matheis JP. 2000. Factors that influence biosynthesis of volatile compounds in apple fruits. *HortScience* 35(6):1026–33.

Fisher C, Scott TR. 1997. *Food Flavours: Biology and Chemistry*. Cambridge, UK: Royal Society of Chemistry, pp. 4–26.

Flath RA, Light DM, Tang EB, Mon TR, John JO. 1990. Headspace examination of volatile emissions from ripening papaya (*Carica papaya* L., Solo variety). *J Agric Food Chem* 38:1060–3.

Guntert M, Bruning J, Emberger R, Hopp R, Kapsel M, Surburg H, Werkhoff P. 1992. Thermally degraded thiamin: A potent source of interesting flavor compounds. In Teranishi R, Takeoka GR, Guntert M (eds.), *Flavor Precursors: Thermal and Enzymatic Conversions. ACS Symposium Series 490*. Washington, DC: American Chemical Society, pp. 140–63.

Hendrich M, Winterhalter P. 1991. 3-Hydroxy-retro-α-ionol: A natural precursor of isomeric edulans in purple passion fruit (*Passiflora edulis* Sims). *J Agric Food Chem* 39:1270–4.

Herderich M. 1999. Mass spectrometry in flavor chemistry: Past and prospect. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer Academic/Plenum Publishers, pp. 239–53.

Hodgson AS, Hodgson LR. 1993. Pineapple juice. In Nagy S, Chen CS, Shaw PE (eds.), *Fruit Juice Processing Technology*. Auburndale, FL: Ag Science, pp. 378–435.

Jones DD, Young AL. 1996. Food and agricultural biotechnology: An overview. In Takeoka GR, Teranishi R, Williams PJ, Kobayashi A (eds.), *Biotechnology for Improved Foods and Flavors*. ACS Symposium Series 637. Washington, DC: American Chemical Society, pp. 1–12.

Karg JE. 1983. Exotic pineapple flavor—History, formulation, and future. *R.A.K., Riechst., Aromen, Kosmet* 33:163–5.

Kishino K, Kobayashi S. 1981. From our memories on study of pineapple flavor. *Koryo* 130:97–106.

Krumbein A, Ulrich D. 1996. Comparison of three sample preparation techniques for the determination of fresh tomato aroma volatiles. In Taylor AJ, Mottram DS (eds.), *Flavour Science: Recent Developments*. Bodmin, UK: The Royal Society of Chemistry, pp. 289–92.

Leahy MM, Roderick RG. 1999. Fruit flavor biogenesis. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer Academic/Plenum Publishers, pp. 275–86.

Lopez MG, Guzman GR, Dorantes AL. 2004. Solid-phase microextraction and gas chromatography-mass spectrometry of volatile compounds from avocado puree after microwave processing. *J Chromatogr A* 1036(1):87–90.

Maarse H, Visscher CA. 1994. *Volatile Compounds in Food. Qualitative and Quantitative Data*. Zeist, The Netherlands: TNO-CIVO Food Analysis Institute.

Martin GJ, Martin ML. 1999. Thirty years of flavor NMR. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer Academic/Plenum Publishers, pp. 19–30.

Misharina TA, Golovnya RV, Beletsky IV. 1994. Comparison of the efficiency of isolation of volatiles from foodstuffs by co-distillation and Likens-Nickerson methods. In Maarse H, van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier Academic Press, pp. 117–20.

Mistry BS, Reineccius T, Olson LK. 1997. Gas chromatography-olfactometry for the determination of key odorants in foods. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Dekker, pp. 265–92.

Murray KE, Shipton J, Whitfield FB. 1972. The chemistry of food flavour. I. Volatile constituents of passion fruit, *Passiflora edulis. Aust J Chem* 25(9):1921–33.

Naf F, Decorzant R, Willhalm B, Velluz A, Winter M. 1977. Structure and synthesis of two novel ionones identified in the purple passion fruit (*Passiflora edulis* Sims). *Tetrahedron Lett* 18(16):1413–6.

Nijssen LM, Visscher CA, Maarse H, Wilemsens LC, Boelens MH. 1996. *Volatile Compounds in Food. Qualitative and Quantitative Data*, 7th Ed. Zeist, The Netherlands: TNO Nutrition and Food Research Institute.

Ohta H, Kinjo S, Osajima Y. 1987. Glass capillary gas chromatographic analysis of volatile components of canned Philippine pineapple juice. *J Chromatogr* 409:409–12.

Paillard NM. 1981. Factors influencing flavor formations in fruits. In Schreier P (ed.), *Flavour '81*. Berlin, Germany: Walter deGruyter, pp. 479–507.

Paillard NM. 1990. The flavor of apples, pears and quinces. In Morton ID, MacLeod AJ (eds.), *Food Flavours Part C: The Flavour of Fruit*. Amsterdam: Elsevier, pp. 1–41.

Parliment TH. 1972. Some volatile constituents of passion fruit. *J Agric Food Chem* 20(5): 1043–5.

Parliment TH. 1997. Solvent extraction and distillation techniques. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Dekker, pp. 1–26.

Pino JA, Almora K, Marbot R. 2003. Volatile components of papaya (*Carica papaya* L., Maradol variety) fruit. *Flav Frag J* 18:492–6.

Purseglove JK. 1968. *Tropical Crops. Dicotyledons*. London: Longmans, pp. 192–8.

Reineccius G. 1994. Flavor analysis. In Reineccius GA (ed.), *Source Book of Flavors*. New York: Chapman and Hall, pp. 24–60.

Reineccius G. 1999. Kinetics of flavor formation during Maillard browning. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer Academic/Plenum Publishers, pp. 346–52.

Reineccius G. 2006. *Flavor Chemistry and Technology*, 2nd Ed. New York: Taylor & Francis, pp. 73–101.

Rodin JO, Himel CM, Silverstein RM, Leeper RW, Gortner WA. 1965. Volatile flavor and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3[2H]-furanone. *J Food Sci* 30:280–4.

Rohrbach KG, Leal F, d'Eeckenbrugge GC. 2003. History, distribution and world production. In Bartholomew D, Pauli RE, Rohrbach KG (eds.), *The Pineapple: Botany, Production and Uses*. Cambridge, MA: CABI Publishing, pp. 1–12.

Samson TA. 1986. *Tropical Fruits*, 2nd Ed. New York: Longman.

Schultz TH, Flath RA, Mon TR, Eggling SB, Teranishi R. 1977. Isolation of volatile components from a model system. *J Agric Food Chem* 25(3):446–9.

Schwab W, Mahr C, Schreier P. 1989. Studies on the enzymatic hydrolysis of bound aroma components from *Carica papaya* fruit. *J Agric Food Chem* 37:1009–13.

Shibamoto T, Tang CS. 1990. Minor tropical fruits—Mango, papaya, passion fruit, and guava. In Morton ID, MacLeod AJ (eds.), *Food Flavours. Part C. The Flavours of Fruits*. Amsterdam: Elsevier, pp. 221–80.

Shiota H. 1991. Volatile components of pawpaw fruit (*Asimia triloba*). *J Agric Food Chem* 39:1631–5.

Sinyinda S, Gramshaw JW. 1998. Volatiles of avocado fruit. *Food Chem* 62(4):483–7.

Spanier AM, Flores M, James C, Lasater J, Lloyd S, Miller JA. 1998. Fresh-cut pineapple (*Ananas* sp.) flavor. Effect of storage. In Contis ET, Ho CT, Mussinan CJ, Parliament TH, Shahidi F, Spanier AM (eds.), *Food Flavors: Formation, Analysis and Packaging Influence in Developments in Food Science*, Vol. 40. Amsterdam: Elsevier, pp. 331–43.

Swisher EH. 1988. Avocado oil from food use to skin care. *J Am Oil Chem Assoc* 65: 1704–6.

Takeoka GR, Buttery RG, Flath RA, Teranishi R, Wheeler EL, Wieczorek RL, Guentert M. 1989. Volatile constituents of pineapple (*Ananas comosus* [L.] Merr.). In Taranishi R, Buttery RG, Shahidi F (eds.), *Flavor Chemistry: Trends and Developments*. Washington, DC: American Chemical Society, pp. 223–37.

Takeoka GR, Buttery RG, Teranishi R, Flath RA, Guntert M. 1991. Identification of additional pineapple volatiles. *J Agric Food Chem* 39:1848–51.

Taranishi R. 1998. Challenges in flavor chemistry: An overview. In Mussinan CJ, Morello MJ (eds.), *Flavor Analysis. Developments in Isolation and Characterization. ACS Symposium Series 705*. Washington, DC: American Chemical Society, pp. 1–6.

Taranishi R, Buttery RG. 1962. Aromagrams, direct vapor analyses with gas chromatography. Reports of the Scientific and Technical Commission. International Federation of Fruit Juice Producers. Symposium on Volatile Fruit Flavors. Bern, Switzerland, pp. 257–266.

Taranishi R, Kint S. 1993. Sample preparation. In Acree TE, Taranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: American Chemical Society, pp. 137–67.

Teai T, Claude-Lafontaine A, Schippa C, Cozzolino F. 2001. Volatile compounds in fresh pulp of pineapple (*Ananas comosus* [L.] Merr.) from French Polynesia. *J Essent Oil Res* 13:314–8.

Ti TC. 2000. The global pineapple economy FAO keynote address. *Acta Hortic* 529:4–52.

Tokitomo Y, Steinhaus M, Buttner A, Schieberle P. 2005. Odor-active constituents in fresh pineapple (*Ananas comosus* [L.] Merr.) by quantitative and sensory evaluation. *Biosci Biotechnol Biochem* 69(7):1323–30.

Tressl R, Rewicki D. 1999. Heat generated flavors and precursors. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry: Thirty Years of Progress*. New York: Kluwer Academic/ Plenum Publishers, pp. 305–26.

Umano K, Hagi Y, Nakahara K, Shoji A, Shibamoto T. 1992. Volatile constituents of green and ripened pineapple (*Ananas comosus* [L.] Merr.). *J Agric Food Chem* 40:599–603.

Wampler TP. 1997. Analysis of food volatiles using headspace-gas chromatographic techniques. In Marsili R (ed.), *Flavor, Fragrance and Odor Analysis*. New York: Dekker, pp. 25–48.

Weber B, Maas B, Mosandl A. 1995. Stereoisomeric flavor compounds. 72. Stereoisomeric distribution of some chiral sulfur-containing trace components of yellow passion fruits. *J Agric Food Chem* 43:2438–41.

Werkhoff P, Guntert M, Krammer G, Sommer H, Kaulen J. 1998. Vacuum headspace method in aroma research: Flavor chemistry of yellow passion fruits. *J Agric Food Chem* 46(3): 1076–93.

Whitfield FB, Last JH. 1986. The flavor of the passion fruit—A review. In Brunke EJ (ed.), *Progress in Essential Oil Research*. New York: Walter de Gruyter, pp. 3–48.

Whitfield FB, Stanley G, Murray KE. 1973. Concerning the structures of edulan I and II. *Tetrahedron Lett* 14(2):95–8.

Willhalm B, Stoll M, Thomas AF. 1965. 5-Dimethyl-4-hydroxy-2,3-dihydrofuran-3-one. *Chem Ind* 18:1692–3.

Williams PJ, Safton MA, Francis IL. 1992. Glycosidic precursors of varietal grape and wine flavor. In Teranishi R, Takeoka GR, Guntert M (eds.), *Flavor Precursors: Thermal and Enzymatic Conversions. ACS Symposium Series 490*. Washington, DC: American Chemical Society, pp. 74–86.

Winter M, Furrer A, Willhalm B, Thommen W. 1976. Identification and synthesis of two new organic sulfur compounds from the yellow passion fruit (*Passiflora edulis* f. *flavicarpa*). *Helv Chim Acta* 59(5):1613–20.

Winter M, Kloti R. 1972. Uber das aroma der gelben Passionfrucht. *Helv Chim Acta* 55:1916–9.

Winter M, Naf F, Furrer A, Pickenhagen W, Giersch W, Meister A, Willhalm B, Thommen W, Ohloff G. 1979a. 4,7-Octadienoic acid ethyl ester und (Z)-butyric acid 3,5-hexadienyl ester, two new aroma components of the purple passion fruit. *Helv Chim Acta* 62:135–9.

Winter M, Schulte-Elte KH, Velluz A, Limacher J, Pickenhagen W, Ohoff G. 1979b. Ubersicht uber die chemische Zusammensetzung und die Inhaltsstoffe einer reihe wichtigen exotischer obstarten. *Helv Chim Acta* 62:131–4.

Winterhalter P. 1990. Bound terpenoids in the juice of the purple passion fruit. *J Agric Food Chem* 38:452–5.

Wu P, Kuo MC, Hartman TG, Rosen RT, Ho CT. 1991. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* [L.] Merr.). *J Agric Food Chem* 39:170–2.

Wu P, Kuo MC, Zhang KQ, Hartman TG, Rosen RT, Ho CT. 1990. Analysis of glycosidically bound 2,5-dimethyl-4-hydroxy-3(2H)-furanone in pineapple. *Perfum Flavor* 15(1):51–3.

Yamaguchi K, Nishimura O, Toda H, Mihara S, Shibamoto T. 1983. Chemical studies on tropical fruits. In Charalambous G, Inglett G (eds.), *Instrumental Analysis of Foods: Recent Progress*, Vol. 2. New York: Academic Press, pp. 93–117.

Yang X, Peppard T. 1994. Solid-phase microextraction for flavor analysis. *J Agric Food Chem* 42:1925–30.

PART V

FLAVORS OF SELECTED DRIED FRUITS

CHAPTER 26

Overview of Flavors and Fruit Dehydration

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INTRODUCTION

Dried fruits have a long and successful history, in spite of their currently unbalanced trade value, and should be considered a worldwide latent commodity, with a brilliant future in an ever-expanding market.

As any other worldwide commercialized food product, nowadays dried fruits exist within a complex supply chain consisting of multifactorial economical operators, and more or less sophisticated processes of perishable and unpredictable raw materials. The careful coordination of these factors will deliver final products perfectly adjusted to the increasingly high standards and expectations of diversified end users and consumers.

Dried vegetables and fruits are important market ingredients, as demonstrated by the European market for dehydrated vegetables, valued for about $8–9 \times 10^8$ kg at EUR 5–6 billion (Torringa et al. 2001). However, changes in consumer behavior and preferences are likely to affect the future dynamics of dried fruit trade.

International commerce and trading, involving exchange of plants or plant products, relies on accurate communication in unambiguous terminology (Heldman 2003; Heldman and Lund 2006), necessitating more precise content terms than something as vague as *dried fruits*.

Botanically, a fruit may be defined as the structure that develops from the ovary wall (pericarp) of the enclosed seed or mature seeds. The systematic classification of fruits is still controversial. A fruit may be succulent (drupes, berries, pomes, etc.) or naturally dry (almonds, nuts, etc.). Despite being fruits, under a botanical perspective, a more common commercial interpretation of "vegetables" is used for eggplants (aubergines), cucumbers, pumpkins, olives, or tomatoes since they are not sensorially perceived as sweet foods (Barbosa-Cánovas and Vega-Mercado 1996).

For clarity, this chapter—unless otherwise stated—only refers to *dried fruits* as those commodities listed by the Commission Regulation (EC) N. 1810/2004 of

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

September 7, 2004, amending Annex I to Council Regulation (EEC) N. 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff—TARIC—Part Two—Schedule of Customs Duties, Section II—Vegetable products, Dried fruit or dried nuts, which may be partially rehydrated, or treated for the following purposes: (1) for additional preservation or stabilization (e.g., by moderate heat treatment, sulfuring, the addition of sorbic acid or potassium sorbate) and (2) to improve or maintain their appearance (e.g., by the addition of vegetable oil or small quantities of glucose syrup), provided that they retain the character of dried fruit or dried nuts and are identified as edible fruit and nuts, peel of citrus fruit, or melons, all of them including in Subheadings of an eight-digit, CN Code, but grouped within in Headings 0813 and 0814.

OVERVIEW OF DRYING PROCESSES

The word "drying" is relative—simply meaning that there is a further reduction in the moisture content, from some initial level, provided by natural or induced mechanical dewatering to a desired or specified lower level.

Drying is a process in which water is removed to halt or slow down the growth of spoilage microorganisms, as well as the occurrence of chemical reactions (Vega-Mercado et al. 2001). *Drying* is usually defined as a reduction of moisture content until equilibrium with the environment is achieved, while the removal of moisture to lower moisture contents is called *dehydration* (Stuchly and Stuchly 1983).

Even from the simplest perspective, there are evident and very well-accepted advantages for drying fruits: increased shelf life, no need of refrigeration, and ease of handling by reduction of weight and volume ranging from 5 to more than 20 times, consequently lowering transport and storage costs. Finally, recent improvements in drying technologies have facilitated the creation of remarkable value-added products from raw, highly sensitive, and perishable fruits.

These characteristics and advantages have associated benefits and challenges. While adding value to the original fresh commodities, even the highly sophisticated drying systems that prevent nutrient loss cannot assure the retention of the aroma, flavor, and texture of the product. Besides, in many cases, improper managing of drying processes leads to brittle structured products, fines, and/or broken pieces during or after drying and/or during handling, packing, shipping, and storage as compared with conventionally dried fruits and vegetables. Such inconveniences arise because fruits are complexly structured, multicomponent edible materials, which require care in terms of circumventing practical difficulties and awareness of basic laws of physics, chemistry, biochemistry, and engineering. A fair amount of practical know-how and expertise is required to assure the consistent delivery of safe, uniform, inexpensive, nutritious, and appealing products in quantity. However, the production of such value-added products may present a challenge in terms of industrial procedure and innovation through shared customers and distributors.

OVERVIEW OF FUNDAMENTALS OF FRUIT DRYING PROCESSES

Drying (dehydration) has been studied widely around the world, yet it is still difficult to understand the subject fully. For instance, wrinkling or buckling is a common

natural phenomenon that occurs in numerous forms on many different length scales in fruit drying processes. Wrinkling and related phenomena in various materials, including fruits, have been studied from the standpoint of micro- and biomechanics systems, which may be why prevention of such phenomena still remains a challenge.

The thermodynamic properties of foods provide an understanding of water properties and energy requirements associated with the sorption behavior (Goula et al. 2008).

Drying processes preserve fruits, or any biological material, by lowering their water activity (a_w). There are important interactions between drying and quality changes in fruits that are nonuniform assemblies (Aguilera 2006).

For instance, water activity (a_w) may simply be defined as the ratio of the equilibrium water vapor pressure of a foodstuff, p (kPa), to the saturated vapor pressure, p_o (kPa), at the same temperature. Actually, it can be estimated by comparing the water pressure of the water content in the material, p, in a specific environment with that of vapor pressure of the free water, p_o, in that same environment: $a_w = p/p_o$. At equilibrium, the chemical potential of liquid water in a food and in its vapor phase is the same.

Water activity is an equilibrium property and the values can be derived from the vapor pressure (Bhandari and Howes 1999; Khalloufi et al. 2000a,b). Usually, water activity increases with temperature, because changes in temperature may cause water migration between food components.

The factors that affect water activity are solute interactions, up-capillary suction forces, and surface force interactions. Water in a food can be either free (independent) or bound (interdependent) to the solid matrix.

Very few food systems are at equilibrium at constant pressure (p) and temperature (T) conditions, and some examples are pure water and water vapor within a food, crystalline materials (ice, salts, sugars, fats), and equilibrium solutions (Aguilera and Lillford 2007; Khalloufi et al. 2000a,b; Roos 2002). In general, foods are often very complex, nonequilibrium systems (supercooled or supersaturated dispersions, suspensions, and solutions) exhibiting time-dependent properties. That is, changes toward equilibrium are observed because during drying processes, physical state and properties are time dependent, and composition and water activity may change with time (Heldman and Lund 2006; Rao and Rizvi 1994; Rao et al. 2005; Roos 2002; Roos and Taylor 1995).

The importance of water activity in controlling shelf life of foods is well established by its interference upon growth of microorganisms, rates of chemical reactions, and enzymatic deterioration (Bhandari and Howes 1999; Khalloufi et al. 2000a; Rao et al. 2005).

The relationship between water activity (a_w) and moisture content at a given temperature is designated as the moisture sorption isotherm. This relationship is complex and unique for each product due to different interactions (colligative, capillary, and surface effects) between the water and the solid components at different moisture contents.

Moisture sorption isotherms are sigmoidal in shape for most foods, although foods that contain large amounts of sugar or small soluble molecules have a J-type isotherm curve shape. There are three types of isotherm curves: adsorption (starting from the dry state), desorption (starting from the wet state), or working (native state).

Differential heat of sorption, often referred as isosteric heat of sorption, is used as an indicator of the state of water adsorbed by the solid particles, and its knowledge is important when designing equipment for dehydration processes (Goula et al. 2008).

Water sorption isotherms and isosteric heat of sorption give information about the water sorption mechanism and interactions between solid components and water content, allow the first approach of energy requirements of drying processes, and can be applied to optimize the drying or rehydration conditions and determine the stability of the product during storage.

The isosteric heat of sorption is a measure of the energy released on sorption and the heat of desorption is the energy requirement to break the intermolecular forces between the molecules of water vapor and the surface of the adsorbent. The isosteric heat of sorption, or "latent heat of vaporization," can be used to estimate the energy requirements of a drying process and to provide crucial information on the state of water in food products (Chen 2006; Siripatrawan and Jantawat 2006).

It is common to present sorption isotherms by mathematical models based on empirical and/or theoretical criteria. There are numerous isotherm models, which, according to Goula and others (2008), can be classified into several categories: (1) kinetic models based on an absorbed monolayer of water (Brunauer–Emmett–Teller [BET] model—Brunauer et al. 1938); (2) kinetic models based on a multilayer and condensed film (e.g., Guggenheim–Anderson–de Boer [GAB] model—Van den Berg and Bruin 1981); and (3) semiempirical (e.g., Halsey model—Halsey 1948) and purely empirical models (e.g., Oswin and Smith models—Oswin 1946; Smith 1947).

Water activity and glass transition temperature are important tools for prediction of available water in food and the physical state of solid foods (Roos 2002; Roos and Taylor 1995).

At constant temperature, water activity alone cannot explain differences in reaction rates, changes in structure and flow, component crystallization rates, and variations in transport properties (retention and diffusion of volatiles, etc.) and is often taken only as a macroscopic property, but variations, in fact, may occur in food microstructure. The surface properties of the drying particles are related to surface viscosity (Downton et al. 1982). This viscosity, as a result of water removal, increases rapidly as the glass transition is approached (Roos 2002).

There is a certain temperature (specific for each amorphous material) called the glass transition temperature, or T_g for short. Glass transition in dried fruits is a property that must be considered because, among other things, it promotes the ability to prevent the loss of small volatile compounds, such as esters during drying and storage (Komes et al. 2005). This is, precisely, the major concern when dealing with many dried fruit products such as leather fruit snacks, fruit powders, and agglomerates. The higher the T_g value, the more stable an amorphous (glass) system should be (Komes et al. 2005). Glass transition is a property of concentrated, amorphous, nonequilibrium food "solids," as the fruits are. T_g defines a second-order phase change temperature at which a solid "glass" is transformed to an immobilized "liquid-like" "rubber" (Goula et al. 2008).

Principal components present in fruits are low-molecular-weight sugars and some organic acids. They have low glass transition temperature (T_g) and are very hygroscopic in their amorphous state, so the dry product becomes sticky (Sonthipermpoon et al. 2006). Water acts as a plasticizer and decreases the glass transition temperature

of the product with the increase in moisture content and water activity. To overcome this problem, ingredients having high T_g value, such as maltodextrin, and food grade anticaking agents should be added to prepare vacuum-dried fruit powders. The relationship between T_g and a_w provides a simple method for prediction of safe storage temperature at different relative humidity environments (Sonthipermpoon et al. 2006).

When the amorphous material is cooled below this temperature, it becomes hard and brittle, like glass. Some amorphous material (dried fruit products) should be used above, while some must be used below its glass transition temperatures.

One should be aware that the collapse of a dehydrating food during freeze-drying, stickiness of the product during spray-drying, and caking and agglomeration of powders during processing and storage are some of the properties that are related to the glass transition temperature (Bhandari and Howes 1999). These defects also lead to structural changes on matrices like stability losses of the coated component due to an increase of the molecular mobility, the diffusivity of oxygen in the matrices, decrease of the matrices' viscosity, and increase of the chemical reactions involving reactants trapped in the glassy matrix (Bhandari and Howes 1999), being, in fruits, many of these reactants are odorant compounds.

Pure components show a single transition and may be only partially amorphous (carbohydrates, e.g., starch and sugars; proteins). Food components are miscible, partially miscible, and immiscible, forming single or several phases (Roos 2002). Glass transition is a change between the solid and liquid-like states of an amorphous phase, and this is represented by a discontinuity in specific heat of the complex substance, at that temperature. Glass transition occurs in cooling and heating and in removal or sorption of a plasticizer, or a solvent or both, like water. The amorphous portions undergo the glass transition only, and the crystalline portion undergoes melting only. Glass transition is time dependent as a result of the nonequilibrium nature of the complex amorphous phase.

The most important changes affecting food behavior are related to the exponential increase of molecular mobility and decrease of viscosity. These changes govern various time-dependent and often viscosity-related structural transformations, such as stickiness, collapse, and crystallization during food processing and storage.

According to Sablani and others (2007), a product is most stable at its monolayer moisture content, that is, a water activity value of about 0.1–0.3, or at or below the corresponding glass transition temperature.

One important property of the materials that must be submitted to drying processes is the thermoplasticity, by which materials that become soft at higher temperatures, harden again as the temperature is reduced. Many flavor products containing sugars or fats exhibit this property. If, for example, the desired fruit products are powders, as their temperature rises, the particles first soften then become sticky. This latter temperature is known as the "sticking temperature."

Many factors are involved in selecting the adequate drying technology, and the type of raw material to be dried, characteristics and specifications of the final product, the product's sensitivity to heat, available upstream processes, capital and processing costs, environmental restrictions, and safety considerations must be taken into account.

Drying process technologies, used to reduce moisture in the materials, are numerous and quite complex but may be classified in three major methods (Hui et al. 2007;

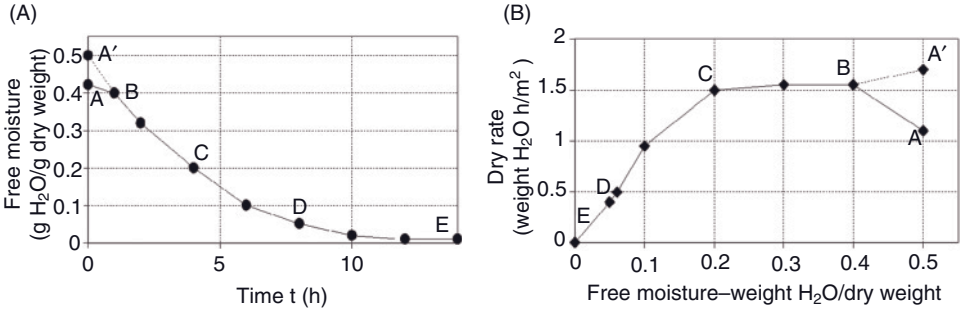

Figure 26.1. (A) Free moisture dynamics. (B) Drying rates versus moisture content. Distinct periods in a drying process (hypothetical data). Adapted from Fellows (2000).

Ibarz and Barbosa-Cánovas 2002; Lozano 2006; Lozano et al. 2000; Sablani et al. 2006; Valentas et al. 1998)—*mechanical dewatering* (where a force is applied), *thermal drying* (air-drying, low air-drying, and modified atmosphere [MA] drying), and *osmotic drying* (based on a differential osmotic pressure).

Water is held by forces whose intensity ranges from the very weak forces retaining surface moisture to very strong chemical bonds.

Also, according to Hui and others (2007), moisture removal may be achieved through several possible mechanisms of liquid-water transfer (capillary flow, surface diffusion, and liquid diffusion) and water-vapor transfer (Knudsen diffusion, Stefan diffusion, Poiseuille flow, and condensation–evaporation).

The drying process occurs along three distinct stages or periods, which are the increasing drying rate period (a short, initial period), the constant drying rate period, and the falling drying rate (Fig. 26.1).

Right after contact between the drying material and the drying medium, the material temperature adjusts until it reaches a steady state.

The material temperature and the rate of drying may increase or decrease to reach the steady-state condition (AB). At steady state, the temperature of the wet solid surface is the wet bulb temperature of the drying medium. The highest rate of drying is normally the constant rate situation; then as drying proceeds, the moisture content falls, and the access of water from the interior of the food to the surface decreases drying rates.

Temperatures within the drying material also tend to equalize the wet bulb temperature of the gaseous phase, but the lag in movement of mass and heat result in some deviation. Once the stock temperatures reach the wet bulb temperature of the gas, they are quite stable and the drying rate also remains constant. This is the constant rate drying period (BC) that ends when the material reaches the critical moisture content, which is fundamental to drying at this stage, since the driving force is the difference between the partial water vapor pressure of the food and the air. Beyond this point, the surface temperature rises, and the drying rate falls off rapidly (CD). In general, increased air velocity and air temperature increase the drying rate, while increased humidity and solid thickness decrease it. Not always distinguishable, there may be another change in the drying rate. If this happens, it is referred to as the second falling rate period. The falling rate periods can take far longer than the constant rate period even though the moisture removal usually is less. The rate-

controlling factors in the falling rate period are complex, depending on the diffusion through the food and on the changing energy-binding pattern of the water molecules. The drying rate approaches zero at a certain equilibrium moisture content, which is the lowest moisture content obtainable within a particular material under the set of drying conditions used.

The increasing rate period, in which only the surface free water is removed, is very important because about half of the possible moisture reduction is accomplished at this stage.

During the constant drying rate period, the wet materials contain high water contents that cause existing liquid surfaces to expose the open surface of free water. The first phase is the constant-rate step, in which moisture rapidly evaporates from the surface and capillary action draws moisture from within the item. The drying rate depends only on the environmental conditions and the total water surface area. Toward the end of the constant drying rate period, moisture has to be transferred from the inside of the material to the surface. The moisture content at which the drying rate ceases to be constant is called the critical moisture content.

After the constant drying rate period, dry spots appear on the surface, and the drying rate decreases. This is called the falling rate period, in which two processes are involved: the movement or migration of moisture within the material (mass transfer) to the surface and then the removal of moisture from the surface (evaporation). When the surface is completely dry, the moisture is transported from the inner parts of the material to the external surface as the result of concentration gradients between the interior of the material and the surface (Mujumdar 1995, 2007). The water that is evaporated from the hotter portions within the drying materials may migrate toward the drier surface region or toward the cooler inner regions and then recondense (Mujumdar 1995). The constant temperature at the middle portions of the materials, during the drying process, indicates that recondensation and evaporation are occurring.

After the drying rates have been experimentally or theoretically estimated, they can be used to calculate drying times, thus, facilitating equipment design.

Generally, the drying rates vary throughout the dryer over time as drying proceeds and as the moisture content of the material changes.

Raising the temperature of the drying air increases the moisture-carrying capacity of the air and decreases the relative humidity. As a general rule of thumb, increasing the air temperature by 10°C doubles the moisture-holding capacity of the gas, hence decreasing by half its relative humidity.

Drying technology began by using wind, direct, or indirect solar energy, and natural successive freezing and thawing processes through simple exposure to open air. The drying processes developed to the present provide multiple options using more or less sophisticated technologies such as solar drying, kiln drying, tray drying, tunnel drying, spray-drying, freeze dehydration, osmotic dehydration, extrusion, fluidization, microwaves (MWs), radio frequency, Refractance Window™, and hurdle technology.

Lamikanra and others (2005) recall that Vega-Mercado and others (2001) summarized the main dehydration technologies in four classifications according to physical and chemical conditions of the raw materials, target characteristics or objectives for the final products, technological mechanisms applied, and, finally, technological sophistication of equipment for each stage (Table 26.1).

TABLE 26.1. Four Main Dehydration Technologies According to Vega Mercado and Others (2001)

	1st	2nd	3rd	4th
Raw materials	Grains Sliced fruits	Slurries and purees	Applied to whole or sliced fruits	Applied to fruit juices, slurries, and purees
Product	Chunked	Dehydrated powders or flakes	Whole or sliced fruits	High value-added products
Dryers	Kilns, trays, rotary flow conveyors, and tunnels	Spray dryers (SD) and Drum dryers (DD)	Freeze-drying (FD) Osmotic dehydration (OD)	Radiant Zone Drying™(RZD) Refraction Window drying (RWD) Microwave-assisted drying (MWAD)
Basic technology	Forced convection of hot airflow over the drying products	In SD—fluid is atomized to fine droplets into a chamber containing a hot drying media.	FD is based on hypobaric water sublimation from previously frozen or ultrafrozen whole or sliced fruits.	RZD—adjust to the change in latent heat of vaporization between initial constant rate drying period and the subsequent falling rate period, preventing thermal damage.
		In DD—a drum heated inside slowly rotate while covered by a layer of food by dripping spraying or spreading. After rotating from 20 s to 30 min, the dried material is bladed from the surface of the drums	OD is based on moisture reduction by immersing fruits in hypertonic solutions, at environment conditions or under vacuum.	

Drying processes are one of the most energy-intensive unit operations. There are a number of approaches to reduce energy consumption in dryers. Drying conditions can be modified or the drying equipment can be modified to increase overall efficiency. Hybrid drying techniques can also be used, such as combining vacuum or convective drying with electro-technologies (MW, radio frequency, infrared heating).

GENERAL OBSERVATIONS ABOUT DRIED FRUITS

Dried fruits are sent to market in various sizes, shapes, and forms: chopped, minced, cubed; as flakes, rings, slices, granules, crystals, and powders; or in concentrated puree form—leather rolls, all according to their planed applications.

Dried fruits may be preserved naturally, or with sulfur dioxide, rolled in rice flour or candied, and can be directly eaten or included as ingredients in baked goods, snack blends, preprepared meals, jellies or marmalades, stuffings, glazes, fillings, gravies, pastries, conserves, confectionery, muffins, muesli, cookies, breads, bagels, breakfast cereals, instant drinks, foods or soup mixes; and as seasonings, added to trail mixtures with other fresh fruits, rectifying the balance of moisture, texture, sweetness, and/or acidity in salads, gravies, sauces, jams and jellies, sherbet, and ice cream. Some dried fruits, like apples, plums, and figs, with high soluble fibers and pectin contents, display thickening properties, binding with water, while malic acid content enhances flavor, giving the final products a smooth, soft, butter-like texture. For example, dried plums—containing high levels of artificially added or naturally occurring sorbitol, an effective humectant—can help keep the finished products soft and moist over an extended shelf life (Castaldi and Degen 2003). Dried fruits in powder are applied to fill capsules of nutrient supplements and in tablets, or may be added as an improved ingredient to powder mixtures or food bars. Dried plums are also an effective bakery humectant. This is due to the combination of fiber (half of which is soluble), sorbitol, and other reducing sugars (glucose and fructose) that retain and then hold moisture.

When rehydrated, dried fruits can be used in fruit salads and jellies, or pureed for fruit soups, sauces, or blended drinks.

Characteristic flavors and aromas of fresh fruits depend on subjective responses to multiple component factors—species, genetic profile, cultivars, location, ripening stage, storage conditions, and fruit maturity at harvest. All of which influence the balance of complex mixtures of nonvolatile and volatile compounds, the prime factor for flavor and aroma perception, some of which are detected at extremely low concentrations. These compounds and/or their relative proportions may be strongly affected during storage stages and processing, leading to a reduction in intensity of original flavor and inducing synthesis of different flavor/aroma compounds. Volatile compounds are also produced by heat, ionizing radiation, oxidation, or enzymatic activity on their constituents (Fellows 2000). The perceived aroma of food detached from complex combinations of many hundreds of compounds, some of which present mutual reactions exhibiting antagonistic, synergistic, additive, potential, or independent effects.

In addition, the perceived flavor of foods is influenced by the rate at which flavor compounds are released during chewing, and hence is closely related to texture resilience of the fruits and with the breakdown rate of the structure disruption during mastication (Fellows 2000).

Regardless of the technological sophistication of the drying equipment or operational control of a dehydration system, in comparison to their original fresh form, dried fruits are necessarily affected by relevant modifications, which may not be beneficial: (1) alteration of flavor and aroma concentration and bouquet composition; (2) change of colors; (3) modifications of texture and microstructures;

TABLE 26.2. Fruit Characteristics for Drying Processes (Extracted from Reynolds 1993)

Fruit	Suitability *for* Drying	Suitability *for* Fruit Leather
Apples	Excellent	Excellent
Apricots	Excellent	Excellent
Avocados	Not recommended (i)	Not recommended
Bananas	Good	Fair to good
Berries with seeds	Not recommended (ii)	Excellent
Blueberries	Fair	Poor unless in combination
Cherries	Excellent	Excellent
Citrus fruits	Not recommended (iii)	Only in combination
Citrus peel	Excellent	Only in combination
Coconuts	Excellent	Only in combination
Crab apples	Not recommended (iv)	Only in combination
Cranberries	Poor	Only in combination
Currants	Good	Not recommended
Dates	Excellent	Only in combination
Figs	Excellent	Only in combination
Grapes	Excellent	Fair to good
Guavas	Not recommended (v)	Only in combination
Melons	Poor	Not recommended
Nectarines	Excellent	Excellent
Olives	Not recommended (vi)	Not recommended
Papayas	Good	Better in combination
Peaches	Excellent	Excellent
Pears	Excellent	Excellent
Persimmons	Fair	Not recommended
Pineapples	Excellent	Excellent
Plums	Good	Good
Pomegranates	Not recommended (vii)	Not recommended
Prune plums	Excellent	Excellent
Quince	Not recommended (viii)	Not recommended
Rhubarb	Good (ix)	Fair
Strawberries	Fair to good	Excellent

(4) alteration of nutritional composition; (5) depletion of vitamins A and C, the most perishable in drying processes; (6) some loss of thiamine, riboflavin, and niacin during blanching; (7) considerable changes in shape or shrinkage also occur; and finally, (8) the enzymes also break down pectin—the glue that holds fruit cells together—softening the fruit by allowing cells to slide past one another. However, a consequence of enzymatic activity is precisely to produce aromatic compounds.

The affinity of each fruit to drying processes differs (Reynolds 1993), as seen in Table 26.2. For instance, apples, apricot, cherries, citrus peel, coconuts, dates, figs, grapes, nectarines, peaches, pears, pineapples, and prune plums are excellent fruits for drying. On the other hand, some other fruits are not suitable or recommend for drying for several reasons, such as (i) high fat content (avocados); (ii) high seed content and slow rate of drying (berries with seeds); (iii) too juicy and pulp lacks firm texture (citrus fruits); (iv) too small and tart; can be combined with other fruit for leather rolls (crab apples); (v) grainy flesh full of seeds (guavas); (vi) high oil content or bitter flavor removable only by long processing (olives); (vii) pulp is full

of seeds (pomegranates); (viii) hard flesh or strongly acidic flavor (quinces); and (ix) leaves contain salts of oxalic acid (rhubard) (Reynolds 1993).

FLAVORS

Flavor is in actuality a psycho-biochemical phenomenon, like the sensation produced by a material taken in one's mouth, or flavor can refer to a perceived attribute of an ingested material (Camelo 2002). For many, the scents are unforgettable—intimately connected to memories and with charged emotional content. Writers have long recognized and made use of this, Marcel Proust being a notable example. At any rate, aromas affect people at many levels and strongly influence consumer behavior. This attribute, normally, results from a subjective sensorial response to the characteristics of the food that triggers a particular sensation of flavor and is only fully perceived through the interaction of aroma receptors in the nose and taste receptors in the mouth.

According to Kraft and Swift (2005) and Goff and Klee (2006), the flavor preferences and repulsion suggest that flavor perception may be linked to the nutritional or health value of foods. The human tongue can detect just five flavors—sweet, sour, salty, bitter, and umami, the latter is also called savory—and scent provides considerable added information about a food. The odors of the odorant compounds are of particular interest because they are a major factor in how taste is perceived.

Studies in rats showed that homeostatic (i.e., eating for physical surviving) and hedonic (i.e., eating just for pleasure) eating motives overlap but are nonetheless separable (Lowe and Levine 2005).

The potential interactions among needing, wanting, and liking are based on several principles of food perception—taste, smell, and orosensation. Furthermore, one must consider food addiction principles, which are tied to food memory and pleasure. These combined characteristics are usually referred to as the food pleasure equation, highlighting the importance of gathering together in a food, both nutrition and sensation effects. Nowadays, description has expanded to seven basic tastes: bitter, salty, sour, astringent, sweet, pungent, and umami (Delwiche 1996).

However, the sense of taste alone is insufficient to describe a food preference. This leads to the consideration of additional sensations: sense of smell, aroma sense, trigeminal sense, orosensation, and brain flavor processing (OFC). Among physiological and psychological relationships to food preferences, the emotional concept of aroma memory must be added (Lyman 1988).

Basic research on perception and human judgment advances the understanding of sensorial function that, in general, induces product-focused development of foods and, of course, is required for new dried fruit product development.

Trigeminal differentiation separated from elementary ingestive movement patterns (biting, licking, and chewing) is still disrupted through the perioral stimuli (Berridge and Fentress 1985).

Touch, smell, and taste are also considered to be emotionally driven, intimate, and trusted senses, as opposed to the somewhat less reliable senses of sight and hearing.

Since 80% of taste is smell, this is a key emotional persuasion factor influencing willingness to try and finally purchase a product. This is economically critical because

(even loyal) consumers can and do switch brands, for example, the Coca-Cola Company's disastrous change in formula a few years ago.

Since the human tongue can detect just five (or perhaps seven) flavors, scent provides crucial information about a food. The odors of the compounds are of particular interest because they are a major factor in how taste of foods is perceived.

Aroma compounds include a large variety of chemical substances—alcohols, carbonyls acids, esters, lactones, and phenols (Heath 1981). Color, acidity, and flavors show similar profile development during fruit ripening (Heath 1981; Maarse 1991).

These compounds suffer from multiple chemical and biochemical modifications. For instance, aldehydes are easily oxidized to acids; amines may complex *bond* with metal ions; in the presence of acids, terpenes rearrange and isomerize; exposure to light may cause photooxidation or reordering; and polymerizations of unsaturated compounds occur.

The physicochemical properties of the volatile compounds when interacting with the chemical nature and structure of the fruit matrix are powerful enough to modify the concentration of the volatile compounds and therefore their perception (Bezman et al. 2003; Chervin et al. 2000).

The odors of the odorant compounds are of particular economic interest because they are a major factor in how the taste of fruits or processed fruits are perceived and evaluated by the consumers.

A typical fruit may have more than a few hundred different volatile components, but in total, these compose only a few parts per million of the entire fruit.

Fruit aromas vary widely. Citrus, such as grapefruit, orange, lemon, and lime, are rich in terpenoids, whereas most non-citrus fruits, such as apple, raspberry, cranberry, and banana, are characterized by esters and aldehydes.

A fruit's quality is frequently attributed to several interrelated factors such as firmness, aroma, sugar and acid metabolism, and appearance (pigments), which are in fact a result of production factors including cultivar, rootstock, mineral nutrition, irrigation, and environmental factors during maturation and storage.

Ripening involves multiple and complex biochemical and physiological reactions, under subtle control of genetic regulation, that leads to softening (Alexander and Grierson 2002; Speirs et al. 1998).

In general, sweetness increases while astringency and tartness decrease during fruit ripening. Ripening is also a process that includes development of color, flavor, and texture (Alexander and Grierson 2002)—mostly, and a softening process, resulting from pectic enzymes starting to break down the pectin of their cell walls.

Building up of the aroma compounds in fruit is a complex, dynamic process during which the concentration of volatiles changes both qualitatively and quantitatively. Some studies about the influence of the degree of maturation in volatile composition have been published (Chapman and Horvat 1990; Lalel et al. 2003).

Fruits that undergo intense metabolic activity during ripening are known as climacteric fruits and may be harvested before fully ripening, because during postharvest storage, their overall quality may be improved by using an indoor appropriate air quality survey, for instance, controlled atmosphere (CA) storage, modified atmosphere packaging (MAP), or with edible coatings. A climacteric fruit shows a distinct increase in respiration, that is, CO_2 production. Respiration rate for a climacteric fruit is about twice the preclimacteric low point. The peak in apples,

for instance, shows up right after the fruit is ripe (Calderon and Barkai-Golan 1990; Saltveit 1993). Furthermore, commodities and or cultivars with higher rates of respiration (strawberry, blackberry, raspberry, cauliflower, lima bean, and avocado) tend to have shorter storage life than those with lower rates of respiration, such as apples, citrus, grapes, and kiwifruits (Saltveit 1996).

At any rate, all postharvest operations and technological manufacture may affect the fruit's flavor (Bezman et al. 2003; Chervin et al. 2000; Derail et al. 1999; Kralj-Cigic and Zupancic-Kralj 1999; Lambert et al. 1999; Lara et al. 2003; Sumitani et al. 1994), leading to the logical conclusion of reducing, as much as possible, the number and the length of the postharvest operations and proceedings, preceding any drying technologies.

Nevertheless, precautions must be taken on account of potential fermentation pathways (Meigh and Reynolds 2006). In all cases, the storage environment must be low in oxygen (O_2) and high in carbon dioxide (CO_2) compared with atmospheric conditions (Barmore et al. 1983; Biale 1946; Roberts et al. 1965). However, low oxygen causes an increase in the ethanol concentration in the juice of the fruit and the development of an undesirable stale flavor (Barmore et al. 1983). The low O_2 (1.5% and 2.0% [v/v]) and high CO_2 concentrations depress ethylene production, which is required to turn on ripening genes that affect color changes, aroma development, and degradation of cell walls. Changes in flavors, aroma, taste, and texture of climacteric fruits are connected with a transitory respiratory peak seemingly related to autocatalytic feedback controlled reaction of the ethylene production (Calderon and Barkai-Golan 1990; Saltveit 1993).

During storage, nonclimacteric fruits, at best, maintain quality equal to that at the moment of harvest, without any further beneficial changes. For instance, apricots picked for drying must not be picked too early or they will lack color and flavor. Late fruits, when overripe, present deficient texture to sustain drying processes (Barrett et al. 2005).

Storage of fresh fruits is a very sensitive task in determining the overall quality of the final dried product, since its success depends on precise requirements for temperature, relative humidity, and ethylene exposure balance throughout the drying processes.

Shelf life may be extended by adding or removing gases resulting in an atmospheric composition different from normal air—MA, or by maintaining a more strict balance in the atmosphere composition—CA or by using hypobaric (low-pressure) chambers. CA reduces metabolic activity of the fruit and the rate of ethylene production, as well as the sensitivity of the fruit to ethylene, which can hasten fruit softening and color change. These procedures, however, must always be accompanied by low storage temperature and not seen as sufficient in themselves. For instance, for apples, a typical atmospheric composition of a CA facility is 7–25% carbon dioxide and 2–4% oxygen. The optimum composition varies with temperature and apple variety, but oxygen does not normally go below 2%, or anaerobic respiration can occur, leading to off-flavors and internal browning. In hypobaric storage, an absolute pressure of 102 mm mercury (0.16 atm) is often used.

The requirements for more sensitive and delicate fruits such as berries are different. For example, the recommended storage of strawberries is at 0°C and 90–95% relative humidity. After a few days in storage, they may lose some color and flavor and shrivel. Berry shelf life can be extended by using 10–30% carbon dioxide in

refrigerated storage, usually during transport. This can be achieved by packing the berries in containers with dry ice or covering pallets with coated fiberboard or heat-shrink polyethylene film. Pallet loads can also be covered with polyethylene bags and injected with carbon dioxide gas; however, in the last case, off-flavors can develop if carbon dioxide levels are above 30%.

An effective guide for some fruit and vegetable storage conditions can be found in Table 26.3.

TABLE 26.3. Fruits at 7 Days Storage—Maximal Environment Conditions: Relative Humidity: 85–95%; Ethylene Concentration: Less Than 1 ppm

Group 1	Group 2	Group 3
Temperature: 0–2°C	Temperature: 7–10°C	Temperature: 13–18°C
Apple	Avocado	Atemoya
Apricot	Babaco	Banana
Avocado	Cactus pear	Breadfruit
Barbados cherry	Calamondin	Canister
Grapes	Carambola	Casaba
Blackberry	Cranberry	Cherimoya
Blueberry	Custard apple	Crenshaw
Boysenberry	Durian	Honeydew melon
Caimito	Granadilla	Jaboticaba
Cantaloupe	Grapefruit[a]	Jackfruit
Cashew	Guava	Mamey
Cherry	Juan Canary melon	Mango
Coconut pear	Kumquat	Mangosteen
Currant persimmon[a]	Lemon[a]	Melon
Cut fruits	Limequat	Melon
Dewberry	Orange	Papaya
Elderberry	Passion fruit	Persian melon plantain
Fig	Pineapple	Rambutan
Kiwifruit[a]	Sugar apple	Sapodilla
Loganberry	Tamarillo	Sapote
Longan	Tamarind	Soursop
Loquat	Tangelo	
Lychee	Tuna mandarin	
Nectarine	Unripe lime[a]	
Peach	Watermelon	
Plum date		
Plumcot		
Pomegranate		
Prune		
Quince		
Raspberry		
Ripe gooseberry		
Strawberry		

[a]Fruits sensitive to damage by ethylene exposure.

Extracted from the Compatibility Chart for Fruits and Vegetables in Short-Term Transport or Storage. University of California, Davis University of California—Division of Agriculture and Natural Resources. Publication 21560 (http://postharvest.ucdavis.edu/Pubs/postthermo.shtml).

Ultimately, the main target of postharvest technology is still reducing respiration rate and other metabolic reactions associated with quality retention by manipulating the external environment. It is generally recognized that respiration is affected by light, chemical stress (e.g., fumigants), radiation stress, water stress, growth regulators, and pathogenic attacks. However, there are postharvest quality depletion factors, before the drying sequence. These vary according to intensity, length, and aggressiveness of harvest procedures and should be reduced as much as possible. Postharvest care, besides minimizing handling and transportation costs, should assure better quality of the raw fruits, therefore, maximizing the investment return even before the drying stage.

RECOMMENDED PRETREATMENTS FOR DRYING FRUITS

Pretreatments prevent fruits from darkening. Many light-colored fruits, such as apples or apricots, darken quickly when cut and exposed to air. If not pretreated, these fruits will continue to darken after they are dried.

Blanching

Water blanching is recommended over steam blanching or blanching in an MW because water blanching achieves a more even heat penetration than the other two methods. Plain water or water with added citric acid may be used. Citric acid acts as an anti-darkening and antimicrobial agent.

Sulfuring

For long-term storage of dried fruit, sulfuring or using a sulfite dip is the best pretreatment. However, sulfites found in the food after either of these treatments have been found to provoke asthmatic episodes in sensitive people. Thus, in some cases, a shorter-term alternative pretreatment may be a better option.

Sulfuring is an old method of retreating fruits. Sublimed sulfur is ignited and burned in an enclosed box with the fruit. The sulfur fumes penetrate the fruit, retarding spoilage and darkening. The sulfur fumes also reduce the loss of vitamins A and C.

Fruits usually sulfured are oranges, apricots, light brown figs, cantaloupe, crystallized ginger, golden raisins, mango, papaya, peaches, pears, and pineapple. Unsulfured dried fruits are brown apricots, blueberries, cherries, cranberries, currants, dates, black mission figs, Turkish figs, prunes, and black raisins.

Food grade bisulfate, sodium sulfite, or sodium metabisulfite dips can achieve the same long-term anti-darkening effect as sulfuring, but quickly and easily.

Ascorbic Acid

Ascorbic acid (vitamin C) mixed with water is a safe way to prevent fruit browning. However, its protection does not last as long as the sulfuring of sulfating. Ascorbic acid reacts easily with oxygen and diminishes oxygen that will be used by phenolase. Ascorbic acid also reduces the *o*-quinones formed by phenolase to the original

o-dihydroxyphenolic compounds. Ascorbic acid is not satisfactory for apples since the internal atmosphere of the fruit contains oxygen.

"Checking"

This procedure is also referred to as "cracking skins" and is used on fruits such as blueberries, cherries, grapes, plums, and a few other fruits with relatively tough skins and a protective waxlike coating. Checking removes this waterproofing substance and cracks open the skin's surface. This promotes drying and prevents rupturing of the fruit.

This procedure is as follows: first, briefly dip the fruit briskly (in and out of) boiling water. Next, immediately immerse fruit in ice cold water for a few seconds. Drain fruit thoroughly and lay it on absorbent toweling. Continue with the next step for drying that particular fruit.

OVERVIEW OF DRYING PROCESSES AND EQUIPMENT

The volume and moisture content of the feeding fruits, its inherent specific surface characteristics, and especially the finished product requirements or legal specifications are determinant factors for a successful selection of drying systems and equipment.

Drying is accomplished by some combination of the basic heat transfer processes: conduction, convection, or radiation.

Nowadays, alternative thermal processing technologies are demonstrating their usefulness and greater future potential: the Ohmic heating technique, radio frequency energies, infrared rays, and the combination of pressure and thermal processes are gaining ground in drying technologies.

Infrared radiation (IR) is a very interesting option because it is the least invasive for fruits' structure.

The majority of artificial drying operations are based on hot air drying, where air is heated by the combustion of fossil fuels prior to being blown around the product. This type of drying requires high energy input, due to the inefficiencies of such dryers. Often, the exhaust air is simply released to the surrounding ambient air. However, some systems allow for the recycling of exhaust heat, which can greatly increase the overall energy efficiency of the dryer.

Naturally Dried Fruits

Fruits may be allowed to naturally dry on trees before they are harvested. This method started in Australia for raisins and is known as dry on vines (DOV). DOV production systems continue to evolve both in Australia and the United States (Christensen et al. 1993; Shaw et al. 1996).

Although raisin production, traditionally, involves a lot of manual labor to cut bunches of green grapes and layer them to dry in the sun on paper trays between rows of grapevines, the new dried-on-the-vine technology (DOV) allows for machine harvesting of the dry raisins and for less tillage (Boriss et al. 2006). Thus, it offers increased yields of high quality, natural, sun-dried raisins that can be machine harvested (Ramming 2006; Shaw et al. 1996; Somogyi 1996).

Dates are technically considered a fresh fruit rather than a dried fruit, as they dry on the trees before harvesting.

Sun-Dried Fruits

The fruits are laid out in the sunshine to dry. Larger fruits should be cut in half so they will dry faster. Fruits typically dried this way are apricots, currants, figs, peaches, pears, prunes, and raisins. If fruits are sun-dried, their enzyme loss will be minimized, provided the fruit is not heated above 48°C.

Solar Drying

Solar drying is an industrial process in many countries where outdoor temperatures reach 45°C or higher. Solar drying is different from "sun-drying" processes, because solar drying uses equipment to collect the sun's rays in a unit designed to ventilate moisture.

The temperature in the solar unit is usually 20–30°C higher than those attained by simple exposure to open sunlight.

Since cool night air condenses and can add moisture back to the food, fruits dried out-of-doors must be covered or brought under shelter at night (Garg 1987).

Conventional Dryers

Drying times in conventional ovens or dehydrators vary considerably, depending on the amount of food dried, its moisture content, and room temperature and humidity (and the use of fans, for oven drying). Some foods require several hours and others may take more than a day. Prolonging drying time (by using lower temperatures) or interrupting drying time may result in spoilage.

It is important to control air temperature and circulation during the drying process. If the temperature is too low or the humidity too high (resulting in poor circulation of moist air) the food will dry more slowly than it should, and microbial growth can occur. Temperatures must be watched closely at the beginning and end of the drying period. If the temperature is too high, at first, a hard shell may develop on the outside, trapping moisture on the inside. This is known as case hardening. Temperatures that are too high at the end of the drying period may cause food to scorch. Temperatures between 49 and 60°C are recommended for drying fruits and vegetables.

Air-Dried or Tunnel-Dried Fruits

The fruit is dried through warm air that is blown over it. Fruits typically dried in this way are apples, coconut, raisins, and tomatoes. Fruit dried this way typically does not oxidize as much as fruit that is sun-dried.

Osmotic Dehydration

The fruit is cut and peeled and then placed in a tub or large container. Water is heated and as much solute (mainly sugars) as possible is dissolved into the water.

The tubs of fruit are then covered with the 30–60°Brix sugar syrup and let set for around 7 days. During these 7 days, the sugar water exchanges with the lower viscosity water in the fruit, and the water from the fruit is thereby extracted.

Once the fruits are partially dried this way, they are then air-dried to complete the drying process. Fruits typically dried by this process are blueberries, cantaloupe, cherries, cranberries, ginger, mango, papaya, pineapple, and strawberries.

For the last few years, several authors have concentrated their attention on improving osmotic dehydration processes (Baroni et al. 2003).

Fruits Dried by Frying

The fruits are fried in oil to cause the water in the fruit to rapidly boil away. Bananas and plantains are dried this way before the fruits have ripened and their starches are turned into sugar.

Drum Dryer

Like no other dryer, the drum dryer can dry highly sticky products and/or highly viscous media, but low-viscous liquids can also be dried in the drum dryer. The drum dryer is heated on the inside and turns continuously. In a full continuous process, the product is applied in a thin film on the outside of the drum and begins to dry immediately. After one rotation, a knife scrapes the dried product off the drum surface as a film or as flakes.

The drum dryer is an indirect dryer. Unlike direct drying methods, where hot air is used to evaporate the product moisture, the drum dryer needs no dust recovery. In addition, the thermal results are more favorable than in other methods because no heat is lost in the hot exhaust air.

MW Drying

MW (MW-related, MW-assisted, or MW-enhanced) combination drying is a rapid dehydration technique that can be applied to specific foods, particularly to fruits and vegetables. Increasing concerns over product quality and production costs have motivated research resulting in increasing industrial adoption of combination drying technologies.

The advantages of MW-related combination drying include the following: shorter drying time, improved product quality, and flexibility in producing a wide variety of dried products. But current applications are limited to small categories of fruits and vegetables due to high start-up costs and relatively complicated technology as compared with conventional convection drying. MW-related combination drying takes advantages of conventional drying methods and MW heating, leading to better processes than MW drying alone (Zhang et al. 2006).

The MW system is not yet generally recommended because the food will partially cook before it dries, imparting an overcooked flavor.

Spray- and Freeze-Drying

These systems are the two main methods most companies use to dry their foods, including fruits, especially those in puree or liquid forms. The most popular is spray-

drying, utilizing temperatures as high as 120°C. Freeze-drying utilizes extreme cold with temperatures as low as –50°C.

Drying occurs in two phases, and air-temperature control is vital to the process. The first phase is the constant-rate step, in which moisture rapidly evaporates from the surface, and capillary action draws moisture from within the particle. In the second, or "falling rate" period, diffusion of water to the surface controls the drying rate. As moisture content drops, diffusion rate also decreases. Removing the last small percentage of moisture in a single-stage dryer is responsible for most of the residence time in the dryer. As a rule, the residence time of the air and the particle in a single-stage, cocurrent dryer are about the same. Since the moisture level is still decreasing toward the end of the process, the outlet temperature must be high enough to continue the drying process.

Spray-drying involves atomization of the liquid and subsequent evaporation of water as the material passes through the drying chamber.

Table 26.4 delineates the general advantages and disadvantages for the most commonly used dryers for value-added fruit products.

Liquid-drying methods present high drying rates, low manufacture costs, and low energy consumption, and preserve initial fresh food quality by reducing additives and preventing oxidation. However, thermal degradation is still a challenge in food processing (Mujumdar 2001; Vega-Mercado et al. 2001).

In spray-drying systems, there is likely the formation of powder deposits on the cone of the dryer, but these can easily be removed after the dryer has cooled down. This is known as thermoplasticity and is a result of the dryer outlet temperature rising above the "sticking point." This problem can be solved by setting a lower outlet temperature in the chamber, on subsequent runs, but this will also give higher powder moisture. It is better then to use an "air broom" inside the drying chamber.

The flat-bottomed spray dryer with a tempered "air broom" is currently the dryer of choice for fruit and vegetable pulps and juices. The correct balance of dryer inlet and outlet temperatures can also reduce energy consumption.

TABLE 26.4. General Advantages and Disadvantages of Spray-Drying and Freeze Systems

Advantages	Disadvantages
Little thermal damage	High drying cost
Good retention of volatile flavors	Damage to certain products
Good vitamin retention	Rapid deterioration unless products are packed and maintained at low humidity
Rapid product rehydration	Product friability (crumbles easily)
Little product shrinkage	Pretreatment sometimes is necessary (e.g., with carrots) to avoid color loss
Long product storage life—if suitably packed	
Good retention of biological activity (with use of cryoprotectants)	
Antioxidant phytochemicals remain a part of the fruit when it is freeze-dried	

Extracted from Mujumdar (2004).

The newer "spin-flash" dryer is about one-third cheaper to build and operate, using much less energy, because with material rapidly dispersed in hot air and short residence time for drying, it is especially applicable for the drying of thermally sensitive substances. However, it is not suitable when a free-flowing spherical particle of a particular size range is required, or when agglomeration is needed.

The applied scope for spin-flash dryers is slurry material with high viscosity or powder material, especially cake-like materials filtrated by plate and frame presses.

Spray-drying, drum drying, MW-assisted drying, freeze-drying, and Refractance Window™ drying produces fruit powders with better quality and at higher productivity.

IR

IR is a form of energy which, unlike other forms, does not use air as a transference medium. Infrared heating elements warm up less than 20% of the air and more than 80% of the objects in a chamber. The big advantage of this method is the fact that the heat penetrates deeply into the object at a moderate temperature, bringing more health advantages and less disadvantages.

Infrared heat is the same heat that the human body itself radiates and is, in principle, identical to solar heat but without harmful UV radiation.

A large proportion of the sun's energy is comprised of infrared energy.

The materials to be dried should have a reasonable transmissivity to avoid excessive heating and material damage (Mujumdar 2007). Materials suffer changes in radiation properties during drying, increasing reflectivity and lowering absorvity at low water contents. Transmissivity decreases with layer thickness, while absorvity increases (Barret et al. 2005).

Refraction Window (RW)

A curious proprietary process is RW—United States Patent 6047484—and the development of another patented process described in U.S. Pat. No. 4,631,837 issued on December 30, 1986, in which an infrared transparent film is supported by the buoyant force of water.

RW consists of the transference of fruit moisture or fruit purees over a hot water bath, on a transparent belt. Infrared energy from hot water dries the fruit materials, and the belt itself prevents thermal energy losses.

The patented RW principle of operation transmits infrared energy in the water directly through the conveyer belt into the fruit or vegetable to be dried, ensuring a gentle drying process. As the fruit or vegetable dries, the conveyor belts' RW closes when the fruit or vegetable reaches 3% moisture. This process reflects back to the water the majority of the infrared rays, leaving only conductive heat as the drying means.

For RW-dried samples, a significant increase in ketone content was observed (Abonyi et al. 2002). This demonstrated that dehydration at elevated temperatures (RW and spray-drying) altered the overall flavor impression in dried samples by enriching ketone and aldehyde flavor notes. RW-dried strawberries had color values close to that measured in freeze-dried samples (Abonyi et al. 2002).

RW drying protects the integrity of the fruits and vegetables by preventing degradation of nutrient content, color, and flavor.

To conclude this summary of drying systems and technologies, it is worth remembering that Kudra and Mujumdar (2001) classified, described, and discussed various new dryers, ranging from laboratory-scale (e.g., acoustic drying, drying of slurries by impinging sprays over a hot surface) to pilot-scale demonstrations (e.g., pulse combustion dryers, ultrasonic spray dryers, impinging stream dryers) to full-scale commercial dryers (e.g., pulsed fluid beds, superheated steam fluid bed/flash dryers, rotary dryers with drying air injected into the rolling bed). Kudra and Mujumdar (2001) also published a comprehensive summary of the numerous new drying concepts and technologies.

Finally, it is advisable to switch energy sources since changing from conventional convective air drying systems to superheated steam dryers can cut 5–10 times the energy requirements (the latter produces 700–1000 kJ/kg of evaporated water) (Mujumdar 2007).

REHYDRATION

It is often required to restore, as nearly as possible, the original moisture level of fruit that has been dried.

Rehydration capacity or rehydration ratio refers to the maximum amount of water that the product is able to absorb upon immersion. This concept is important to products that are to be used after a total or partial reconstitution (Oliveira and Oliveira 1999).

Shrinkage and case hardening adversely influence rehydration characteristics (Moreira and Sereno 2003). Freeze-drying is used to dehydrate high-added-value fruits and has a high success rate concerning shrinkage and case hardening prevention, which in turn indirectly influence, up to a point, flavor preservation.

Stirring or agitation of the viscous immersion media also improves the rehydration rate.

When considering rehydration, two aspects must be taken into account: rehydration rate and rehydration capacity (Oliveira and Oliveira 1999). Rehydration rate usually decreases with time of water absorption rate. A low rehydration rate is required for products that should remain crispy (Oliveira and Oliveira 1999).

Most of the rehydration analyses on vegetables are based on Fick's laws of diffusion and the appropriate equations (Engels et al. 1986; Hsu 1983; Mazza and LeMaguer 1980).

At any rate, a two parameter, non-exponential, empirical equation was proposed by Peleg (1988), which seems to successfully locate the optimal dried vegetable rehydration conditions: $1/(M_t - M_o) = K_1 + K_2 t$, where M_t = moisture content (mc, % db) at time t; M_o = initial moisture content (% db); t = rehydration time (min); K_1 = constant (min per % mc db); K_2 = constant (reciprocal of % mc db); and M_e = equilibrium moisture content (% db).

The effect of the extrinsic rehydration variables (process temperature and solution composition and physical characteristics) on the mass transfer between fruit and solution and on the physicochemical characteristics of the rehydrated products are also reported and discussed by Oliveira and Oliveira (1999). Through varying

the concentration of solutions used for rehydration medium and the nature of the solute, it is possible to adjust the water and soluble solids transfer, in order to obtain products with the desired water activity, freezing point, freezable water content, and firmness (Mastrocola et al. 2005). Rehydration in aqueous sugar solution could thus improve the stability, technological functionality, and sensory quality of fruit pieces used as ingredients in food to be consumed at room, chilling, or subfreezing temperatures.

Krokida and Marinos-Kouris (2003) used a first-order kinetic model, in which the rate constant and the equilibrium moisture content are functions of the process variable (water temperature), in which the rate constant and the equilibrium moisture content may be expressed as functions of the process variable (water temperature). In fact, both rehydration constants (K1 and K2) of the Peleg model increase with the increase of the drying temperature (Planinicacute et al. 2005).

Simultaneously, the paramount role of thermal physical properties of the food particles (e.g., pore-size distribution, heterogeneity, tortuosity, contact angle) and the embedding liquid (e.g., density, viscosity, temperature) should also be considered in order to overcome what seems to date a poor integration of elements in the process model. Hence, size and geometry of dried fruits affect dehydration rate and final moisture retention capacity.

Temperature and composition of the immersion media play an important role in the rehydration rate. Temperature effects may be described by an Arrhenius-type relation. Furthermore, not only is the fruit anisotropic, but also its geometry, volume porosity, and structure changes with moisture uptake. Rigorously mathematical phenomenological modeling of rehydration of dried vegetables and fruits is still a very difficult task. However, while difficulties in rehydration processes are being overcome, flavor loss and texture modifications are yet to be adequately dealt with.

THE FUTURE OF DRIED FRUITS

Dried fruits are becoming more and more imaginative—tend to be intense in flavor and easier to store, but are also higher in caloric content by weight due to their low moisture content, not to mention that they can be sugar infused and/or coated with oils to prevent sticking. They can also lose essential vitamins through heat exposure and may become discolored during the drying process. Industrial measures may mitigate some of these problems.

As with any other product, there are two major strategies to develop and increase the dried fruits market: (1) lower-cost production and shipping; and (2) differentiated producer/shipper marketing, by promoting a premium product or a product with identifiably preferred characteristics commercially perceived and valued.

Many of the products marketed as “natural gourmet” and “specialty organics” are perceived as products of uncommon quality. This appears to be a consumer-driven trend, since more and more consumers seem willing to pay a premium price for such labels.

According to Howard and Guile (1992), innovation is defined as follows: “a process that begins with an invention, proceeds with development of the invention, and results in the introduction of new product, process or service in the marketplace.”

In 1886, E. Spawn, an American inventor, patented the first mechanized dehydration machine—the "Climax Fruit Evaporator"—consisting of trays of fruit that revolved slowly in an upward current of hot air. Since then, a consistent nonstop evolution of drying technologies has been applied to improve productivity and quality. None of the developed technologies proved harmful in any way (not to be taken lightly in the history of food processing) but represent steady and progressive improvement.

A respected researcher and opinion maker of the art/science (art because aesthetics *are* important in what we choose to eat) of drying systems, Mujumdar, in 2004, in his Plenary Lecture, at the International Drying Symposium Series (IDS), Sao Paulo, Brazil stated that "new products, new processes, higher production rates, more stringent environmental regulations, increased safety concerns, etc. often demand better performance levels at lower costs than is possible with traditional dryers. This need has led to some innovation in drying technologies."

In the same lecture, the author outlined a checklist for near future developmental tasks concerning drying technologies: new products and processes in the works; greater capacities than today's technology permits; better quantity and quality control than currently feasible; reduced environmental impact; safer operation; increased efficiency; lowering overall cost (i.e., lower investment and running costs). Table 26.5 lists a set of expected developments.

Concerning product innovation, an ever-increasing number of consumers have less time for cooking and eating at home, so home preparation and cooking is on the decline. This has led to the significant growth in easily prepared food products. Packaging innovations have helped food products to become more easily portable and storable (Mintel 2004—MSU Product Center, *category review*, *fruits*).

The confluence of social changes, economic trends, and technological innovation spells grand opportunities for growth and dried fruit products to fill an ever-expanding gap in the commodity markets. All that is required are joint ventures between producers, processors, and researchers; some interdisciplinary expertise, vision, and methodology; and, of course, the courage to run calculated risk.

TABLE 26.5. Examples of New Drying Technologies Developed through Technology Push and Market Pull

Technology *Originally Developed* for *Other Applications Applied* to *D*rying—"*Push Technologies*"	Developed to *Meet Current* or *Future Market Demand*—"*Pull Technologies*"
Microwave/RF/induction/ultrasonic drying	Superheated steam
Heat pump dryers	Impulse drying/Condebelt drying
Pulse combustion drying	Combined spray-fluid bed dryers—to improve economics of spray-drying
Vibrating bed dryers	Intermittent drying—enhance efficiency by reducing energy consumption and/or allowing use for multichamber, multiproduct designs
Impinging streams (opposing jets)	

Extracted from Mujumdar (2004). RF, radiofrequency.

In conclusion, while no one can foresee future market prices for dried fruit products nationally or globally, no one can deny that the future of dried fruit production and technology will continue to advance through human ingenuity, and it should certainly be a sunny future indeed.

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However, all the still remaining imprecision or errors in the present chapter will stay under author's full property copyrights.

REFERENCES

Abonyi BI, Feng H, Tang J, Edwards CG, Chew BP, Mattinson DS, Fellman JK. 2002. Quality retention in strawberry and carrot purees dried with Refractance Window™ System. *J Food Sci* 67(2):1051–6.

Aguilera JM. 2006. Food product engineering: Building the right structures. *J Sci Food Agric* 86:1147–55.

Aguilera JM, Lillford P (eds.). 2007. *Food Materials Science. Principles and Practice*. New York: Springer-Verlag.

Alexander L, Grierson D. 2002. Ethylene biosynthesis and action in tomato: A model for climacteric fruit ripening. *J Exp Bot* 53(377):2039–55.

Barbosa-Cánovas GV, Vega-Mercado H. 1996. *Dehydration of Foods*. New York: Springer.

Barmore CR, Purvisa C, Fellers PJ. 1983. Polyethylene film packaging of citrus fruit: Containment of decaying fruit. *J Food Sci* 48(5):1558–9.

Baroni AF, Sereno AM, Hubinger MD. 2003. Thermal transitions of osmotically dehydrated tomato by modulated temperature differential scanning calorimetry. *Thermochim Acta* 1–2:295–308.

Barrett DM, Somogyi LP, Ramaswamy HS. 2005. *Processing Fruits: Science and Technology*. New York: CRC Press.

Berridge KC, Fentress JC. 1985. Trigeminal-taste interaction in palatability processing. *Science* 228(4700):747–50.

Bezman Y, Mayer F, Takeoka GR, Buttery RG, Ben-Oliel G, Rabinowitch HD, Naim M. 2003. Differential effects of tomato (*Lycopersicon esculentum* Mill) matrix on the volatility of important aroma compounds. *J Agric Food Chem* 51:722–6.

Bhandari BR, Howes T. 1999. Implication of glass transition for the drying and stability of dried foods. *J Food Eng* 40:71–9.

Biale JB. 1946. Effect of oxygen concentration on respiration of the Fuerte avocado fruit. *Am J Bot* 33(5):363–73.

Boriss H, Brunke H, Kreith M. 2006. *Commodity Profile: Raisins (Dried Grapes)*. Agricultural Issues Center, University of California. http://aic.ucdavis.edu/profiles/Raisins-2006.pdf (accessed March 13, 2010).

Brunauer S, Emmett PH, Teller E. 1938. Adsorption of gases in multimolecular layers. *J Am Chem Soc* 60:309–20.

Calderon M, Barkai-Golan R. 1990. *Food Preservation by Modified Atmospheres*. Boca Raton, FL: CRC Press.

Camelo AFL. 2002. Manual for the preparation and sale of fruits and vegetables. FAO Agricultural Services Bulletin 151.

Castaldi P, Degen JM. 2003. Dried plums: Natural solutions to improve the quality, nutrition, and safety of foods for space travel. 031CES-328—Document number: 2003-01-2377. Bulletin no. 228—September 1993.

Chapman GW, Horvat RJ. 1990. Changes in nonvolatile acids, sugars, pectin and sugar composition of pectin during peach (cv. Monroe) maturation. *J Agric Food Chem* 38:383–7.

Chen C. 2006. Obtaining the isosteric sorption heat directly by sorption isotherm equations. *J Food Eng* 74:178–85.

Chervin C, Speirs J, Loveys B, Patterson BD. 2000. Influence of low oxygen storage on aroma compounds of whole pears and crushed pear flesh. *Postharvest Biol Technol* 19:279–85.

Christensen P, Studer H, Rumming D. 1993. "UC Researching DOV Concept." *Grape Grower* 11:20–1.

Delwiche JF. 1996. Are there "basic" tastes? *Trends Food Sci Technol* 7:411–5.

Derail C, Hofmann T, Schieberle P. 1999. Differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure. *J Agric Food Chem* 47:4742–5.

Downton DP, Flores-Luna JL, King CJ. 1982. Mechanism of stickiness in hygroscopic, amorphous powders. *Ind Eng Chem Fundam* 21:447–51.

Engels C, Hendrickx M, DeSamblanx S, DeGryze I, Tobback P. 1986. Modeling water diffusion during long-grain rice soaking. *J Food Eng* 5:55–74.

Fellows P. 2000. *Food Processing Technology: Principles and Practice*. Cambridge, UK: Woodhead Publishing.

Garg HP. 1987. *Advances in Solar Energy Technology*, Vol. 3. Dordrecht: D. Reidel.

Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311(5762):815. doi: 10.1126/science.1112614.

Goula A, Karapantsios T, Achilias D, Adamopoulos K. 2008. Water sorption isotherms and glass transition temperature of spray dried tomato pulp. *J Food Eng* 85(1):73–83.

Halsey G. 1948. Physical adsorption on non-uniform surfaces. *J Chem Phys* 16:931–93.

Heath HB. 1981. *Source Book of Flavors*. New York: Springer.

Heldman DR. 2003. *Encyclopedia of Agricultural, Food, and Biological Engineering*. New York: Marcel Dekker.

Heldman DR, Lund DB. 2006. *Handbook of Food Engineering*, 2nd Ed. New York: CRC Press.

Howard WG, Guile BR. 1992. *Profiting from Innovation: The Report of the Three-Year Study from the National Academy of Engineering*. New York: The Free Press.

Hsu KH. 1983. A diffusion model with a concentration dependent diffusion coefficient for describing water movement in legumes during soaking. *J Food Sci* 48:618–22.

Hui YH, Clary CD, Farid MM, Fasina OO, Noomhorm A, Welti-Chanes J. 2007. *Food Drying Science and Technology: Microbiology, Chemistry, Applications*. Lancaster, PA: DEStech Publications.

Ibarz A, Barbosa-Cánovas GV. 2002. *Unit Operations in Food Engineering*. New York: CRC Press.

Khalloufi S, Giasson J, Ratti C. 2000a. Water activity of freeze dried mushrooms and berries. *Canadian Agricultural Engineering* 42(1):51–6.

Khalloufi S, El-Maslouhi Y, Ratti C. 2000b. Mathematical model for prediction of glass transition temperature of fruit powders. *J Food Sci* 65:842–5.

Komes D, Lovrić T, Kovačević Ganić K, Gajdoš Kljusurić J, Banović M. 2005. Trehalose improves flavour retention in dehydrated apricot puree. *Int J Food Sci Technol* 40:425–35.

Kraft P, Swift KAD. 2005. Perspectives in flavor and fragrance research. *Helv Chim Acta* 250.

Kralj-Cigic I, Zupancic-Kralj L. 1999. Changes in odour of Bartlett pear brandy influenced by sunlight irradiation. *Chemosphere* 38:1299–303.

Krokida MK, Marinos-Kouris D. 2003. Rehydration kinetics of dehydrated products. *J Food Eng* 57(1):1–7.

Kudra T, Mujumdar AS. 2001. *Advanced Drying Technologies*. New York: CRC Press.

Lalel HJD, Singh Z, Tan SC. 2003. Aroma volatiles production during fruit ripening of "Kensington Pride" mango. *Postharvest Biol Technol* 27:323–36.

Lambert Y, Demazeau G, Lagerteau A, Bouvier JM. 1999. Changes in aromatic composition of strawberry after high pressure treatment. *Food Chem* 67:7–16.

Lamikanra O, Iman SH, Ukuku DO. 2005. *Produce Degradation: Pathways And Prevention*. New York: CRC Press.

Lara I, Miró RM, Fuentes T, Sayez G, Graell J, López ML. 2003. Biosynthesis of volatile aroma compounds in pear fruit stored under long-term controlled-atmosphere conditions. *Postharvest Biol Technol* 29:29–39.

Lowe MR, Levine AS. 2005. Eating motives and the controversy over dieting: Eating less than needed versus less than wanted. *Obes Res* 13:797–806.

Lozano JE. 2006. Processing of fruits: Elevated temperature, nonthermal and miscellaneous processing. In *Fruit Manufacturing Scientific Basis, Engineering Properties, and Deteriorative Reactions of Technological Importance*. New York: Springer, pp. 55–72.

Lozano JE, Anon C, Parada-Arias E, Barbosa-Canovas GV. 2000. *Trends in Food Engineering*. New York: CRC Press.

Lyman B. 1988. *A Psychology of Food: More Than a Matter of Taste*. New York: Kluwer Academic Publishers.

Maarse H. 1991. *Volatile Compounds in Foods and Beverages*. New York: CRC Press, p. 763.

Mastrocola D, Sacchetti G, Pittia P, Di Mattia C, Dalla Rosa M. 2005. Rehydration of dried fruit pieces in aqueous sugar solutions: A review on mass transfer and final product characteristics. *Ital J Food Sci* 17(3):131–254.

Mazza G, LeMaguer M. 1980. Dehydration of onions: Some theoretical and practical considerations. *J Food Technol* 15:181–94.

Meigh DF, Reynolds J. 2006. Effect of low concentrations of oxygen on the production of ethylene and on other ripening changes in apple fruit. *J Sci Food Agric* 20(4):225–8.

Mintel. 2004. MSU Product Center—The Strategic Marketing Institute Working Paper No 1-102605. Category review, fruits and vegetables: Fruit, December, MSU Product Center for Agricultural and Natural Resources, Michigan State University Extension.

Moreira R, Sereno AM. 2003. Evaluation of mass transfer coefficients and volumetric shrinkage during osmotic dehydration of apple using sucrose solutions in static and non-static conditions. *J Food Eng* 57:25–31.

Mujumdar AS. 1995. *Handbook of Industrial Drying*. New York: Marcel Dekker.

Mujumdar AS. 2001. Industrial drying technologies: Current status and future trends. In Mori YH, Ohnishi K (eds.), *Energy and Environment Technological Challenges of the Future*. Tokyo: Springer-Verlag, pp. 112–25.

Mujumdar AS. 2004. Drying 2004—Proceedings of the 14th International Drying Symposium (IDS 2004), pp. 101–18, August 22–25, 2004, Vol. A, São Paulo, Brazil.

Mujumdar AS. 2007. *Handbook of Industrial Drying*. New York: CRC Press.

Oliveira FAR, Oliveira JC. 1999. *Processing Foods: Quality Optimization and Process Assessment*. New York: CRC Press.

Oswin CR. 1946. The kinetics of packing life. III. The isotherm. *J Soc Chemic Indust* 65(12):419–21.

Peleg M. 1988. An empirical model for the description of moisture sorption curves. *J Food Sci* 53:1216–9.

Planinicacute M, Velicacute D, Tomas S, Bilicacute M, Bucicacute A. 2005. Modelling of drying and rehydration of carrots using Peleg's model. *Eur Food Res Technol* 221(3–4):221–5.

Ramming DW. 2006. Plum and plum hybrids. *HortScience* 41(50):1120–2.

Rao MA, Rizvi SSH. 1994. *Engineering Properties of Foods*. New York: Marcel Dekker.

Rao MA, Rizvi SSH, Datta AK. 2005. *Engineering Properties of Foods*, 3rd Ed. New York: CRC Press.

Reynolds S. 1993. Drying fruits. In So Easy to Preserve. Cooperative Extension Service, University of Georgia. College of Agricultural and Environmental Sciences, 3rd Ed. National Food Safety Database. http://www.clemson.edu/extension/hgic/food/food_safety/preservation/hgic3084.html (accessed March 13, 2010).

Roberts EA, Wills RBH, Scott KJ. 1965. The effects of change in concentration of carbon dioxide and oxygen on storage behaviour of Jonathan apples. *Aust J Exp Agric Anim Husbandry* 5(17):161–5.

Roos YH. 2002. Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait* 82:475–84.

Roos YH, Taylor S. 1995. *Phase Transition in Foods*. San Diego, CA: Academic Press.

Sablani SS, Datta AK, Rahman S, Qaboos S, Mujumdar AS. 2006. *Handbook of Food and Bioprocess Modeling Techniques*. New York: CRC Press.

Sablani SS, Kasapis S, Rahman MS. 2007. Evaluating water activity and glass transition concepts for food stability. *J Food Eng* 78:266–71.

Saltveit ME. 1993. *Physiological Basis of Postharvest Technologies*. Oxford, UK: International Society for Horticultural Science Acta Horticulturae.

Saltveit ME. 1996. Physical and physiological changes in minimally processed fruits and vegetables. In Tomás-Barberán FA (ed.), *Phytochemistry of Fruit and Vegetables*. Oxford, UK: University Press, pp. 205–20.

Shaw I, Hayes R, Bucik M, Clingeleffer P, Skinner R. 1996. Drying for Profit: The Shaw Swingarm Trellis—Mechanizing Dried Vine Fruit Production. Pamphlet prepared by Riverlink Research Agencies through the Drying for Profit Program, Sunraysia-Riverland, Australia.

Siripatrawan U, Jantawat P. 2006. Determination of moisture sorption isotherms of Jasmine rice crackers using BET and GAB models. *Food Sci Technol Int* 12(6):459–65.

Smith SE. 1947. The sorption of water vapour by high polymers. *J Am Chem Soc* 69:646–51.

Somogyi LP. 1996. *Processing Fruits: Science and Technology*. New York: CRC Press.

Sonthipermpoon W, Suwonsichon T, Wittaya-Areekul S, Wuttijumnong P. 2006. Effect of maltodextrin on glass transition temperature and water activity of production banana flake. *Kasetsart J (Nat Sci)* 40:708–15.

Speirs J, Lee E, Holt K, Yong-Duk K, Steele N, Loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117:1047–58.

Stuchly SS, Stuchly MA. 1983. Microwave drying: Potential and limitations. In Mujumdar AS (ed.), *Advances in Drying*, Vol. II. Washington, DC; New York; London: Hemisphere Publishing Corporation, pp. 53–71.

Sumitani H, Suekane S, Nakatani A, Tatsuka K. 1994. Changes in composition of volatile compounds in high-pressure treated peach. *J Agric Food Chem* 42:785–90.

Torringa E, Esveld E, Scheewe I, Van den Berg R, Bartels P. 2001. Osmotic dehydration as a pre-treatment before combined microwave-hot-air drying of mushrooms. *J Food Eng* 49:185–91.

Valentas KJ, Rotstein E, Singh RP. 1998. *Handbook of Food Engineering Practice*. New York: CRC Press.

Van den Berg C, Bruin S. 1981. Water activity and its estimation in food systems. In Rockland LB, Stewart GF (eds.), *Water Activity: Influences on Food Quality*. New York: Academic Press, pp. 147–77.

Vega-Mercado H, Gongora-Nieto MM, Barbosa-Canovas GV. 2001. Advances in dehydration of foods. *J Food Eng* 49:271–89.

Zhang M, Tang CJ, Mujumdar AS, Wang S. 2006. Trends in microwave-related drying of fruits and vegetables. *Trends Food Sci Technol* 17(10):524–34.

CHAPTER 27

Flavors of Dried Apples

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DEHYDRATED APPLE PRODUCTS

The world production of apples (2004/2005) is estimated at about 42 million tons (Dobrzański et al. 2006), more than 70% of which is consumed as fresh fruit, while about 20% is processed into value-added products, of which 65% are juice concentrate; the balance is made into other products, which include apple juice, apple wine and cider, apple purees and jams, and dehydrated apple products.

The dried and dehydrated apple market offers various types of products differing both in shape (flakes, dices, rings, wedges, slices, grind, chips, powder) and final moisture: high moisture, 22–26% (lowered to 17–21% for unsulfured apples), intermediate moisture, 13–17%, and low moisture, 3–5%, referred to also as *evaporated*, *semievaporated*, and *dried low moisture* apples, respectively. The overall quality of dehydrated apple derivatives may vary to a large extent, according to raw material and process conditions, but for all kinds of products, flavor plays a major role among the sensorial properties defining quality.

APPLE FLAVORS IN RELATION TO DEHYDRATION PROCESSES

Detailed investigations of the volatile loss and volatile changes occurring during dehydration of apples and apple derivatives are not easily found in the literature, while a large amount of experimental data and theoretical interpretations can be found with reference to the basic chemical–physical mechanisms of aroma release and retention, which are associated with the removal of water from food matrices.

In very general terms, aroma loss, retention, changes, and formation are reported to be related to both the drying methods (hot air, freeze concentration, freeze-drying, vacuum drying, osmotic dehydration, membrane separation, etc.) and the specific process parameters, mainly temperature and time.

With regard to dewatering effect on apple flavor, a generalized classification of drying techniques can be suggested as in Figure 27.1, with evaporative methods

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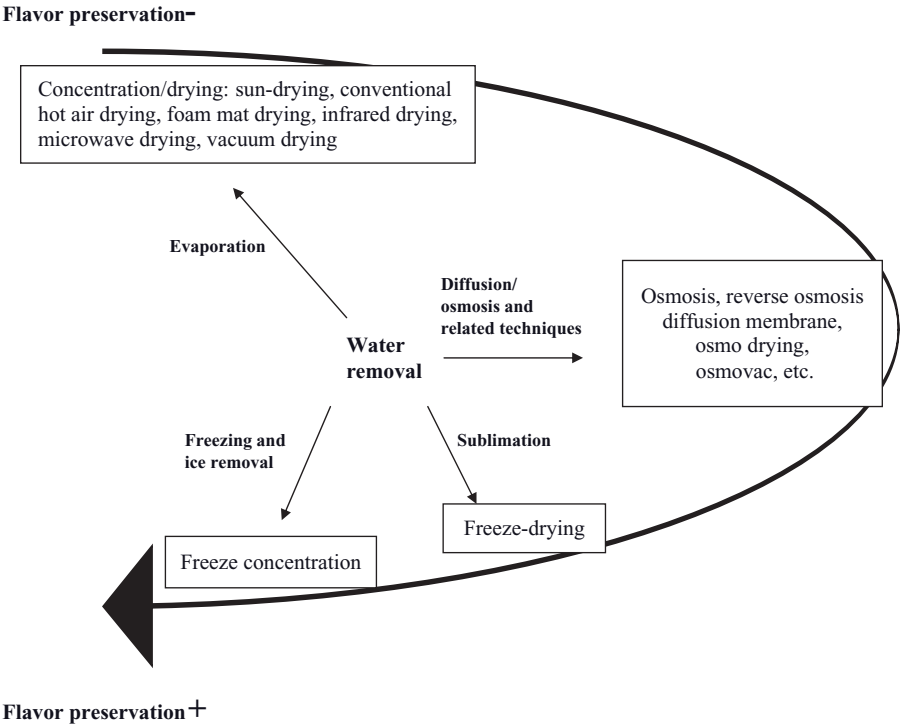

Figure 27.1. Schematic representation of water removal methods and influence on flavor preservation.

causing less aroma preservation, followed by membrane processing, freeze-drying, and freeze concentration, the last method allowing practically a full natural flavor retention.

The flavor of raw apples is influenced by variety, ripening stage, and agronomical practices (Mehinagic et al. 2006). More than 200 components have been identified as volatiles in different apple cultivars (Dimick and Hoskin 1981), apple aroma being the result of a complex mixture that includes esters, aldehydes, ketones, and alcohols. In particular, butyl acetate, 2-methylbutyl acetate, hexyl acetate, and hexyl hexanoate have been identified as being responsible for the overall apple aroma in several cultivars (Mehinagic et al. 2006; Young et al. 1996). Cunningham and others (1986) stated that flavor in fresh apples should not be caused by the same compounds in all cultivars; nevertheless, a generalized description of apple odor showed β-damascenone (International Union of Pure and Applied Chemistry [IUPAC] name: *trans*-1-(2,6,6-trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one, which is generally formed from an odorless precursor under postharvest treatments and contributes to odor prior to processing) and butyl, isoamyl (IUPAC name: 3-methylbutyl hexanoate), and hexyl hexanoates, along with ethyl, propyl, and hexyl butanoates, to be important to the aroma of most of the 40 cultivars considered. Table 27.1 shows some odor-active volatiles of fresh and dried apples, and their sensory descriptions, which have been derived from a review (Dimick and Hoskin 1981) reporting more than 250 volatile components isolated from apples, together with data from Teule

TABLE 27.1. Some Fresh and Dried Apple Volatile Compounds and Their Sensory Description

Volatiles	Odor Descriptor	Reference
Fresh apple		
γ-Hexalactone	Sweet, powerful, warm-herbaceous, coumarin-caramel	Dimick and Hoskin (1981)
γ-Undecalactone	Sweet, oily-fruity, peach like	
2-/3-Methylbutylacetate	Sweet-fruity, banana, pear like	
2-/3-Methylbutanol	Whiskey, malt, burnt,[a] highly diluted-pleasant, fruity-winey	
Acetaldehyde	Pungent ethereal odor, diluted, coffee or wine	
cis and *trans*-Linalool oxide	Powerful sweet-woody, penetrating odor	
cis-3-Hexenol	Powerful and intensely green, grassy	
Ethanol	Sweet-ethereal, mild	
Ethyl 2-methylbutanoate	Powerful diffusive, green-fruity, pungent	
Ethyl acetate	Pleasant, ethereal-fruity, brandy like, pineapple[a]	
Ethyl isobutanoate	Diffusive, sweet-ethereal, fruity	
Ethyl *n*-butanoate	Powerful, ethereal-fruity, banana, pineapple, apple[a]	
Ethyl *n*-propanoate	Ethereal, fruity-rum like	
Hexanol	Very penetrating, fatty-green grassy odor	
Isobutanol	Mild, chemical, sweet, harsh when diluted	
n-Butanal	Penetrating, pungent-irritating odor, diluted fruity, banana like, green fresh	
n-Butanol	Mild "fusel"-like odor	
n-Butyl acetate	Very diffusive, ethereal-fruity, pungent, pear	
n-Heptyl acetate	Fruity, fatty-green, slightly floral	
n-Hexanal	Very powerful, penetrating, fatty-green, grassy	
n-Hexyl 2-methylbutanoate	Powerful, fresh-green fruity	
n-Hexyl acetate	Sweet, fruity, slightly floral	
n-Propanol	Alcoholic-nauseating, sweet	
Propanal	Penetrating, suffocating diluted roasted coffee	
trans-2-Hexenal	Powerful, green-fruity, pungent vegetable like	
trans-2-Hexenol	Powerful, fruity, green, slightly caramel like	

TABLE 27.1. ***Continued***

Volatiles	Odor Descriptor	Reference
β-Damascenone	Apple[a]	Cunningham and others (1986)
1-Octen-3-one	Mushroom, metal[a]	
6-Methyl-5-heptene-2-one	Citrus, strawberry,[b] fruity, green	
Butyl 2-methylbutanoate	Fruit, cocoa[a]	
Butyl hexanoate	Fruity[a]	
Butyl octanoate	Fruity[a]	
Ethyl decanoate	Grape[a]	
Ethyl hexanoate	Fruity, apple peel[a]	
Ethyl octanoate	Fruit, fat[a]	
Ethyl valerate	Yeast, fruit[a]	
Heptyl 2-methylbutanoate	Apple[a]	
Hexyl 2-methylbutanoate	Strawberry[a]	
Hexyl butanoate	Fruity, apricot, banana, apple peel[a]	
Hexyl hexanoate	Apple peel, peach[a]	
Isoamyl hexanoate	Fruity	
Methyl 2-methylbutanoate	Apple[a]	
Methyl butanoate	Ether, fruit, sweet[a]	
Methyl decanoate	Wine[a]	
Methyl nonanoate	Coconut[a]	
Pentyl 2-methylbutanoate	Apple[a]	
Pentyl butanoate	Banana[a]	
Propyl butanoate	Pineapple, solvent[a]	
Propyl propanoate	Pineapple[a]	
α-Farnesene	Apple[a]	Birch and others (2004)
Dried apple		
δ (2E)-undecenal	Fatty, fruity	Teule and Crouzet (1994a,b)
2,5-Dimethyl pyrazine	Peanut	
2,6-Dimethyl pyrazine	Grilled (roasted), rancid	
2-Acetyl furane	Almond, smoke	
2-Amyl furane	Smoke, grilled	
2-*trans*, 4-*cis*-Decadienal	Cucumber	
2-*trans*, 4-*trans*-Decadienal	Cucumber	
2-*trans*, 6-*cis*-Nonadienal	Cucumber, fatty	
2-*trans*-Decenal	Fatty, green	
2-*trans*-Heptenal	Green, fatty	
2-*trans*-Hexenal	Apple, green	
2-*trans*-Nonenal	Green, fatty	
5-Methyl furfural	Cooked, burnt	
6-Methyl-5-heptene-2-one	Fruity, green	
Benzaldehyde	Bitter almond	
Butyl acetate	Candy, fruity	
Ethyl-2-methyl butanoate	Green apple	
Hexyl acetate	Green apple	
Hexyl butanoate	Fruity, apricot, banana, apple peel[a]	
Octanal	Fruity, fatty	
Phenylacetaldehyde	Flowers	
Pyridine	Cooked, burnt	

TABLE 27.1. ***Continued***

Volatiles	Odor Descriptor	Reference
α-Farnesene	Apple	Birch and others (2004)
1-Octene-3-ol	Mushroom[a]	
1-Penten-3-ol	Butter, pungent[a]	
2 or 3-hexenal	Powerful, green-fruity, pungent vegetable like, green[b]	
2,4-Heptadienal	Nut, fat, fried[a]	
2-Decenal	Fatty, green	
2-Heptenal	Green, fatty	
2-Methyl-butanol	Wine, onion[a]	
2-Octenal	Green[a]	
3-Hydroxy-2-butanone	Butter, cream[a]	
3-Methyl-butanol	Mint, alcohol[b]	
3-Octen-2-one	Nut, crushed bug[a]	
Acetic acid	Sour[a]	
Butanal	Penetrating, pungent, irritating odor, diluted fruity, banana like, green fresh	
Butanol	Mild "fusel" like	
Butyrolactone	Caramel, sweet[a]	
Decanoic acid	Rancid, fat[a]	
Dimethyl sulfide	Cabbage, sulfur, gasoline[a]	
Dodecanal	Lily, fat, citrus[a]	
Ethanol	Sweet-ethereal, mild	
Ethyl 3-hydroxybutanoate	Marshmallow[a]	
Ethyl acetate	Pleasant, ethereal-fruity brandy like	
Ethyl butanoate	Fruity, apple, strawberry[b]	
Ethyl decanoate	Grape[a]	
Ethyl dodecanoate	Leaf[a]	
Ethyl octanoate	Fruit, fat[a]	
Furfural	Cooked, smoke	
Hexanal	Very powerful, penetrating, fatty-green, grassy, green[b]	
Hexanoic acid	Sweat[a]	
Hexanol	Very penetrating, fatty-green, grassy, fresh, green[b]	
Hexyl-2-methylbutanoate	Strawberry[a]	
Nonanal	Fat, citrus, green[a]	
Octanoic acid	Sweat, cheese[a]	
Pentanal	Almond, malt, pungent[a]	
Phenylethyl acetate	Rose, honey, tobacco[a]	
Phenylethyl alcohol	Honey, spice, rose, lilac[a]	
Propanal	Penetrating, suffocating odor, diluted roasted coffee	
Propanol	Alcoholic-nauseating, sweet	
Toluene	Paint[a]	

[a]Acree and Arn (2004).
[b]Mehinagic and others (2006).

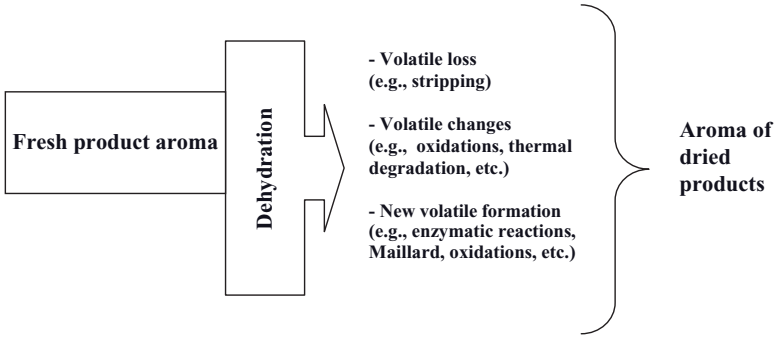

Figure 27.2. Summary of dehydration effects on aroma.

and Crouzet (1994a,b), Cunningham and others (1986), Birch and others (2004), and Mehinagic and others (2006). It can be noted that fruity, green, sweet, apple, grassy, and so on are among the most recurring odors in fresh apples, while grilled, nut, smoke, cooked, burnt, almond, and so on odors are often encountered in dried apples.

As previously stated, volatile loss, volatile generation, and volatile changes may occur during drying (Fig. 27.2). While volatile losses are associated with stripping and changes are mainly caused by oxidation and thermal degradation, nonvolatile precursors can lead to the formation of new flavor components, frequently undesired, as in the case of Maillard reaction products (MRPs).

High vapor pressures characterize volatile compounds compared with water, which are liable to reduction or disappearance during processing, depending on drying conditions. In very general terms, the "harder" the drying process, the more the aroma is affected. Lee and others (1967) reported a decrease of carbonyl compounds following conventional hot air drying from 3.2 mg/kg in fresh apple (cv. Golden Delicious) to 0.8 mg/kg in the dried sample (values converted to fresh fruit basis). Furthermore, several peaks from headspace gas–liquid chromatography, which occurred in the fresh samples, did not appear in the hot-air-dehydrated apples.

In early stages of heating, several esters, mostly responsible for fruity, floral apple aroma, were present, but no evidence of their later evolution was obtained (Nursten and Woolfe 1972). Following an exaggerated cooking time of apples of 6.5 h, Nursten and Woolfe (1972) found that alcohols were prominent and were accompanied by varying amounts of hexanal, phenylacetaldehyde, damascenone, and farnesenes (IUPAC names α-farnesene: 3,7,11-trimethyl-1,3,6,10-dodecatetraene; β-farnesene: 7,11-dimethyl-3-methylene-1,6,10-dodecatriene). Similarly, during drum drying of Golden Delicious apple puree at 140°C for 15 s, Teule and Crouzet (1994a) found aldehydes and alcohols responsible for green odor, esters accounting for fruity, floral, grass, and apple odors, other than α-farnesene, conferring a typical apple flavor, to decrease (Table 27.2) with α-farnesene concentration shifting from 2.6 to 0.5 mg/kg. In contrast, thermal degradation of volatile/nonvolatile precursors, oxidation, and Maillard reaction lead to the formation of new compounds (heterocyclic compounds, saturated and unsaturated aldehydes), some of which cause off-flavor development. For example, 2-furfural (IUPAC name: furan-2-carbaldehyde), benzaldehyde, 5-methyl-2-furfural (IUPAC name: 5-methylfuran-2-carbaldehyde), and 2,4-decadienal were not detected in fresh apples but contributed increasingly during

TABLE 27.2. Decreasing and Increasing Amount of Volatile Compounds following Drum Drying of Golden Delicious Apple Puree at 140°C for 15 s

Decreasing Compounds	Increasing Compounds
Butyl acetate	2,5-Dimethyl pyrazine
Ethyl 2-methylbutanoate	2,6-Dimethyl pirazine
Heptyl 2-methylpropanoate	2-Acetyl furane
Heptyl isobutanoate	2-Amylfurane
Hexan-1-olo	5-Methyl furfural
Hexanal	6-Methyl-5-hepten-2-one
Hexyl acetate	Benzaldehyde
Hexyl butanoate	Furfural
Isoamyl acetate	Octan-1-ol
Propyl butanoate	Octanal
trans-2-Hexenal	Phenylacetaldehyde
α-Farnesene	Pyridine
	trans, *cis*-2,4-Decadienal
	trans, *cis*-2,6-Nonadienal
	trans, *trans*-2,4-Decadienal
	trans-2-Decenal
	trans-2-Heptenal
	trans-2-Nonenal
	δ*trans*-2-Undecenal

Data from Teule and Crouzet (1994a,b).

time, 2-furfural finally becoming the predominant volatile in cooked apples (Nursten and Woolfe 1972). Following drum drying of apple puree, Teule and Crouzet (1994a,b) observed an increase in the concentration of phenylacetaldehyde and benzaldehyde, whose precursor is phenylalanine (IUPAC name: 2-amino-3-phenyl-propanoic acid) (Nursten and Woolfe 1972), which is also involved in the furfural formation by Maillard reaction. Table 27.2 summarizes volatile compounds whose concentrations have been found to decrease or increase during drum drying of apple puree (data from Teule and Crouzet 1994a,b).

It should be considered that the most important odor-active volatiles may not correspond with the major chemical components. With regard to Golden Delicious apple puree, hexanal, (2)-hexenal, ethyl 2-methylbutanoate, hexan-1-ol, isoamyl acetate (IUPAC name: 3-methyl-1-butyl acetate), hexyl acetate, and α-farnesene mostly account for the perceived aroma. In turn, after dehydration, hexanal; *trans*-2-heptenal; *trans*, *cis*-2,6-nonadienal; *trans*, *trans*-2,4-decadienal; and α-farnesene were identified as predominant flavored volatiles in apple flakes by Teule and Crouzet (1994a).

Teule and Crouzet (1994a,b) reported that the flavor quality of conventional hot-air-dried apple strongly depends on drying temperature. Besides, the newly formed volatiles responsible for cooked apple odor showed different relations with air-drying temperatures, namely:

- the amount of volatiles formed increases linearly with raising air temperature from 120 to 170°C, as in the case of furfural (low to medium cooked apple odor);

- a different behavior characterizes β-ionone (IUPAC name: *trans*-4-(2,6,6-trimethyl-1-cyclohexenyl)but-3-en-2-one), damascenone, and 2,6-dimethyl pyrazine whose concentrations versus air-drying temperature showed a slope increase at temperatures higher than 140°C; and
- in the case of 2-amyl furane (giving strong to very strong odor of cooked apple), *trans*, *cis*-2,6-nonadienal; *trans*-2-decenal; *trans*, *trans*-2,4-decadienal; and phenylacetaldehyde, sharp slope increase was observed in the temperature range of 140–160°C, followed by a less important slope change at higher temperatures.

The authors concluded that the losses of aroma compounds as well as the generation of thermally induced volatiles are reduced at air-drying temperatures below 140–150°C.

Drying kinetics of both volatiles and moisture have been studied by Krokida and Philippopoulos (2006) who found most of the flavor compounds losses occurring during the early stages of drying. A first-order kinetic model, involving a drying constant as a function of drying temperature, indicated volatile (methylanthranilate; IUPAC name: methyl 2-aminobenzoate and ethyl butanoate) removal rates to be higher than that of moisture and both varying with process temperatures. Lower drying temperatures lead to a strong apple taste as reported by Birch and others (2004), who used 60°C drying temperature under air or nitrogen. In these samples, α-farnesene, responsible for the strong apple aroma, was a predominant volatile (with best retention in the nitrogen-dried sample), while, in comparison, commercially dried apples had almost no α-farnesene. On the contrary, furfural; hexanoic acid; 2,4-heptadienal; 3-octene-2-one (IUPAC name: oct-3-en-2-one); octanoic acid; 2-decenal (IUPAC name: dec-2-enal); and ethyl dodecanoate, mostly undesirable in dried apple, have not been found in the low-temperature-treated samples. Besides the data reported above, in our knowledge, no other data on single apple volatile changes, with reference to specific drying techniques, are reported in the literature.

In conventional drying, convective heating supplies the energy for evaporation, but novel electro-technologies, such as microwaves, infrared, and radio-frequency techniques, stirred up interest in view of some related advantages (Barbosa-Canovas and Juliano 2004; Piyasena et al. 2003). Studies on electro-technologies focused the attention on drying rate/drying time and temperatures, rehydration capacity, dielectric properties of foods, and apples as well (Barbosa-Canovas and Juliano 2004; Funebo and Ohlsson 1998; Nowak and Lewicki 2004; Piyasena et al. 2003, among others). Little reported works regard the influence of these novel methods on aroma so far. With reference to infrared drying, Timoumi and coworkers (2007) determined the effect of dehydration time and radiation temperature on the total volatile components of apples.

Better flavor preservation arises from milder drying methods as the osmovac process, a combined technique consisting of immersing the fruit pieces in concentrated sugar solutions prior to vacuum drying. Dixon and Jen (1977) found that osmose treatment improved the retention of fresh fruit flavor. An excellent flavor was attributed both to the increased sugar-to-acid ratio and to sugar's ability in preventing volatile losses during drying. Jezek and Smyrl (1980) used freeze-drying to remove water from osmosed apples and found, during the drying stage, a 100% volatile retention, which was attributed to the beneficial influence of increased dissolved solids in the apple slices.

With regard to apple juice concentration, frequently applied prior to drying, Bolin and Salunkhe (1971) tested freeze concentration, diffusion membrane, osmosis, and reverse osmosis methods up to soluble solids content of 47%, 37%, 29%, and 32%, respectively. The average volatile retention, as total peaks area, of fruit juices was 63%, 8%, 12%, and 16% for freeze concentration, diffusion membrane, osmosis, and reverse osmosis, respectively. Freeze concentration resulted in the smallest chromatographic peak change with a maximum overall retention and no detectable flavor changes. Diffusion-membrane processes did not cause flavor changes. Both osmosis and reverse osmosis were associated with volatile loss and with an increase of ethyl butanoate, possibly due to a chemical reaction. Regarding reverse osmosis, the retention of flavor volatiles relates to the types of membranes and the operating conditions; for apple juice concentration from 10 to 20°Brix, Chou and others (1991) found a substantial loss of volatiles due to evaporation, thermal degradation, and/or membrane "capture."

AROMA RETENTION AND RELEASE IN APPLE DRYING

Let us now mind about volatile retention in dried apple products starting from freeze–drying, which, in the early 1970s, allowed the mechanisms of volatile retention to be elucidated. It is worth noting that freeze-drying gives optimum flavor preservation in terms of both quality and quantity. High volatile retention was unexpected considering the low process temperatures and operating pressures and the high vapor tension of volatiles compared with that of water. Actually, the retention does not seem to be directly related to the relative volatility of the component.

Briefly, in freeze-drying the water is removed from the frozen matrix by sublimation of ice, which requires operating conditions to be set below the triple point of water. Highly concentrated amorphous solutions in earlier stages are rapidly achieved, thanks to water crystallization. The water removal, supported by heat, produces low moisture or dried layers, having both an increase in viscosity and in the glass transition temperature (T_g).

In earlier studies on volatile retention during concentration and drying, Chandrasekaran and King (1971) observed that apple juice flavors, ethyl acetate, *n*-hexanal, 2-hexanal, and *n*-hexyl acetate were best retained during freeze-drying and slush drying (a combined technique where partially frozen juice is dehydrated by simultaneous sublimation of the ice and evaporation of unfrozen water), with respect to conventional drying (evaporation at atmospheric pressure at surface temperature of 55–60°C). The authors observed that flavor retention in freeze- and slush drying was strongly affected by initial solid concentrations. For these low temperature methods, volatile retention increased with increasing initial solid content, which, by converse, did not affect the retention during conventional drying. Hence, in slush and freeze-drying, the retention was related to both a locking action of sugars and a selective diffusion of chemical components rather than to equilibrium considerations, also according to Thijssen (1971). Saravacos and Moyer (1968) reported that in the processing of fruit/apple juices, the losses of flavor compounds can be substantially reduced by first concentrating the juice, using a non-evaporative process (e.g., freeze concentration) and then freeze-drying the fruit concentrate.

Since low moisture products (and frozen material for freeze-drying) may be considered kinetically controlled systems, the physical structure and the amorphous glassy concepts have been related to volatile retention on several carbohydrate model and food systems. The microregion theory was postulated by Flink and Karel (1970), among others, which stated the presence of small, independent regions of concentrated solutes (with particular reference to carbohydrates), which orient during freezing and drying, thus entrapping substantial quantities of volatiles. The "entrapment mechanisms" may be extended to all drying processes when the residual moisture in the product is low enough to determine the formation of highly concentrated, viscous amorphous phases, which are close to the glassy state at the operating temperatures. Clearly, medium-high temperature dehydration techniques reach such conditions in the later stage of drying, at very low water contents, when most volatiles have already been lost. For a drop of concentrated juice, the faster the drying process, the better the volatile retention, for example, spray-drying performed at selected optimal conditions according to King (1990). Following these considerations, many techniques tend to increase heat and mass transfer, by means of the production of foams, where the liquid phase quickly reaches high concentration and viscosity. A relation between fruit serum (juice) viscosity and drying ability has been found for apples, apricots, and peaches by Maltini and others (1992), who reported that the power law consistency index adjustment of juice concentrates allow vacuum belt drying to be used without the need of "drying aids." In particular, a controlled depolymerization of high-molecular-weight pectins leads to suitable viscosities between non-foaming (too high viscosity) and collapse-susceptible (too low viscosity) juices.

During storage or hydration the entrapped volatiles are released by microregion rupture when both the temperature and the plasticizer content (water) increase. Molecular mobility and diffusion increase with water uptake, and some swelling and bridging of the particles can occur, possibly associated with crystallization, leading to volatile escape. With increasing water content or temperature, plasticization and collapse occur at the "collapse temperature" (T_c), determining porosity loss and reduced diffusion through the matrix. In some cases, collapse can result in reencapsulation of the aroma compound (Labrousse et al. 1992). Structural collapse is related to the glass transition temperature, with T_c 10–20°C higher than T_g, according to Roos (1995). In medium/low moisture, kinetically controlled systems, T_g and state diagrams represent a suitable tool for glass-transition-related physicochemical changes to be predicted. The difference ($T - T_g$), which includes both the effects of water content (which lowers T_g) and storage/processing temperature (T), indicates how far the system is from physical/structural stability. Glass transition curve may be related to sorption isotherm by a "critical a_w," that is, the a_w at which T_g drops below ambient or operating temperature (Fig. 27.3) (redrawn from Roos 1995).

With regard to dry apple juice (cv. Golden Delicious), the main quantitative constituents are fructose, glucose, and sucrose, whose average concentration and T_{gdry} (T_g of anhydrous sugars) are reported in Table 27.3, other than soluble pectins, which do not influence T_g. Ponting and others (1973) reported that to preserve the quality of juice powders for a long time (e.g., 6 months) at fairly high storage temperatures, moisture should be in the neighborhood of 1% or below, while Monzini and Maltini (1989) reported a suitable water activity (a_w) less than 0.11 for a 25°C temperature storage. From the data in Table 27.3, a T_{gdry} of the mixture of about

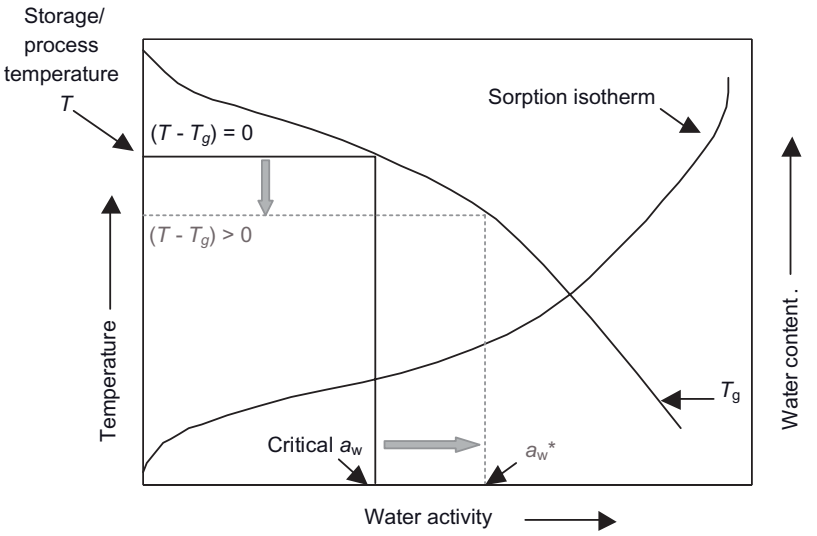

Figure 27.3. Schematic representation of glass transition curve, sorption isotherm and critical a_w · $(T - T_g)$ is zero at the "critical a_w"; $(T - T_g)$ becomes >0 at increased a_w's (e.g., a_w^*). Modified from Roos (1995).

TABLE 27.3. Dry T_g of Fructose, Glucose, and Sucrose and Their Average Concentrations in a Golden Delicious Juice Powder

	Fructose	Glucose	Sucrose
T_{gdry} (°C)	5	31	62
Concentration (g/100 g d.b.)	64%	21%	15%

Data from Monzini and Maltini (1989).

19°C may be calculated, which should represent for the specific apple cultivar considered, the upper storage temperature limit for best quality and flavor to be retained. The upper limit will drop in more hydrated samples (e.g., commercial dehydrated juice whose moisture content is close to 6%) according to the mechanisms described in Figure 27.3.

While T_g and state diagrams allow prediction of physical changes in relatively homogeneous systems such as fruit juices, some complications arise when multidomain, multiphase systems are concerned, as in the case of fruit pieces.

Actually, raw apple tissue is a multicomponent system whose structural elements may be described, according to Maltini and others (2003), as:

- a water-soluble phase, consisting of both low-molecular-weight (sugars, organic acids, salts) and high-molecular-weight (hydrocolloids) components;
- an insoluble phase consisting of cellular matrix biopolymers (insoluble pectins, hemicelluloses, cellulose, and sometimes lignins); and
- a gas phase of intracellular air spaces, present in parenchymous tissue, which may be considered as true structural elements, having a very characteristic influence on the perceived texture.

The soluble low-molecular-weight components interact with water, lowering its vapor pressure mainly by polar binding, while the insoluble ones may hold water through surface and capillary effects. With regard to T_g, low molecular species will have a strong effect on transition temperatures, while hydrocolloids and insoluble matrix will have only negligible effects. Actually, the glass transition temperature (T_g) of apple tissue almost coincides with that of the juice (Aguilera et al. 1998), while no T_g appears for the separated cellular matrix (Venir et al. 2007). Hydrocolloids, mainly soluble pectins, will have a reduced effect on T_g and a_w but will strongly increase the macroviscosity of the soluble fraction, thus reducing the tendency to collapse.

Structure-related changes during moistening of freeze-dried Golden Delicious apple tissue, assumed as basic plant food structure, have been considered by Venir and others (2007). Figure 27.4 shows the glass transition (T_g) and collapse (T_c) temperature curves, the sorption isotherms of both whole apple and separated cell wall (CW) cubes, obtained by washing raw apple cubes under flowing water. T_c is a measure of structural collapse obtained by thermal mechanical analysis (TMA) determinations revealing the temperature at which matrices start to soften while subjected to a constant load. In the same figure are reported physical changes (volatile release, shrinkage, and consistency/strain) associated with a gradual hydration performed at different relative humidity after 20 days at 25°C. As the T_{gdry} of the product was 18°C, according to the glass transition concepts, 25°C storage temperature should have led to substantial volatile losses even in the anhydrous material. Nevertheless, little volatile release was observed up to $a_w = 0.31$, while a maximum was found at $a_w = 0.41$, well above the glass transition temperature, with $(T - T_g) \cong 50$°C. Hydration levels higher than 0.41 led to reduced amounts of volatiles in the headspace, possibly owing to a readsorption by the constant humidity saturated solutions used for moistening. Otherwise, volatile release could be hindered in the collapsed matrix ($a_w > 0.4$–0.5) if kinetic of collapse is faster than that of moistening. Actually, during hydration at $a_w = 0.75$, well above the critical a_w range, a maximum was observed after about 70 h hydration time (Fig. 27.5) (E. Venir and E. Maltini, unpublished data), suggesting that volatiles gradually released in the headspace were subsequently readsorbed by the saturated solution (NaCl), according to thermodynamic vapor/liquid partition.

As reported in Figure 27.4, collapse (T_c) and glass transition temperature (T_g) curves paralleled up to about $a_w = 0.40$, while they diverged at higher a_w's. It may be evidenced a threshold a_w range (about 0.40–0.50) below which collapse can be viewed as a microstructural change directly related to T_g (as in simple sugar solutions), while at higher a_w's, collapse occurs on a macroscopic scale and is associated with softening of the insoluble matrix and tissue shrinkage. Separated CWs did not shrink, and deformation was only 1/6 (strain in Fig. 27.4) with respect to whole apple tissue. CW was found to exert a supporting effect, which delayed shrinkage of whole apples at higher critical a_w's. Similarly, the shift of volatile release at higher a_w's could be attributed to the insoluble matrix, which may act as a further barrier hindering diffusion. It appeared that, where a CW network is present, the relationships among T_g and/or a_w and physical changes may be different with respect to that of homogenous systems, where no separated phases are present. The insoluble fraction may strongly affect the dependence of volatile release and other time-dependent physical changes, from both the glass transition temperature and the water activity of the soluble phase.

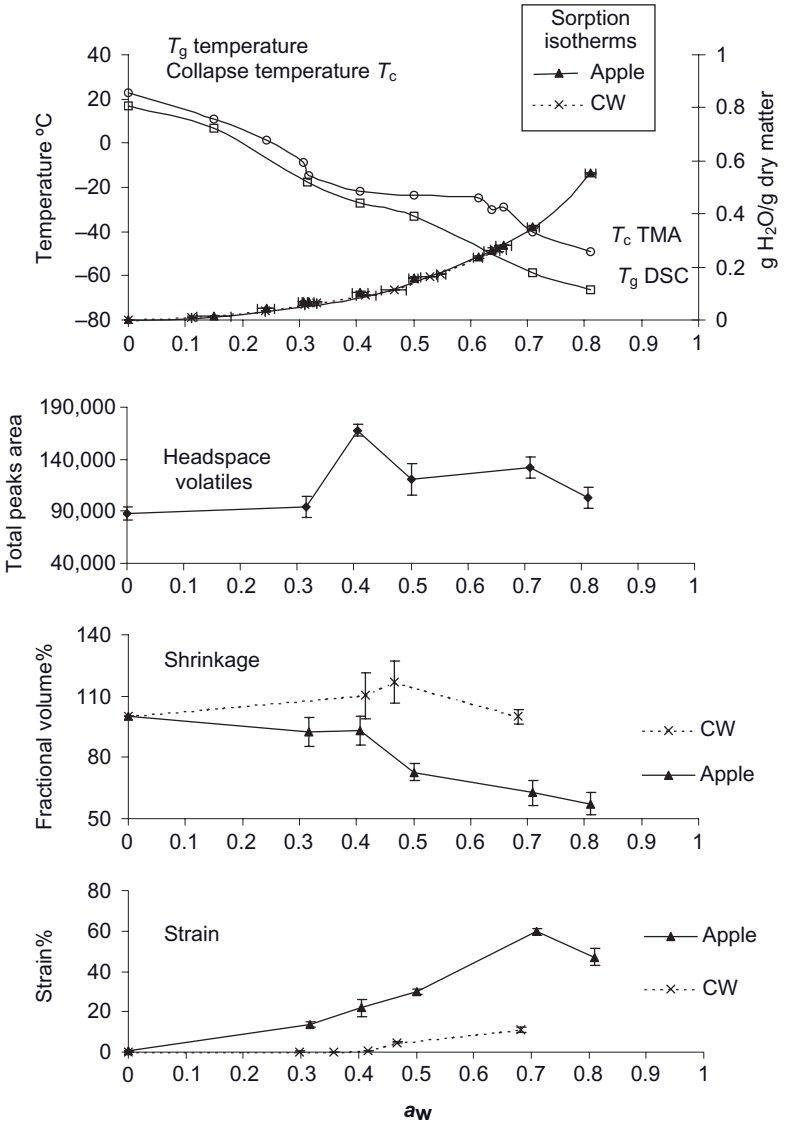

Figure 27.4. Apple and cell walls (CWs) sorption isotherms, glass transition and collapse temperatures, headspace volatiles, and shrinkage and strain of apple cubes at different water activities. Shrinkage and strain of CW are also reported. Figure modified from Venir and others (2007).

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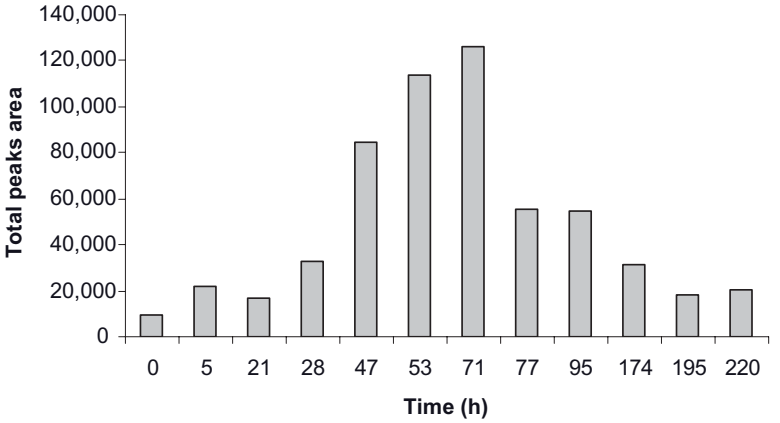

Figure 27.5. Total peak area of headspace volatiles in freeze-dried apple dices during hydration at a_w = 0.75 (E. Venir and E. Maltini, unpublished data).

REFERENCES

Acree TE, Arn H. 2004. Flavornet and human odor space. http://www.flavornet.org.

Aguilera JM, Cuadros TR, del Valle JM. 1998. Differential scanning calorimetry of low-moisture apple products. *Carbohydr Polym* 37:79–86.

Barbosa-Canovas GV, Juliano P. 2004. Adaptation of classical processes to new technical developments and quality requirements. *J Food Sci* 69(5):240–50.

Birch J, McNab R, Lim M, Dufour JP, Carrington G. 2004. Assessment of apple quality following dehydration using a heat pump dehumidifier system under conventional and modified atmosphere conditions. *Food New Zealand* 4(4):29–34.

Bolin HR, Salunkhe DK. 1971. Physicochemical and volatile flavor changes occurring in fruit juices during concentration and foam-mat drying. *J Food Sci* 36:665–8.

Chandrasekaran SK, King CJ. 1971. Retention of volatile flavor components during drying of fruit juices. *Chem Eng Prog Symp Ser* 67(108):122–30.

Chou F, Wiley RC, Schlimme DV. 1991. Reverse osmosis and flavor retention in apple juice concentration. *J Food Sci* 56(2):484–7.

Cunningham DG, Acree TE, Barnard J, Butts RM, Braell PA. 1986. Charm analysis of apple volatiles. *Food Chem* 19:137–47.

Dimick PS, Hoskin JC. 1981. Review of apple flavor—State of the art. *CRC Crit Rev Food Sci Nutr* 18(4):387–409.

Dixon GM, Jen JJ. 1977. Changes of sugar and acid of osmovac dried apple slices. *J Food Sci* 42(4):1126–31.

Dobrzański B, Rabcewicz J, Rybczyński R. 2006. *Handling of Apple. Transport Techniques and Efficiency, Vibration, Damage and Bruising Texture, Firmness and Quality*. Lublin, Poland: Dobrzański B Institute of Agrophysics of Polish Academy of Sciences.

Flink J, Karel M. 1970. Retention of organic volatiles in freeze-dried solutions of carbohydrates. *J Agric Food Chem* 18(2):295–7.

Funebo T, Ohlsson T. 1998. Microwave-assisted air dehydration of apple and mushroom. *J Food Eng* 38:353–67.

Jezek E, Smyrl TG. 1980. Volatile changes accompanying dehydration of apples by the osmovac process. *Can Inst Food Sci Technol J* 13(1):43–4.

King CJ. 1990. Spray drying food liquids and the retention of volatiles. *Chem Eng Prog* 86(6):33–9.

Krokida MK, Philippopoulos C. 2006. Volatility of apples during air and freeze drying. *J Food Eng* 73:135–41.

Labrousse S, Roos Y, Karel M. 1992. Collapse and crystallization in amorphous matrices with encapsulated compounds. *Sci Aliments* 12:757–69.

Lee CY, Salunkhe DK, Nury FS. 1967. Some chemical and histological changes in dehydrated apple. *J Sci Food Agric* 18:89–93.

Maltini E, Nani R, Bertolo G. 1992. Role of serum viscosity and of pulp content in the vacuum belt drying of pure fruit juices. *Int J Food Sci Technol* 27:531–9

Maltini E, Torreggiani D, Venir E, Bertolo G. 2003. Water activity and the preservation of plant foods. *Food Chem* 82:79–86.

Mehinagic E, Royer G, Symoneaux R, Jourjon F, Prost C. 2006. Characterization of odor-active volatiles in apples: Influence of cultivars and maturity stage. *J Agric Food Chem* 54:2678–87.

Monzini A, Maltini E. 1989. Storage characteristics of fruit juice powders prepared by continuous vacuum drying. *Industria delle bevande* 18(102):284–8.

Nowak D, Lewicki PP. 2004. Infrared drying of apple slices. *Innov Food Sci Emerg Technol* 5:353–60.

Nursten HE, Woolfe ML. 1972. An examination of the volatile compounds present in cooked Bramley's seedling apples and the changes they undergo on processing. *J Sci Food Agric* 23:803–22.

Piyasena P, Dussault C, Koutchma T, Ramaswamy HS, Awuah GB. 2003. Radio frequency heating of foods: Principles, applications and related properties—A review. *CRC Crit Rev Food Sci Nutr* 43(6):587–606.

Ponting JD, Stanley WL, Copley MJ. 1973. Fruit and vegetable juices. In Van Arsdel WB, Copley MJ, Morgan AI (eds.), *Food Dehydration*, 2nd Ed., Westport, CT: The Avi Publishing, pp. 199–245.

Roos YH. 1995. *Phase Transitions in Foods*. San Diego, CA: Academic Press.

Saravacos GD, Moyer JC. 1968. Volatility of some flavor compounds during freeze-drying of foods. *Chem Eng Prog Symp Ser* 64(86):37–42.

Teule S, Crouzet J. 1994a. Modification de l'arôme au cours du séchage des purées de pomme. *Sci Aliments* 14:655–62.

Teule S, Crouzet J. 1994b. Effect of drying temperature on the flavour characteristics of dehydrated apple products. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research.* Amsterdam: Elsevier, pp. 233–9.

Thijssen HAC. 1971. Flavor retention in drying preconcentrated food liquids. *J Appl Chem Biotechnol* 21:372–7.

Timoumi S, Mihoubi D, Zagrouba F. 2007. Shrinkage, vitamin C degradation and aroma losses during infra-red drying of apple slices. *Lebensm Wiss Technol* 40:1648–54.

Venir E, Munari M, Tonizzo A, Maltini E. 2007. Structure related changes during moistening of freeze dried apple tissue. *J Food Eng* 81:27–32.

Young H, Gilbert JM, Murray SH, Ball RD. 1996. Causal effects of aroma compounds on Royal Gala apple flavours. *J Sci Food Agric* 71:329–36.

CHAPTER 28

Flavors of Dried Apricots

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INTRODUCTION

Apricot (*Prunus armeniaca* L.) is an important fruit crop believed to have originated in China and to have been brought to Europe at the beginning of the Roman era (Crouzet et al. 1990).

Apricots are members of the Rosaceae family. They belong to the subfamily Prunoideae and the subgenus *Prunus* of the genus *Prunus*. Botanically, apricots are stone fruits, like plums, peaches, and cherries, in which an outer fleshy part (exocarp and mesocarp) surrounds a hard stone (endocarp) with a seed inside. The most common varieties of apricots are Blenheim (Royal), Tilton, Perfection, Moorpark, and Early Golden. Their production is mainly located in the Mediterranean countries, which collectively account for 40% of global production. Turkey grew 14.15% of the world production of 3.25 MT in 2006 (FAOSTAT 2006). Turkey is the main producer of dried apricots, accounting for approximately 85% of world production.

The apricot fruit is smaller than a peach, colored orange-yellow when ripe, and with a drier flesh. The apricot has a distinctive taste, and no other fruit has a flavor to match it. It is marketed fresh, as well as dried and packed. Large quantities are canned or pulped for jam making. The most important market for apricot fruit developed from the exceptional qualities observed when the aromatic apricot is dried.

The moisture content of fresh apricots is 85.5% on average, and it drops to 10–25% when dried. The moisture content of dried apricots is of paramount importance because of the preservation effect of moisture reduction on the fruit. Several methods are available for the determination of moisture in fresh or dried apricots (Witherspoon and Jackson 1995).

Flavor is an important part of the quality of many food products, especially fruits. Preservation is needed to keep fruit for long periods of time. Dehydration or drying is one of the major preservation techniques for foods, and it is often linked with

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reduced product quality resulting from the degradative loss of the original aroma compounds (Pozderovic and Lovric 1986; Singh and Heldman 2001). Earlier studies have shown the complexity of the apricot flavor. This flavor cannot be attributable to a single compound but most probably to various mixture of compounds. Terpenic alcohols, lactones, and carbonyl compounds are important in the apricot's flavor, but many esters might also contribute to it (Komes et al. 2005). Butyl butanoate, butyl hexanoate, hexyl butanoate, ethyl hexanoate, ethyl butanoate, ethyl 2-methyl butanoate, 2-phenylethyl acetate, and butyl acetate are responsible for the fruity taste of apricot (Gomez et al. 1993; Guichard et al. 1990; Takeoka et al. 1990). Terpenic alcohols such as linalool, α-terpineol, terpinen-4-ol, nerol, and geraniol are responsible for the floral notes (rose, violet, and orange flower) (Guichard and Souty 1988). Lactones are very common in apricots and give a fruity taste (Guichard and Souty 1988). Benzaldehyde gives a very strong almond aroma and is also a typical compound found in apricots (Guichard and Souty 1988).

Carotenoids are important compounds of fruits and vegetables because they give specific coloration to fruit and provide some protection against a variety of degenerative diseases in humans (Van den Berg et al. 2000). β-Carotene gives a specific color to apricots and has been found to be the most abundant carotenoid (Karabulut et al. 2007). Hot air drying at 50°C causes great losses of β-carotene with or without sulfurization (Karabulut et al. 2007).

EFFECT OF DRYING ON APRICOTS

Drying is a very important step in food processing. Drying of food products is mainly carried out for preservation reasons and aims at removing water from solids to a level at which microbial spoilage and chemical reactions are minimized. Apricot is one of the most important, popular, and delicious fruits grown in Turkey. Due to its very short season, it is common to dry these apricots, and it is desirable for them to possess high-quality attributes in terms of physicochemical and flavor characteristics. During the drying of apricots, there are a number of chemical degradation reactions that may influence the taste of the final product. There is potential for too much chemical change leading to loss in flavor, and nutritional and commercial value. Apricot is very low in fat and high in sugar content. There are four major sugars in apricot, namely sucrose, glucose, fructose, and maltose. Sugars are very important in the quality of dried apricots. The most important chemical reactions affecting sugars during drying are acid hydrolysis, Maillard reactions, and caramelization. These reactions have a decisive influence on the quality of the dried product and have been shown to occur during the drying process depending on the pretreatment and drying method used, the temperature, and also the sugar composition of the fruit (Forni et al. 1997; Gogus et al. 2007; Karabulut et al. 2007; Koç and Alpaslan 2003; Komes et al. 2005; Mahmutoğlu et al. 1996).

Dried apricots have a greater nutritional value (especially vitamin A and minerals) than fresh ones because all the nutrients are concentrated (Guclu et al. 2006). Apricots are often treated with sulfur dioxide, used as a synthetic antioxidant, before being dried. Guclu and others (2006) found the antioxidant capacity of Malatya apricots to be higher than that found in literature. Blanching and sulfurization were effective in reducing the loss of lycopene and carotenoids in the dried apricot pulp

(Sabry 1961). Sulfurization is the most common commercially applicable method for preventing loss of quality in foods. Both enzymatic and nonenzymatic browning and microbial activity are prevented by using low concentration sulfides. Dried apricots have shown microbial loads under allowable limits. Depending on storage conditions, there is no essential change in total aerobic and yeast–mold counts for a storage period of up to 8 months (Mahmutoğlu et al. 1996). At high levels of sulfurization (4000 ppm of sulfur dioxide), the taste of sulfur is detectable. A high level of SO_2 in dried apricots can cause health problems such as asthmatic reactions. It has been reported that drying the pulp to a 10% instead of a 25% moisture content produced a slight off-flavor (Karabulut et al. 2007; Taylor et al. 1986). Apricots that have not been treated with SO_2 become darker with a caramelized almost fig-like color (Guclu et al. 2006).

The effects of pretreatments, SO_2 gas, prior to sun-drying of apricots were studied by Mahmutoğlu and others (1996). The pretreatment of fresh apricots caused a pH decrease and acidity increase due to dissolution of SO_2. They also found that the dried apricots compared with fresh ones had lower Hunter L (lightness), a (redness), and b (yellowness) values. The results indicated a darkening of color and the occurrence of browning reactions. They concluded that these changes were dependent on the initial SO_2 concentration, pH, maturity of the fruit, drying method, and external factors such as temperature and relative humidity.

Koç and Alpaslan (2003) investigated physical and chemical properties of microwave-finished dried apricots. They found that redness increased significantly with the increase in microwave power intensity. The taste and overall acceptance scores of the microwave-finished dried apricots were lower when high (over 400 W) levels of microwave power were used.

High-performance liquid chromatography (HPLC) has often been used to analyze nonvolatile compounds of apricot. Ascorbic acid, sugars (glucose, fructose, and sorbitol), and β-carotene have been analyzed using HPLC (Forni et al. 1997). Karabulut and others (2007) studied the effects of different hot air drying temperatures (50, 60, 70, and 80°C) and sun-drying on color and β-carotene content of Hacihaliloglu variety apricots. They observed a darkening in color for both sun- and hot air drying. However, it was reported that color and β-carotene content of the hot-air-dried samples were favorable in comparison to the sun-dried ones.

ANALYSIS METHODS OF APRICOT FLAVORS

The analysis of volatiles is generally accomplished by an extraction step, followed by concentration, chromatographic separation, and subsequent detection. Well-established methods of extraction of volatiles from apricot include steam distillation, vacuum steam distillation, solvent extraction, purge and trap, liquid–liquid extraction, and dynamic headspace techniques (Kok et al. 1987; Miszczak et al. 1995; Takeoka et al. 1990; Tang and Jennings 1967). An overview of sample preparation methods is provided by Pillonel and others (2002) for food volatiles and by Wilkes and others (2000) for food flavors and off-flavors. Methods based on the use of solvents have severe drawbacks, such as possibility of sample contamination, the loss of volatiles during the concentration process, and environmental problems related to the use of large amount of organic solvents (Grigonis et al. 2005;

Solis-Solis et al. 2007b). The chromatographic profile will vary depending on the method of sample preparation employed, and it is not uncommon to produce artifacts during this step.

The dynamic headspace technique is a very popular method for analyzing volatile compounds in plant materials (Esteban et al. 1993; Ozel et al. 2006). Solid phase microextraction (SPME) has been often used to analyze apricot volatiles (Guillot et al. 2006; Riu-Aumatell et al. 2005; Solis-Solis et al. 2007a,b). However, it requires liquid samples. Direct thermal desorption (DTD) is one version of a dynamic headspace technique with cryogenic trapping post desorption used to enrich the analytes prior to separation and involves solid samples. DTD has important advantages over the other methods such as the ability to be directly coupled to gas chromatography (GC)–mass spectrometry (MS), the requirement of only a small amount of sample, and, of course, the fact that it is a rapid method. DTD also has disadvantages, for example, the thermal decomposition of thermolabile compounds and the adverse effects of any water in the sample. According to previous literature, the DTD method has been applied to various systems such as plant material (Ozel et al. 2006) and sugar (Marsili et al. 1994). A great future strength of the technique, however, is that it may allow the elimination of the traditional sample preparation stages in a number of areas. The analytic technique of DTD coupled with GC is a very viable one. It is suitable especially for rapid qualitative compound analysis.

The volatile constituents of dried apricot samples have been isolated by thermal desorption with a cryo-focusing trap and analyzed using GC-time of flight (TOF)/MS (Gogus et al. 2007). The optimization of the DTD temperature has been studied previously, and 150°C was found to be the best from the 100–250°C range studied (Ozel et al. 2004). As has been discussed by Grimm and others (2002), sample preparation is the crucial step in the further analysis of compounds. Pillonel and others (2002) described some of the advantages of the thermal desorption technique as follows: (1) analysis of 100% of the trap content (instead of an aliquot part), (2) no solvent peak, (3) no waste, and (4) no contamination.

THE VOLATILE FLAVOR COMPOUNDS OF DRIED APRICOTS

Flavor compounds have a range of chemical group characteristics. The main apricot flavor compounds are esters, some terpenoids, alcohols, aldehydes, and lactones. There are over 100 flavor compounds in apricot. Many of these have been identified qualitatively and quantitatively since the invention of GC. With the addition of a mass spectrometer as a GC detector, it became much more popular in the determination of volatile compounds. However, many of the peaks on the GC of foods do not correlate to flavor. For example limonene, found in apricot, has a weak taste but a powerful odor. Oxygenated terpenes present in food have a major impact on the flavor. Benzaldehyde, linalool, and esters are characteristic volatile compounds of apricot (Guichard and Souty 1988; Guichard et al. 1990; Takeoka et al. 1990). β-Ionone, linalool, and carbonyl compounds could be responsible for the floral character of apricot odors, and lactones provide peach-like and coconut-like odors present in apricots (Guichard et al. 1990; Takeoka et al. 1990).

Most ketones are relatively rich flavor compounds. Aldehydes play an important role in providing flavor characteristics of a wide range of foods. The unsaturated

aliphatic aldehydes tend to produce stronger aromas. Benzaldehyde is reminiscent of bitter almonds and is associated with cherry flavor. The odor of citrus comes from aliphatic aldehydes and oxygenated terpenes like terpineol and citral.

The odor threshold of alcohols is considerably higher than that of the corresponding aldehydes, so they are normally less important to flavor profiles. Several terpenic alcohols have been found in the different stages of ripening. Linalool, terpineol, and geraniol appeared at high concentration in apricot (Gomez and Ledbetter 1997).

Lactones mostly have a low odor threshold but are rich in flavor. Lactones are internally formed esters and in chemical equilibrium with their corresponding acids; 4-hydroxy acids transform into γ-lactones and 5-hydroxy acids transform into δ-lactones. γ-Lactones have been described as fruity, peach-, or coconut-like (Furia and Bellanca 1981). Tang and Jennings (1967) were the first to identify γ-lactones, among other volatile compounds, in Blenheim variety apricots. According to Issanchou and others (1989), δ-octalactone in apricot gives a fruity taste. Guichard (1995) identified various γ-lactones (C6–C12) in apricot. Lactones possess two optical isomers. However, Guichard (1995) determined the R-enantiomer to be predominant in apricot.

There are studies on the volatile components of various varieties of apricot (Gogus et al. 2007; Guichard and Souty 1988; Guichard et al. 1990; Solis-Solis et al. 2007a,b; Takeoka et al. 1990). However, most of these studies concentrated on fresh apricots, and there are not much data found in the literature on the volatiles of dried apricot. Table 28.1 shows the compounds identified in dried apricot, using various methods. The number of identified compounds in dried apricot was significantly lower than that of fresh samples recorded in literature (Aubert and Chanforan 2007; Riu-Aumatell et al. 2005). The overall number of identified compounds was 86: 7 hydrocarbons, 9 esters, 2 lactones, 21 alcohols, 14 aldehydes, 7 ketones, 2 acids, 17 terpenes, and 7 miscellaneous (Table 28.1).

Azodanlou and others (2003) reported lactones, terpenes, and ketones as the main compounds of six varieties of apricots, which are the degradation products of C6-lipids. A range of hydrocarbons, esters, lactones, aldehydes, ketones, and acids have been seen in the volatiles of apricot (Gogus et al. 2007). The major components identified in desiccator-dried apricot are limonene (16.33%), (*E*)-2-hexenal (9.32%), γ-decalactone (7.89%), butyl acetate (6.94%), β-ionone (5.96%), acetic acid (4.83%), and isobutanal (4.78%) (Gogus et al. 2007). It was reported by Guillot and others (2006) who studied six varieties of fresh apricot that limonene and (*E*)-2-hexenal were among the major components. Takeoka and others (1990) also reported for fresh apricots that (*E*)-2-hexenal, γ-decalactone, and β-ionone were among the major contributors to apricot aroma of the Blenheim variety. However, they found that esters (>85%) were the dominant constituents using headspace analysis. In another study, ethyl acetate, butyl acetate, and hexyl acetate with a total percentage of 10.52% were the only esters found in desiccator-dried apricots of the Şekerpare variety. Isobutanal, tridecanol, and 1-pentadecanol were also reported as apricot constituents for the first time (Gogus et al. 2007).

Gogus and others (2007) reported that the method of drying the apricot samples results in a change in compositions of their volatiles. The number of components of volatile fractions obtained for desiccator-, sun-, hot-air-, and microwave-dried samples were 32, 28, 32, and 30, respectively. The number of identified components

TABLE 28.1. Various Volatile Constituents of Apricot Using Different Drying Techniques

Volatile Constituent	Type	Reference	Drying Method
Acetic acid	Acid	1,3	S, M, H, D
Hexanoic acid	Acid	3, 4	H
2,3-Butanediol	Alcohol	1	H
1-Octen-3-ol	Alcohol	1	H, D
2-Ethylhexanol	Alcohol	1, 3, 4	S, M, H, D
Furaneol	Alcohol	1	M, H
2-Decen-1-ol	Alcohol	1	D
Nonanol	Alcohol	1	S, D
Decanol	Alcohol	1	H, D
Tridecanol	Alcohol	1	S, M, H, D
1-Pentadecanol	Alcohol	1	S, M, D
cis-3-Hexen-1-ol	Alcohol	2, 3, 4	H, F, FM
1-Hexanol	Alcohol	2, 4	F, FM
2-Phenylethanol	Alcohol	2	F, FM
2,6-Dimethylcyclohexanol	Alcohol	3, 4	H
2,6-Dimethyl-7-octen-2,6-diol	Alcohol	3	H
1,3-Dimethylcyclohexanol	Alcohol	3, 4	H
Benzyl alcohol	Alcohol	3	H
2-Butyl-1-octanol	Alcohol	3	H
4-Vinyguaiacol	Alcohol	3	H
Butanol	Alcohol	3, 4	H
Octanol	Alcohol	3, 4	H
2,4-Dimethylcyclohexanol	Alcohol	3	H
Isobutanal	Aldehyde	1	S, D
Methylbutanal	Aldehyde	1	S, M
Pentanal	Aldehyde	1	S, M, H
Hexanal	Aldehyde	1, 2, 3	S, M, H, D, F FM
Furfural	Aldehyde	1, 3	S, M, H, D,
(*E*)-2-Hexenal	Aldehyde	1, 2, 3, 4	S, M, H, D, F FM
Benzaldehyde	Aldehyde	1, 2, 3	S, M, H, D, F FM
5-Methylfurfural	Aldehyde	1	M, H
Phenylacetaldehyde	Aldehyde	1, 3	S, M, H
(*E*,*Z*)-2,4-nonadienal	Aldehyde	1	D
Decanal	Aldehyde	1	S, M, H, D
5-HMF	Aldehyde	1	S, M, H, D
Nonanal	Aldehyde	3, 4	H
Heptanal	Aldehyde	4	H
Ethyl acetate	Ester	1, 3, 4	H, D
Butyl acetate	Ester	1, 2	S, H, D, F, FM
Hexyl acetate	Ester	1, 2, 3, 4	S, M, H, D, F FM
Ethyl-2-methylbutanoate	Ester	2	F, FM
n-Amyl acetate	Ester	2	F, FM
Butyl butanoate	Ester	2	F, FM
Ethyl hexanoate	Ester	2	F, FM
Ethyl octanoate	Ester	2	F, FM
Ethyl butanoate	Ester	2	F, FM
1-Nonene	Hydrocarbon	1	M, H, D
Decane	Hydrocarbon	1	S, M, H, D
Dodecane	Hydrocarbon	1	S, M, H, D
Tetradecane	Hydrocarbon	1	S, M, H, D
Hexadecane	Hydrocarbon	1	S, M, H, D

TABLE 28.1. ***Continued***

Volatile Constituent	Type	Reference	Drying Method
Methyl cyclopentane	Hydrocarbon	3	H
3,7-Dimethyl-1,6-octadiene	Hydrocarbon	4	H
Hydroxyacetone	Keton	1	D
6-Methyl-5-heptenone	Keton	1, 3, 4	S, M, H, D
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	Keton	1	S, M, H, D
geranyl acetone	Keton	1, 3, 4	H
Cyclohexanone	Keton	3	H
Isopropyl myristate	Keton	3	H
Fernesyl acetone	Keton	3	H
Butyrolactone	Lactone	1	S, M, H, D
γ-Decalactone	Lactone	1, 2, 3, 4	S, M, H, D, F FM
α-Pinene	Terpene	1	S, M
Limonene	Terpene	1, 3, 4	S, M, H, D
α-Terpineol	Terpene	1, 2, 3, 4	S, M, H, D, F FFFFFF,FM
β-Ionone	Terpene	1, 3, 4	S, M, H, D
Myrcene	Terpene	2	F, FM
Linalool	Terpene	2, 3, 4	S, F, FM
Nerol	Terpene	2, 3	S, F, FM
Geraniol	Terpene	2, 3	S, F, FM
Eugenol	Terpene	2	F, FM
cis-Linalool oxide	Terpene	3	H
Linalool oxide	Terpene	3	H
Epoxilinalool	Terpene	3	H
8-Hydroxy linalool	Terpene	3	H
5-Hydroxy linalool	Terpene	3	H
β-Cyclocitral	Terpene	3	H
β-Pinene	Terpene	3, 4	H
Thymol	Terpene	4	H
Pentylfuran	Miscellaneous	1	S, M, H
Cyclohexylisothiocyanate	Miscellaneous	3, 4	H
Pyrazine	Miscellaneous	3	H
Pyridine	Miscellaneous	3	H
2-Ethylaniline	Miscellaneous	3	H
p-Cresol	Miscellaneous	3, 4	H
Diethylphtalate	Miscellaneous	3, 4	H

1, Gogus and others (2007); 2, Komes and others (2005); 3, Solis-Solis and others (2007a); 4, Solis-Solis and others (2007b).
S, sun-drying; M, microwave; H, hot air; D, desiccator; F, freeze-drying; FM, foam mat drying.

common to all apricot samples was 21. Ethyl acetate, hydroxyacetone, and 2-decen-1-ol were found only in the desiccator-dried apricot samples (Gogus et al. 2007).

The major volatile components of apricot change drastically when dried by commercial techniques (sun, hot air, and microwaves) (Gogus et al. 2007). The main components identified using these drying techniques were 5-hydroxymethylfurfural (5-HMF), 2,3-dihydro-4-H-pyran-4-one, and furfural. These compounds together with other minor ones (5-methyl furfural, pentyl furan, furaneol) are known to be

the result of browning reactions. It is known that the heat treatment of fruits and vegetables often reduces the number of original volatile flavor compounds while, at the same time, introducing additional ones through the autoxidation of unsaturated fatty acids and thermal decomposition and/or initiation of caramelization and/or Maillard reactions. Maillard reaction products may negatively affect the flavor of the dried product, whereas it is desirable to preserve some naturally present compounds such as β-ionone, γ-decalactone, and butyl acetate, which positively enhance the flavor. Sulfurization extends the storage life of the dried apricot but does not prevent the occurrence of browning. Absence of oxygen in the storage pack as well as a reduction in storage temperature offers some protection against browning changes (Sabry 1961).

Apricots with their very low fat and high sugar content particularly tend to produce browning reaction products. Sugars are very important in the quality of dried apricots. There are four major sugars in apricot, namely sucrose, glucose, fructose, and maltose. Riu-Aumatell and others (2005) found glucose to be the main sugar present in apricot juice. Forni and others (1997) found sucrose to be the main sugar (75%) in cubes of apricot fruit, and they also found no difference in sugar composition, pH, and total titratable acidity between the apricot cubes before and after air-drying.

Gogus and others (2007) found browning reaction products to be only 5% in desiccator-dried apricot samples; however, they were 53.20%, 62.24%, and 64.83% for sun-, microwave-, and hot-air-dried samples of apricot, respectively. Komes and others (2005) found that the retention of apricot aroma after foam-mat drying (7.3% moisture content) and freeze-drying (2% moisture content) was only 17.81% and 43.23%, respectively, of the original.

Gogus and others (2007) found over 87% of the volatiles defined were the same whether sun-, hot air, or microwave drying was used, but, also that hot air and microwave drying made a difference to the volatile profiles. For example 5-methyl furfural, phenylacetaldehyde and furaneol only appeared in the hot-air- and microwave-dried samples. These degradation products were found to be 2.33% and 4.51% in microwave-dried and hot-air-dried samples, respectively. Phenylacetaldehyde has previously been found to be one of the major degradation products of the oxidation of phenylalanine (Adamiec et al. 2001). Furaneol and 5-methyl furfural have also been found as Maillard reaction products in dried prunes (Crouzet et al. 1990). Among the three commercial techniques studied, sun-drying has been found to be the best in that fewer degradation products were produced, and the components of the resulting volatiles were more like the desiccator-only dried sample (Gogus et al. 2007).

Gogus and others (2007) showed that DTD is an extremely useful technique. Its main advantage is that it does not require a sample preparation stage unlike other methods. This means it is more rapid and easily carried out, and compounds are not lost or contamination is not produced during sample preparation. Isobutanal, tridecanol, and 1-pentadecanol have been reported as apricot constituents for the first time.

The application of DTD coupled with GC-TOF/MS could be useful in industry to monitor the quality of the drying process of apricots. Samples could be rapidly and easily analyzed without the need for costly and time-consuming sample preparation stages. However, the drying process in the desiccator should be considered

as a time factor. Batches of dried apricots could be analyzed to check their levels of degradation products. This technique could even be used during the drying process itself to determine the end point of drying. Drying could be halted, therefore, when the level of degradation products (or loss of the desirable flavor compounds) became unacceptably high.

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REFERENCES

Adamiec J, Rössner J, Velisek J, Cejpek K, Savel J. 2001. Minor Strecker degradation products of phenylalanine and phenylglycine. *Eur Food Res Technol* 212:135–40.

Aubert C, Chanforan C. 2007. Postharvest changes in physicochemical constituents of apricot (*Prunus armeniaca* L). Characterization of 28 cultivars. *J Agric Food Chem* 55:3074–82.

Azodanlou R, Darbellay C, Luisier JL, Villettaz JC, Amado R. 2003. Development of a model for quality assessment of tomatoes and apricots. *Lebensm Wiss Technol* 36:223–33.

Crouzet J, Etievant P, Bayonove C. 1990. Stoned fruit: Apricot, plum, peach, cherry. In Morton ID, McLeod AJ (eds.), *Food Flavors. Part C. The Flavor of Fruit*. Amsterdam: Elsevier, pp. 43–92.

Esteban JL, Martinez-Castro I, Sanz J. 1993. Evaluation and optimization of the automatic thermal desorption method in the gas chromatographic determination of plant volatile compounds. *J Chromatogr A* 657:155–64.

FAOSTAT. 2006. Agricultural production data, ProdSTAT module on crops. FAO on-line and multilingual database. http://faostat.fao.org/ (accessed April 2008).

Forni E, Sormani A, Scalise S, Toreggiani D. 1997. The influence of sugar composition on the color stability of osmodehydrofrozen intermediate moisture apricots. *Food Res Int* 30:87–94.

Furia TE, Bellanca H. 1981. *Fenaroli's Handbook of Flavor Ingredients, Chemical Rubber*. Cleveland, OH: CRC Press.

Gogus F, Ozel MZ, Lewis AC. 2007. The effect of various drying techniques on apricot volatiles analysed using direct thermal desorption-GC-TOF/MS. *Talanta* 73:321–5.

Gomez E, Ledbetter CA. 1997. Development of volatile compounds during fruit maturation: Characterization of apricot and plum x apricot hybrids. *J Sci Food Agric* 74:541–6.

Gomez E, Ledbetter CA, Harstell PL. 1993. Volatile compounds in apricot, plum and their interspecific hybrids. *J Agric Food Chem* 41:1669–76.

Grigonis D, Venskutonis PR, Sivik B, Sandahl M, Eskilsson CS. 2005. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloe odorata*). *J Supercrit Fluids* 33:225–35.

Grimm CC, Llloyd SW, Miller JA, Spanier AM. 2002. *Flavor, Fragrance, and Odor Analysis*, Marsili R (ed.). New York: Marcel Dekker.

Guclu K, Altun M, Ozyurek M, Karademir SE, Apak R. 2006. Antioxidant capacity of fresh, sun- and sulphited-dried Malatya apricot (*Prunus armeniaca*) assayed by CUPRAC, ABTS/TEAC and folin methods. *Int J Food Sci Technol* 41:76–85.

Guichard E. 1995. Chiral γ-lactones, key compounds to apricot flavor. In Rouseff RL, Leahy MM (eds.), *Fruit Flavors: Biogenesis, Characterization and Authentication. ACS Symposium Series 596*. Washington, DC: American Chemical Society, pp. 258–67.

Guichard E, Schlich P, Issanchou S. 1990. Composition of apricot aroma: Correlations between sensory and instrumental data. *J Food Sci* 55:735–8.

Guichard E, Souty M. 1988. Comparison of the relative quantities of aroma compounds found in fresh apricot (*Prunus armeniaca)* from six different varieties. *Eur Food Res Technol* 186:301–7.

Guillot S, Peytavi L, Bureau S, Boulanger R, Lepoutre JP, Crouzet J, Schorr-Galindo S. 2006. Aroma characterization of various apricot varieties using headspace-solid phase microextraction combined with gas chromatography-mass spectrometry and gas chromatography-olfactometry. *Food Chem* 96:147–55.

Issanchou S, Schlich P, Guichard E. 1989. Odour profiling of the components of apricot flavour. Description by correspondence analysis. *Sci Aliments* 9:351–70.

Karabulut I, Topcu A, Duran A, Turan S, Ozturk B. 2007. Effect of hot air drying and sun drying on color values and β-carotene content of apricot. *Lebensm Wiss Technol* 40:753–8.

Koç K, Alpaslan M. 2003. Microwave finish drying to improve physical and chemical properties of apricots. *Acta Aliment* 32:311–5.

Kok MF, Yong FM, Lim G. 1987. Rapid extraction method for reproducible analysis of aroma volatiles. *J Agric Food Chem* 35:779–81.

Komes D, Lovric T, Ganic KK, Kljusuric JG, Banovic M. 2005. Trehalose improves flavour retention in dehydrated apricot puree. *Int J Food Sci Tech* 40:425–35.

Mahmutoğlu T, Saygı YB, Borcaklı M, Özay G. 1996. Effects of pretreament-drying method combinations on the drying rates, quality and storage stability of apricots. *Lebensm Wiss Technol* 9:418–24.

Marsili RT, Miller N, Kilmer GJ, Simmons RE. 1994. Identification and quantitation of the primary chemicals responsible for the characteristic malodor of beet sugar by purge and trap GC-MS-OD techniques. *J Chromatogr Sci* 32:165–71.

Miszczak A, Fornet CF, Prange RK. 1995. Development of aroma volatiles and color during postharvest ripening of "Kent" strawberries. *J Am Soc Hortic Sci* 120:650–5.

Ozel MZ, Gogus F, Hamilton JF, Lewis AC. 2004. The essential oil of *Pistacia vera* L. at various temperatures of direct thermal desorption using GCxGC-TOF/MS. *Chromatographia* 60:79–83.

Ozel MZ, Gogus F, Lewis AC. 2006. Determination of *Teucrium chamaedrys* volatiles by using direct thermal desorption-comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J Chromatogr A* 1114:164–9.

Pillonel L, Bosset JO, Tabacchi R. 2002. Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review. *Lebensm Wiss u-Technol* 35:1–14.

Pozderovic A, Lovric T. 1986. Investigation of the retention of aromatic substances during freeze-drying of apple puree using headspace analysis. *Prehram Tehnol Biot* 24:101–8.

Riu-Aumatell M, Lopez-Tamames E, Buxaderas S. 2005. Assessment of the volatile composition of juices of apricot, peach, and pear according to two pectolytic treatments. *J Agric Food Chem* 53:7837–43.

Sabry ZI. 1961. Browning in dried fruid products: Non-enzymatic browning and its effect on the carotenoids in Qamareddeen, a dried apricot pulp. *J Agric Food Chem* 9:53–5.

Singh RP, Heldman DR. 2001. Dehydration. In Taylor SL (ed.), *Introduction to Food Engineering*. London: Academic Press, pp. 557–8.

Solis-Solis HM, Calderon-Santoyo M, Gutierrez-Martinez P, Schorr-Galindo S, Ragazzo-Sancheza JA. 2007a. Characterization of aroma potential of apricot varieties using different extraction techniques. *Food Chem* 105:829–37.

Solis-Solis HM, Calderon-Santoyo M, Gutierrez-Martinez P, Schorr-Galindo S, Ragazzo-Sancheza JA. 2007b. Discrimination of eight varieties of apricot (*Prunus armeniaca*) by electronic nose, LLE and SPME using GC-MS and multivariate analysis. *Sens Actuators B* 125:415–21.

Takeoka GR, Flath RA, Mon TR, Teranishi R, Guntert M. 1990. Volatile constituents of apricot (*Prunus armeniaca*). *J Agric Food Chem* 38:471–7.

Tang CS, Jennings WC. 1967. Volatile components of apricot. *J Agric Food Chem* 15:24–8.

Taylor SL, Higley NA, Bush RK. 1986. Sulfites in foods: Uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity and hypersensitivity. *Adv Food Res* 30:1–76.

Van den Berg H, Faulks R, Fernando GH, Hirschberg J, Olmedilla B, Sandman G. 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *J Sci Food Agric* 80:880–912.

Wilkes JG, Conte ED, Kim Y, Holcomb M, Sutherland JB, Miller DW. 2000. Sample preparation for the analysis of flavors and off-flavors in foods. *J Chromatogr A* 880:3–33.

Witherspoon JM, Jackson JF. 1995. Analysis of fresh and dried apricot. In Linskens HF, Jackson JF (eds.), *Fruit Analysis*. New York: Springer, pp. 111–31.

CHAPTER 29

Flavors of Dried Bananas

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INTRODUCTION

Banana is a productive fruit in the tropical and subtropical zones. Although the processing of banana is considerably less than that of orange, apple, and pineapple, some dry products such as banana chips, snacks, and powder are produced.

Dried banana can be produced using various drying methods. Schirmer and others (1996) published their experimental investigation into the performance of the solar tunnel dryer for drying banana. Generally, air-drying (AD) is widely applied to banana slices. Preferences for different air conditions in a two-stage heat pump dryer, AD, and convective drying for banana pieces have been reported during the past few years (Boudhrioua et al. 2002; Chua et al. 2001; Jannot et al. 2004; Mariani et al. 2008). Separate or combined hot air drying (including high and low temperature), vacuum, microwave, freeze-drying (FD) and osmotic drying to produce dehydrated bananas have also been studied (Krokida et al. 2001; Maskan 2000; Mui et al. 2002; Prachayawarakorn et al. 2008). Chu and others (2001) referred to the frying of banana chips. Besides banana slices, the production of banana powder by foam-mat drying has been investigated (Sankat and Castaigne 2004; Siliha et al. 1994). Chen and others (2006) compared the processing of banana powder dehydrated by vacuum belt drying (VBD), FD, and AD. Hervas and Polit (1985) examined a mixture of banana and soybean dehydrated in a drum dryer.

IDENTIFICATION METHODS FOR BANANA VOLATILES

Solid Phase Microextraction (SPME) Gas Chromatography–Mass Spectrometry (GC-MS)

GC-MS is widely used in odor analysis. SPME, a relatively new treatment technique, is applied as a way of extracting volatiles, especially fruit and vegetable volatiles.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Many reports have analyzed banana flavors using headspace (HS) SPME (Agelopoulos and Pickett 1998; Ibanez et al. 1998; Liu and Yang 2002).

HS–Programmed Temperature Vaporizer (PTV)–GC

Ibáñez and others (1999) introduced a method for the analysis of fruit. HS using a PTV gas chromatographic injector as an intermediate trap was proposed. The method consists of a purge of the volatile compounds into the PTV glass liner filled with a packing material of high surface area. The trapped compounds are thermally desorbed in the PTV injector and transferred to the gas chromatographic column.

GC–Olfactometry (GC-O)

Besides GC-MS, GC-O has been used to determine the aromatic composition and active aroma components of commercial banana essence and fresh banana fruit paste (Jordán et al. 2001). In addition, volatiles from banana passa were captured by static cryogenic HS. The extract was investigated on a GC-O-multidimensional-MS system, and aroma extract dilution analysis was applied (Miranda et al. 2001).

Micro Steam Distillation–Solvent Extraction (SDE)

The operation of an SDE device in two different modes, namely at reduced pressure and involving the concentration of the dynamic HS resulting from purging the sample with an inert gas, was investigated in a study by Blanch and others (1993).

Breath-By-Breath Analysis

Mayr and others (2003) reported on the *in vivo* breath-by-breath analysis of volatiles released in the mouth during the eating of ripe and unripe banana. The air exhaled through the nose and nosespace (NS) was directly introduced into a proton transfer reaction mass spectrometer, and the time–intensity profiles of a series of volatiles were monitored online.

Isotopic Techniques

Salmon and others (1996) determined the molecular composition and the ^{13}C content of some of the main constituents of banana aroma using GC, GC-MS, and ^{13}C GC-combustion furnace-isotope ratio (C-IR) MS in fruits, commercial nectars, and industrial aromas.

AROMA OF DRIED BANANA

A great number of studies have reported on the aroma of fresh ripe and unripe banana and banana during ripening. However, few studies have focused on dried banana. Several studies by Wang and others (2007), Boudhrioua and others (2002), Mui and others (2002), and Miranda and others (2001) referred to the odor of dehydrated banana. Flavors reported in these articles are summarized in Table 29.1.

TABLE 29.1. Volatiles of Dried Bananas

Product	Volatiles	Dry *M*ethods	Reference
Banana powder	3-Methylbutyl acetate Butanoic acid 3-methylbutyl ester 3-Methylbutanoic acid 3-methylbutyl ester Isoamyl butyrate Butanoic acid 1-methylhexyl ester Hexyl isovalerate 2-Heptanol acetate Isobutyl isoval ester Eugenol Elemicin	Vacuum belt drying; freeze-drying; air-drying	Wang and others (2007)
Dehydration banana	Isoamyl alcohol Isoamyl acetate Butyl acetate Elemicine N1 N2 N3	Convective air-drying at 40, 60, and 80°C	Boudhrioua and others (2002)
Banana chips	Ethyl butanoate Isoamyl buyanoate Isobutyl acetate Butyl acetate Isoamyl acetate Isobutyl butanoate 3-Methyl butanal	A combination of air-drying and vacuum microwave drying	Mui and others (2002)
Banana passa	4-Hydroxy-2,5-dimethyl-3(2H)-furanone Isovaleric Ethyl butanoate 2-Methylpropan-1-ol 3-Methylbutan-1-ol (*Z*)-3-hexen-1-ol Heptan-2-ol 3-Nonen-2-ol Eugenol Ethylcinnamate Elimicine	Dried at 50°C with forced ventilation	Miranda and others (2001)

It is said that most of the volatiles in green banana are aldehydes; however, the prominent odors in yellow banana are esters (Zhu et al. 2007). It is shown in Table 29.1 that esters, alcohols, ketones, and other flavors are found in dried banana, whereas esters play a key role in banana fruit aroma. A large number of the esters are derived from acetate and butanoate.

Wang and others (2007) compared the volatiles in banana powder dehydrated using VBD, FD, and AD. The major volatiles of the three types of banana powder were the same; however, the quantities of the components were different. The relatively high drying temperature of AD may damage some of the original compounds while causing the formation of others. 3-Methylbutanoic acid 3-methylbutyl ester, 3-methybutyl acetate, and butanoic acid 3-methylbutyl ester, which are thought to

give the ripe banana's fruity odor, have been separated and identified by SPME GC-MS in banana powder. The former two compounds were found to be the major components, while the latter compound was a minor component in the powder. Eugenol and elemicin, responsible for the typical mellow aromas, were also detected in banana powder. The aroma of banana powder was particularly associated with compounds such as 3-methyl, 1-methylhexyl, 2-methylpropyl, 3-methylbutyl, isoamyl, and hexyl esters of acetic, butanoic, and isovalerate acids. It was inferred that FD and VBD are better ways of protecting the original banana flavors than the AD process.

Boudhrioua and others (2002) studied the changes in aromatic compounds of Cavendish banana during ripening and AD at three different temperatures below 100°C. Twelve flavors (two alcohols, nine esters, and one phenol) in fresh banana, which are regarded as the most significant compounds related to banana smell, were identified by GC-MS. Seven odors, four among the aforementioned 12 flavors (isoamyl alcohol, isoamyl acetate, butyl acetate, and elemicin) and three other unidentified compounds were selected by olfactometric analysis as characteristic of banana smell. These seven aromatic compounds were analyzed during AD at 40, 60, and 80°C. During AD, a strong decrease in these seven compounds during the first 2 h, an increase during the second and the sixth hour of drying at 60 and 80°C, then a slight decrease at the end of the drying period at 60°C were noted. With regard to 80°C, a continuous decrease was observed for isoamyl acetate, isoamyl alcohol, and butyl acetate, while some of the unidentified compounds increased or seemed to form at the end of the drying process. Elemicin was found to be the most thermally resistant compound. A loss in aromatic content due to water vapor entrainment was found to be the dominant process at the start of the drying period whatever the air temperature and cooking method and Maillard reactions developed at the end of drying at 80°C.

Mui and others (2002) reported the behavior of 16 volatile banana compounds during a combination of AD and vacuum microwave drying (VMD) of banana chips. It was found that samples that underwent more VMD had significantly lower levels of volatile compounds, which was attributed to the decreased formation of an impermeable solute layer on the surface of the chips. High water solubility and relative volatility of the compounds correlated with losses during VMD; however, additional factors appeared to influence the behavior of the compounds during VMD. The combination of AD and VMD yielded crisper banana chips with significantly higher volatile levels and sensory ratings than chips dried by AD alone.

Miranda and others (2001) published the odor-active compounds of banana passa identified by aroma extract dilution analysis. Banana was dehydrated at 50°C with forced ventilation to produce banana passa. Banana passa volatiles were captured by static cryogenic HS, and the extract was then investigated using a GC-O-multidimensional-MS system and the application of aroma extract dilution analysis. The authors concluded that the extremely sweet and caramel note, characteristic of banana passa, was associated with 4-hydroxy-2,5-dimethyl-3(2H)-furanone. As this compound was not detected in the natural banana extract, it is probably generated during the thermal dehydration process, especially as its origin has already been associated with the thermal degradation of sugars. Although numerous short chain esters were identified, only ethyl butanoate was identified as an impact odor compound with a fruity note, even though it is commonly found in fruits with no specific fruity-like aroma. Ethyl cinnamate was responsible for the most

characteristic note of banana passa, with the fermented and cooked notes being associated with 2-methylpropan-1-ol and 3-methylbutan-1-ol.

CONCLUSION AND DISCUSSION

Banana is usually dehydrated to produce banana chips, snacks, powder, and passa. There are several methods available to identify fruit volatiles. GC-MS is the most widely used measure for the identification of aromatic compounds. SPME is a comparatively new method for flavor extraction. Other techniques, such as HS-PTV-GC, GC-O, GC-O-multidimensional-MS, SDE, *in vivo* breath-by-breath analysis, and isotopic methods are also used.

With regard to the volatiles of dried banana, the esters are the most predominant odor. The aroma of dried banana is particularly associated with the compounds butyl, isobutyl, isoamyl, ethyl esters of acetic acid, and butanoic acid. Isoamyl acetate, which is regarded as the main aroma constituent in fresh banana flavor, and eugenol, which represents the sweet, phenolic aroma, are detected in most dried banana products. Dehydration, usually by thermal processes, tends to destroy the thermally unstable aromatic compounds and induces some thermal reaction odors. However, elemicin, responsible for the typical full-bodied and mellow aromas, was found to be the most thermally resistant component (Boudhrioua et al. 2002).

Research on dried banana has concentrated on the drying techniques, parameters, and dynamics of banana processing, and the physical–chemical, sensory, and microbiological aspects of dehydrated banana products. Investigations into banana flavors have concentrated on fresh fruit and banana cultured in different regions, and information on dried banana volatiles is very limited. Therefore, further studies on the aroma of dehydrated banana are necessary.

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REFERENCES

Agelopoulos NG, Pickett JA. 1998. Headspace analysis in chemical ecology: Effects of different sampling methods on ratios of volatile compounds present in headspace samples. *J Chem Ecol* 24(7):1161–72.

Blanch GP, Herraiz M, Reglero G, Tabera J. 1993. Preconcentration of samples by steam distillation-solvent extraction at low temperature. *J Chromatogr A* 655(1):141–9.

Boudhrioua N, Michon C, Cuvelier G, Bonazzi C. 2002. Influence of ripeness and air temperature on changes in banana texture during drying. *J Food Eng* 55:115–21.

Chen RR, Wang J, Gong L, Li YZ. 2006. Comparison of vacuum belt, freeze and air-drying methods of processing banana puree. *Transactions of the Chinese Society for Agricultural Machinery* 37(8):159–62.

Chu BS, Ghazali HM, Lai OM, Che Man YB, Yusof S, Yusoff MSA. 2001. Performance of a lipase-catalyzed transesterified palm kernel olein and palm stearin blend in frying banana chips. *Food Chem* 74:21–33.

Chua KJ, Mujumdar AS, Hawlader MNA, Chou SK, Ho JC. 2001. Batch drying of banana pieces—Effect of stepwise change in drying air temperature on drying kinetics and product colour. *Food Res Int* 34:721–31.

Hervas OM, Polit CP. 1985. Production of a food mixture of banana and soybean dehydrated in a drum drier. Part II. *Politecnica* 10(4):151–66.

Ibanez E, Lopez-Sebastian S, Ramos E, Tabera J, Reglero G. 1998. Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem* 63(2):281–6.

Ibáñez E, Tabera J, Ramos E, López-Sebastián S, Reglero G. 1999. Analysis of volatile components of fruits by HS-PTV-GC. *J Sci Food Agric* 79:1275–9.

Jannot Y, Talla A, Nganhou J, Puiggali J-R. 2004. Modeling of banana convective drying by the drying characteristic curve (DCC) method. *Dry Technol* 22(8):1949–68.

Jordán MJ, Tandon K, Shaw PE, Goodner KL. 2001. Aromatic profile of aqueous banana essence and banana fruit by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). *J Agric Food Chem* 49(10):4813–7.

Krokida MK, Maroulis ZB, Saravacos GD. 2001. The effect of the method of drying on the colour of dehydrated products. *Int J Food Sci Technol* 36(1):53–9.

Liu TT, Yang TS. 2002. Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *J Agric Food Chem* 50(4):653–7.

Mariani VC, Lima AGB, Coelho LS. 2008. Apparent thermal diffusivity estimation of the banana during drying using inverse method. *J Food Eng* 85:569–79.

Maskan M. 2000. Microwave/air and microwave finish drying of banana. *J Food Eng* 44:71–8.

Mayr D, Märk T, Lindinger W, Brevard H, Yeretzian C. 2003. Breath-by-breath analysis of banana aroma by proton transfer reaction mass spectrometry. *Int J Mass Spectrom* 223–224:743–56.

Miranda EJF, Nogueira RI, Pontes SM, Rezende CM. 2001. Odour-active compounds of banana passa identified by aroma extract dilution analysis. *Flav Frag J* 16:281–5.

Mui WW, Durance TD, Scaman CH. 2002. Flavor and texture of banana chips dried by combinations of hot air, vacuum, and microwave processing. *J Agric Food Chem* 50(7):1883–9.

Prachayawarakorn S, Tia W, Plyto N, Soponronnarit S. 2008. Drying kinetics and quality attributes of low-fat banana slices dried at high temperature. *J Food Eng* 85:509–17.

Salmon B, Martin GJ, Remaud G, Fourel F. 1996. Compositional and isotopic studies of fruit flavors. Part I. The banana aroma. *Flav Frag J* 11(6):353–9.

Sankat CK, Castaigne F. 2004. Foaming and drying behaviour of ripe bananas. *Lebensm Wiss Technol* 37(5):517–25.

Schirmer P, Janjai S, Esper A, Smitabhindu R, Mühlbauer W. 1996. Experimental investigation of the performance of the solar tunnel dryer for drying banana. *Renewable Energy* 7(2):119–29.

Siliha H, El-Zoghbi M, Askar A. 1994. Foam-mat drying of enzyme treated banana pulp. *Flussiges Obst* 61(8):4–5, 210–2.

Wang J, Li YZ, Chen RR, Bao JY, Yang GM. 2007. Comparison of volatiles of banana powder dehydrated by vacuum belt drying, freeze-drying and air-drying. *Food Chem* 104: 1516–21.

Zhu H, Chen YF, Li XP, Li J, Han DF, Chen WX. 2007. Determination of volatiles in harvested banana fruit by HS-SPME and GC-MS. *Acta Hortic Sin* 34(2):485–8.

CHAPTER 30

Volatile Aroma/Flavor Components of Raisins (Dried Grapes)

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INTRODUCTION

Over at least the last 3000 years, humans have been drying grapes (usually sun-drying) to produce a relatively stable, storable product. There are a number of different types of dried grapes; the major types in the United States are referred to as raisins, golden raisins, and zante currants. Both types of raisins from dried Thompson seedless grapes and the zante currants from a small seedless grape in the United States are called Black Corinth. The only significant studies carried out on dried grape aroma/flavor volatiles have been with the common dark-colored, sun-dried raisins, and this review will be largely confined to this type. To the author's knowledge, no aroma/flavor volatile studies have been carried out on golden raisins that are obtained by treating the grapes with sulfite during drying.

The simplest way to dry grapes is to put them out in the sun, and this is essentially the method that is still used in California, which is one of the major producers of raisins. Besides the untreated sun-drying of grapes, there are a number of other different ways in which they can be dried. One variation is to treat the grapes with an emulsion of ethyl oleate and potassium carbonate (or similar mixture) before sun-drying, which increases the rate of drying. Raisins produced in this way have a lighter color. Other methods involve drying in an oven at about 65°C. Usually, the grapes are dried to a moisture content of about 10–14%.

ATTEMPTS TO OBTAIN COMPREHENSIVE ANALYSES OF AROMA/FLAVOR VOLATILES

Although grape volatile aroma/flavor compounds have been extensively studied (Nijssen et al. 1996; Schreier et al. 1976), there are very few studies on the volatile aroma/flavor compounds of raisins. In fact, the published attempts to analyze the total

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flavor volatiles of fresh raisins amounts to only three studies. One of these was carried out in Australia (Ramshaw and Hardy 1969), another in the United States (Buttery et al. 1981), and a third in France (Joulian and Fourniol 1990). The studies in both the United States and France were with Californian raisins from Thompson seedless grapes. These are normally obtained by sun-drying the grapes between the grape rows without any other treatment. Those used in the Australian work were from a similar seedless variety (called Sultana), but one sample of the two studies was dipped in an emulsion of C14–C18 fatty acid ethyl esters in a potassium carbonate solution before drying. Some very limited observations were made by Ramshaw and Hardy comparing the volatiles in the raisins produced by the different methods of drying.

The volatile flavor compounds found in these studies have been summarized in Table 30.1. The second column lists which of the researchers identified each particular compound (see footnote). Although a thorough quantitative study was not carried out, some rough semiquantitative data (from Buttery et al. 1981) is included in the table in the third column. In these studies, compounds found in highest concentration included the aliphatic acids hexanoic, heptanoic, octanoic, and nonanoic acids along with the aliphatic aldehydes nonanal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, and (*E*,*E*)-2,4-decadienal. These aliphatic compounds are all well-known as being derived from unsaturated fatty acid oxidative degradation.

Other major volatiles identified included phenylacetaldehyde and 2-hexyl-3-methylmaleic anhydride. Phenylacetaldehyde likely results from phenylalanine via the Maillard reaction, which occurs during drying and storage.

2-Hexyl-3-methylmaleic anhydride is an unusual compound that had not been previously reported in any other food, although it was found (Buttery et al. 1980) also in the dried hulls of almonds, which might be considered as a type of dried fruit. Although present in reasonable amounts in the raisins, this compound had essentially no odor and is unlikely to be of any importance to the total aroma.

Odor thresholds, when available, of the compounds in water solution are also included in the fourth column of Table 30.1. This can give some idea of the contribution of each compound to the total raisin aroma. Compounds that are present below their odor threshold have a very low probability to contribute, whereas those with a concentration many times their threshold have a high probability of contributing. From the data in Table 30.1, it can be seen that the compounds nonanal, decanal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*)-2-octenal, (*E*,*E*)-2,4-decadienal, (*E*,*E*)-2,4-nonadienal, 1-octen-3-ol, 1-octen-3-one, and phenylacetaldehyde all have a reasonable probability of contributing to the total aroma/flavor. The aliphatic acids, despite their high concentrations, occur at concentrations less or a little above their threshold and so have a lower probability of contributing to the aroma/flavor. This consideration is, however, for water solutions. Raisins are a relatively dry product, and it is difficult to determine what the contribution would be when considering the aroma/flavor of the original raisins. Only when the raisin is chewed in the mouth and mixed with saliva would an approximate aqueous system apply.

Studies on Specific Compounds and Off-Flavors

Besides the general studies outlined above, other studies have been carried out, looking for specific compounds, which are not necessarily isolatable by the simpler

TABLE 30.1. Volatile Compounds Reported in Raisins

Compound	Reported by	Approx. Conc. µg/kg[a]	Odor Thresh. nL/L (ppb)[b]
Aliphatic aldehydes			
Pentanal	R	—	—
Hexanal	R, B	75	4.5
Heptanal	B	15	3
Octanal	B	10	0.7
Nonanal	B	270	1
Decanal	B, J	60	0.1
Monounsat. aliphatic aldehydes			
(*E*)-2-hexenal	B	5	17
(*E*)-2-heptenal	R, B	110	13
(*E*)-2-octenal	B	140	3
(*E*)-2-nonenal	B	85	0.08
(*E*)-2-decenal	B	100	0.3
(*E*)-2-undecenal	B	60	—
Diunsaturated aliphatic aldehydes			
(*E*,*E*)-2,4-heptadienal	B	85	38
(*E*,*E*)-2,4-nonadienal	B	20	—
(*E*,*Z*)-2,4-decadienal	B	10	—
(*E*,*E*)-2,4-decadienal	B	100	0.07
Aliphatic ketones			
Biacetyl (2,3-butandione)	R	—	3
Acetoin (2-hydroxybutan-3-one)	R, J	—	39,000
4-Hydroxy-4-methyl-2-pentanone	J	—	—
1-Octen-3-one	B	10	0.05
6-Methyl-3,5-heptadien-2-one	B	5	380
Geranylacetone	B, J	70	60
Aliphatic alcohols			
Butanol	R	—	500
3-Methylbutanol	J	—	250
Pentanol	R	—	4000
1-Octen-3-ol	B, J	130	1
Octanol	B	85	—
Nonanol	B	80	50
Decanol	B	15	—
Aliphatic acids			
Acetic	J	—	22,000
Butyric	J	—	240
Hexanoic	B, J	25–220	3000
Heptanoic	B	25–220	3000
Octanoic	B, J	220–420	—
Nonanoic	B, J	150–280	—
Decanoic	B, J	50–95	10,000
Dodecanoic	J	—	6,100
Benzene and furan derivatives			
Furfural	R, B, J	25	3000
Furfuryl alcohol	J	—	—
2-Pentylfuran	B	170	6
2-Acetylfuran	R, B	50	10,000

TABLE 30.1. ***Continued***

Compound	Reported by	Approx. Conc. µg/kg[a]	Odor Thresh. nL/L (ppb)[b]
5-Methylfurfural	R, B, J	110	500
Methylacetylfuran	R	—	—
Benzaldehyde	R, B	40	350
Phenylacetaldehyde	R, B	200	4
Acetophenone	R	—	—
Benzyl alcohol	R	—	119
2-Phenylethanol	R	—	1100
4,5-Dihydro-2-methyl-2*H*-furan-3-one	J	—	—
Terpenoids			
α-Terpineol	R, B	20	330
Esters			
Methyl propionate	J	—	—
Methyl furoate	J	—	—
Ethyl octanoate	R	—	—
Methyl salicylate	J	—	40
Ethyl nonanoate	R	—	—
Lactones			
γ-Butyrolactone	R, J	—	>1000
α-Angelicalactone	J	—	—
β-Angelicalactone	R	—	—
γ-Hexalactone	R	—	—
4-Methyl-2-butenolide	J	—	—
4-Ethyl-2-butenolide	J	—	—
γ-Octalactone	R	—	8
γ-Nonalactone	J	—	2
Miscellaneous			
3-ethyl-2,5-dimethylpyrazine	J	—	9
N-methyl-2-formylpyrrole	R	—	37
N-ethyl-2-formylpyrrole (1-Ethyl-2-pyrrolecarbaldehyde)	B, J	50	—
2-Hexyl-3-methylmaleic anhydride	B, J	250	—

[a]Rough data only. Calculated from relative percentage and total amount of volatile oil. No internal standard used.
[b]Threshold data in water solution from the author's own files. Reported in many of the author's publications. Data for decanoic and dodecanoic acids from Meilgaard (1975).
R, Ramshaw and Hardy (1969); B, Buttery and others (1981); J, Joulain and Fourniol (1990).

methods commonly used for flavor volatiles such as steam distillation (including SDE) or porous polymer adsorbent trapping (e.g., using dynamic headspace or SPME). One of these was 5-hydroxymethyl-2-furaldehyde, which, because of its high water solubility, has a low volatility in aqueous medium, which exists in many foods or where steam distillation methods are used. Palma and Taylor (2001) isolated this compound from raisins using supercritical carbon dioxide extraction and found a concentration of 128,000 µg/kg (128 ppm), which is far higher than that of any other volatiles found in raisins. 5-Hydroxymethyl-2-furaldehyde is a very weak odorant with an odor threshold of 1000 mg/kg (1000 ppm) in beer (Meilgaard 1975) and is usually not considered important in food flavor studies. Its concentration in raisins (although extremely high) would be below this threshold.

Diacetyl, acetoin, and 2,3-butandiol occur in many foods and were identified in raisins using colorimetric methods of analysis in a general study of a number of foods (Peynaud and Lafon 1951). In a similar type of study, benzoic acid was identified in a number of foods including raisins (Nagayama et al. 1983).

Chloroanisoles, well-known to cause off-flavor in a number of foods, were identified as contaminants in Australian raisins (and other dried fruits) in 1987 (Tindale 1987). The presence of chloroanisoles in Californian raisins was also studied by Aung and others (1996) who found ~35 μg/kg of 2,4,6-trichloroanisole in some samples of raisins. They also found evidence that using methods of sterilization of the raisins (to reduce microorganisms) did not reduce the concentrations of chloroanisoles found. Most studies of off-flavor caused by chloroanisoles in various foods have indicated that the chloroanisoles come by vapor transfer from materials with which the food has come in contact (e.g., Tindale 1987).

COMPARISON WITH GRAPE VOLATILES

Some years ago an extensive list of compounds were already known to occur in grapes (Nijssen et al. 1996; Schreier et al. 1976). Many of the compounds found in raisins had been found in grapes. These included most of the aliphatic aldehydes, alcohols, and ketones formed by oxidative degradation of unsaturated fatty acids. In fresh grapes, these were probably formed by an enzyme-catalyzed oxidation. One major difference between the fresh grape volatiles and the raisin volatiles noted by Ramshaw and Hardy (1969) was the almost complete loss of the major fresh grape volatiles hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenal in the raisins. It seems likely that the compounds originally present in the grapes would be largely lost in the drying. A lower moisture content would enhance chemical lipid autoxidation to produce many of the same group of compounds but with some differences particularly in the relative concentrations of components. Exposure to sunlight, during drying, would also enhance such a free radical process.

Some of the more water-soluble compounds would have a lower volatility and may show considerably less loss in drying. These could include compounds such as 2-phenylethanol, benzyl alcohol, and 2,3-butandiol.

Volatiles in Partially Dried Grapes

For certain sweet dessert wines, grapes are partially dried to increase their sugar content before being fermented. The crushed juice (must) from such partially dried grapes has been reported to have a "raisiny" aroma (Franco et al. 2004). Volatiles have been analyzed from such crushed juice (Franco et al. 2004), and 36 compounds were identified. Many of the identified compounds, in the partially dried grapes, are the same as those identified in raisins but (in that study) the relative concentrations of components are considerably different.

Volatiles Expected but Not Yet Identified

The three early general studies all involved the isolation of volatiles by steam distillation (or simultaneous distillation–extraction [SDE]) under reduced pressure,

which would have missed very water-soluble volatiles such as the important odorants furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and sotolon (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone) and related compounds, which are commonly found in food products containing high concentrations of sugars. Special methods, such as direct solvent extraction, are required to isolate these compounds.

A different group of compounds are easily isolated by steam distillation methods, but were little known when the previous raisin studies were carried out. One such compound, which is very likely present, is the lipid oxidative breakdown product 4,5-epoxy-(*E*)-2-decenal. Other compounds that are possibly present are norisoprenoids such as β-damascenone, a very potent odorant, easily missed in complex mixtures. The method of aroma extract dilution analysis (AEDA) (e.g., Schmid and Grosch 1986) would pinpoint these compounds rather easily but was not commonly used when the three main raisin studies were carried out. Two norisoprenoids that were identified were geranylacetone and 6-methyl-3,5-heptadien-2-one.

POSSIBLE ORIGINS OF AROMA/FLAVOR VOLATILES

As discussed earlier, most raisin volatiles are likely formed by two main processes during the dehydration. One of these processes is autoxidation of the unsaturated fatty acids. From the United States Department of Agriculture (USDA) nutrition data (Gebhardt et al. 1982), raisins from Thompson seedless grapes contain ca. 0.3 g of total fatty acids in 100 g of raisins. Linoleic acid occurs at ca. 0.1 g/100 g raisins, linolenic acid at ca. 0.03 g/100 g, and oleic acid at ca. 0.02 g/100 g.

In the case of fatty acid autoxidation with raisins, however, the effect of such long exposure to intense sunlight may involve different conditions than these processes in most other foods. The oxidation of the unsaturated fatty acids may be largely initiated by free radicals generated by the sunlight exposure. This may possibly result in somewhat different pathways of formation than with other foods.

The other main process is the Maillard reaction, which involves reaction between the sugars and amino acids. Californian raisins contain total amino acids at a concentration of ca. 2.9 g/100 g (Bolin and Petrucci 1985) of which phenylalanine occurs at a concentration of ca. 0.097 g/100 g. Volatiles formed by the Maillard reaction are well-known and have been studied extensively in regard to other foods. The Maillard reaction has generally been studied with foods that are cooked, but it is known that it can also occur with long storage at close to room temperatures. The temperature of the grapes may get well above the air temperature in field sun-drying. A study has been carried out on the nature of the nonvolatile Maillard reaction products of raisins such as Amadori compounds (Sanz et al. 2001).

One volatile compound that is unusual and does not seem to be formed by these two main processes is 2-hexyl-3-methylmaleic anhydride. It had also been found (Buttery et al. 1980) in the dried hulls of almonds, which are essentially also a dried fruit. It is interesting that a related compound (2,3-dimethylmaleic anhydride) was identified in the volatiles of "pickled prunes" (Chen et al. 1986), which are dried by salting. The origin of this raisin compound is difficult to understand. One remote possible pathway is that methylmaleic anhydride is first formed by dehydration of citric acid (this is a known reaction in the laboratory) and that this reacts with a hexyl free radical from the lipid autoxidation.

CONCLUSIONS

Only a few studies have been carried out on the volatile aroma/flavor components of raisins. Much of the work was carried out more than 25 years ago. Although 67 volatile compounds have been identified in raisins, quantitative data are very limited. It is felt also that a number of important aroma/flavor compounds have been missed. Future work should use more comprehensive methods of isolating volatiles such as the solvent-assisted flavor evaporation (SAFE) method to ensure that water-soluble volatiles are isolated efficiently as well as the more easily isolated less polar compounds. In addition, it will be important to apply modern sensory approaches using methods such as AEDA. Raisins have a distinct, much appreciated flavor and aroma and a better knowledge of their important aroma/flavor components would benefit both the raisin industry and the consumer.

REFERENCES

Aung LH, Smilanick JL, Vail PV, Hartsell PL, Gomez E. 1996. Investigations into the origin of chloroanisoles causing musty off-flavor of raisins. *J Agric Food Chem* 44:3294–6.

Bolin HR, Petrucci V. 1985. Amino acids in raisins. *J Food Sci* 50:1507.

Buttery RG, Seifert RM, Ling LC. 1981. Raisin and dried fig volatile components: Possible insect attractants. In Teranishi R, Barrera-Benitez H (eds.), *Quality of Selected Fruits and Vegetables of North America. ACS Symposium Series 170*. Washington, DC: American Chemical Society, pp. 29–41.

Buttery RG, Soderstrom EL, Seifert RM, Ling LC, Haddon WF. 1980. Components of almond hulls: Possible navel orangeworm attractants and growth inhibitors. *J Agric Food Chem* 28:353–6.

Chen C-C, Kuo M-C, Liu S-E, Wu C-M. 1986. Volatile components of salted and pickled prunes. *J Agric Food Chem* 34:140–4.

Franco M, Peinado RA, Medina M, Moreno J. 2004. Off-vine grape drying effect on volatile compounds and aromatic series in must from Pedro Ximénez grape variety. *J Agric Food Chem* 52:3905–10.

Gebhardt SE, Cutrufelli R, Matthews RH. 1982. *Composition of Foods: Fruits and Fruit Juices; Raw, Processed, Prepared. Agric. Handbook No. 8-9*. Washington, DC: US Department of Agriculture, Human Nutrition Information Service.

Joulain D, Fourniol J-P. 1990. "Craisins" versus raisins—The flavor standpoint. In Bessiere Y, Thomas AF (eds.), *Flavour Science and Technology*. Chichester, UK: John Wiley and Sons, pp. 45–8.

Meilgaard M. 1975. Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. *MBAA Tech Quart* 12:151–68.

Nagayama T, Nishijima M, Yasuda K, Saito K, Kamimura H, Ibe A, Ushiyama H, Nagayama M, Naoi Y. 1983. Natural benzoic acid in agricultural products and processed foods. *Shokuhin Eiseigaku Zasshi* 24:416–22 (CA 99, 193420n).

Nijssen LM, Visscher CA, Maarse H, Willemsens LC, Boelens MH (eds.). 1996. *Volatile Compounds in Food. Qualitative and Quantitative Data*, 7th Ed. Zeist, The Netherlands: TNO.

Palma M, Taylor LT. 2001. Supercritical fluid extraction of 5-hydroxymethyl-2-furaldehyde from raisins. *J Agric Food Chem* 49:628–32.

Peynaud E, Lafon M. 1951. Presence and significance of diacetyl, acetoin and 2,3-butandiol in alcoholic beverages. *Ann Falsif Fraud* 44:263–83 (CA 46: 2234e).

Ramshaw EH, Hardy PJ. 1969. Volatile compounds in dried grapes. *J Sci Food Agric* 20:619–21.

Sanz ML, del Castillo MD, Corzo N, Olano A. 2001. Formation of Amadori compounds in dehydrated fruits. *J Agric Food Chem* 49:5228–31.

Schmid W, Grosch W. 1986. Identification of highly aromatic compounds from cherries. *Z Lebensm Unters Forsch* 182:407–12.

Schreier P, Drawert F, Junker A. 1976. Identification of volatile constituents from grapes. *J Agric Food Chem* 24:331–6.

Tindale CR. 1987. Shipping container floors: A potential source of chloroanisole contamination in packaged dried fruit. *Chem Ind* 1987:458–9.

CHAPTER 31

Flavors of Dried Pears

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INTRODUCTION

A pear is a pomaceous fruit produced by plants from genus *Pyrus* (Maloideae, Rosaceae). It is one of the most popular and delectable fruits, while its particular aroma, determined by volatile compounds, is a key quality factor. The genus *Pyrus* consists of more than 22 primary species and cultivars and are divided in two major groups: Western (European or French pears) and Eastern cultivars (Asian, Chinese, or Japanese pears). Western cultivars combine a buttery juicy texture with rich flavor and aroma, while Eastern cultivars possess a crisp texture and sweet but subacid flavor (Rapparini and Predieri 2003).

Pear fruits are popular among consumers due to their sweetness, crispness, characteristic fragrance, and delectable aroma. According to commercial practice, most of the pear fruits are consumed in fresh state when fully mature. However, an increase in the proportion of processed pear products has been witnessed recently. Some of the most common processing techniques of pears include drying; production of juice, soft drinks, and alcoholic beverages; and preservation in syrup, jellies, jams, and purees for use in nectars and yogurts (Ashoor and Kanox 1982).

Advances in dehydration techniques and development of novel drying methods in recent years have enabled the preparation of a wide range of dehydrated products and convenient foods from fruits and vegetables meeting the quality, stability, and functional requirements coupled with economy. Dried fruit has a long shelf life and therefore can provide a good alternative to fresh fruit, allowing availability of fruit even out of season. Dried fruit is more widely available in different forms, including whole dried, cut, diced, and powdered fruit (Guiao 1964).

Demand for dried pears has increased in recent years, along with the popularity of dried fruit in general, both as a snack by itself and as an admixture in different products such as compotes, salads, sauces, trail mixes, cereals, bakery products (muffins, scones, fruitcakes), homemade ice cream, gravies, garnishments, puddings, and food for infants and children. It is interesting to mention that in the southern

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region of Brazil, iced and sweetened dried pear tea is a commonly consumed beverage (Park et al. 2002a).

Even though this is not an exhaustive list, it certainly implies that the application possibilities of dried pears, that is, dried fruit in general, are numerous indeed.

Although pear is one of the most important and most popular existing species of the fruit, and pear processed products are widely commercialized throughout the world, there is, unfortunately, a lack of comprehensive data regarding the flavor of processed, especially dried, pears.

PEAR FLAVOR COMPOSITION

Pear flavor depends on a delicate balance of sugars, acids, phenolics, and aromatic compounds (Bell et al. 1996). Caffeoylquinic acid, (+)-catechin, (–)-epicatechin, and proanthocyanidins are the most important phenolic compounds in pear, in which their content in plants can be affected by the process of germination, fruit development, ripening, storage, and processing (Ayaz et al. 1997; Hanna et al. 1991; Maga 1978). Phenolic compounds may contribute to the dark color, bitter taste, and objectionable flavor of fruit (Lee and Nagy 1990; Maga 1978; Makkar et al. 1991). Although there have been many studies on pear, most of these are limited to examination of physiology and nonvolatile chemical constituents (Chen et al. 2007).

Aroma sensed through olfactory receptors that bind volatiles emitted by food plays a dominant role in flavor delineation (Kays and Wang 2000; Tucker 1993), and in most temperate fruits, flavor is closely related to aroma.

The typical aroma of pear is determined by a complex mixture of many volatile compounds, which are produced though metabolic pathways, during ripening, harvest, postharvest, and storage and are influenced by many factors related to species, variety, and technological treatments (Rizzolo et al. 1995).

The first chemical investigation on pear volatiles was published by Harley and Fisher (1927). This and some later research on pear fruit volatile components were initially focused on "Bartlett" (syn. "Williams," "Williams Bon Chretien") (Creveling and Jennings 1970; Heinz and Jennings 1966; Heinz et al. 1964; Jennings 1961; Jennings and Creveling 1963; Jennings and Sevenants 1964; Jennings and Tressl 1974; Jennings et al. 1960, 1964; Romani and Ku 1966), a major cultivar in many pear production areas due to its particularly fine aroma. To date, more than 50 cultivars have been studied, mostly "Comice," "Packham's Triumph," "Bosc," "Doyenne du Comice," "Magness," "Kieffer," "Passe-Crassane," and "Anjou" (Jaeger et al. 2003; Komes et al. 2007; Russell et al. 1981; Suwanagul and Richardson 1998).

The three varieties "Bartlett," "Magness," and "Kieffer" have been classified respectively as highly, intermediate, and poorly flavored (Russell et al. 1981), while the ripe European pears ("Comice," "Packham," and "Bosc") have high levels of sweetness and cooked pear odor and flavor. With respect to texture, "Comice," "Packham," and "Bosc" are soft and juicy. Ripe "Bosc" is less juicy, more firm, crunchy, and grainy than the ripe "Comice" and ripe "Packham" (Jaeger et al. 2003).

During the last three decades, physicochemical methods of analysis, such as spectrophotometry, nuclear magnetic resonance, and especially gas–liquid chromatography coupled to mass spectrometry allowed the isolation and identification of more

than 300 aroma compounds from various pear cultivars at different maturity stages and postharvest conditions (Rapparini et al. 2005).

The volatile profiles of pears are characterized by volatile compounds like esters, alcohols, hydrocarbons, aldehydes, and ketones, which are usually present in extremely low concentration (parts per million, billion, or trillion) relative to the total fruit weight (Suwanagul and Richardson 1998; Takeoka et al. 1992). Pear volatiles typically comprise a wide variety of esters, and those formed from even-numbered carboxy chains such as acetic, butanoic, hexanoic, octanoic, decanoic, or dodecanoic acid, and ethyl, butyl, or hexyl alcohol are more typical than esters containing odd-numbered chains. Suwanagul (1996) reported that all odor-active compounds identified by solid phase microextraction (SPME) sampling of "Bartlett," "Doyenne du Comice," and "Anjou" pears were esters. Additionally, in various European cultivars, esters also accounted for as little as 60% to as high as 99% by dynamic headspace analysis (Rapparini and Predieri 2003; Suwanagul and Richardson 1998).

The methyl to hexyl esters of (*E*,*Z*)-2,4-decadienoic acid and hexyl acetate are character-impact compounds of "Bartlett" pear, but other esters including hexyl acetate, 2-methylpropyl acetate, butyl acetate, ethyl butanoate, butyl butanoate, pentyl acetate, and ethyl hexanoate also contribute to pear aroma (Berger 1991; Jennings and Sevenants 1964).

The aromatic ester 2-phenylethyl acetate was noticed to contribute a sweet, rose, honey-like aroma of pear. Additional compounds, ethyl octanoate and ethyl-(*E*)-2-octenoate, contribute to floral, sweet, or fruity aromas (Suwanagul 1996).

Straight-chain alcohols with two to eight carbons constitute the second largest category of pear volatile compounds. Depending on the cultivar, alcohols account for 1.5–14% of the total volatiles collected by headspace sampling of pear fruits (Suwanagul and Richardson 1998).

In the study of Riu-Aumatell and others (2005), hexanal, cinnamaldehyde, and farnesenes were additionally detected as the pear constituents.

The synthesis and emission of aroma volatiles, especially esters, was found to be greater in skin than in flesh or whole unpeeled fruit (Berger 1991; Chervin et al. 2000; Guadagni et al. 1971; Paillard 1990). It is evident that esters other than acetates are located mainly in the pear skin, as they were not detected in the crushed flesh that lacked the skin (Chervin et al. 2000).

The high aroma production in the epidermis as compared with the parenchymatic tissues corresponds with a higher content of fatty acid substrates, which are precursors of aroma volatiles, especially esters (Russell et al. 1981; Song and Bangerth 2003), and with an enhanced enzymatic activity in the peel tissue (Berger 1991; Kolesnik et al. 1989). The varieties, which contained high amounts of unsaturated fatty acids or those having enzyme systems favoring this type, are likely to produce esters with a high degree of unsaturation, and yield a large number of esters, more than those varieties that contained less unsaturated fatty acids or contained mainly saturated fatty acids (Suwanagul and Richardson 1998).

When fruit is cut, crushed, homogenized, or blended, certain enzymatic processes may be activated, some of which are extremely rapid once cellular disruption begins. This leads to the production of many volatiles, which normally occur only in trace amounts or not at all in intact cells (Heath and Reineccius 1986; Takeoka and Full 1997).

DRYING CHARACTERISTICS

Drying Methods

Fruit drying has a long tradition and is a major preservation technique for foods and food materials. Like many other evaporative concentration processes, it is often linked with reduced product quality resulting from large losses of volatile flavor components (Bruin 2000).

Pears, like other fruits, can be dried whole, in halves, or as slices, or alternatively can be chopped after drying (Park et al. 2002a; Phoungchandang and Woods 2000; Togrul and Pehlivan 2003).

Several types of dryers and drying methods are commercially used to remove moisture from a wide variety of food products, including fruit. These processes can be divided into three basic types:

- sun-drying and solar drying,
- atmospheric drying including batch (kiln, tower, and cabinet dryers) and continuous (tunnel, belt, belt trough, fluidized bed, explosion puff, foam mat, spray, drum, and microwave), and
- subatmospheric dehydration (vacuum shelf/belt/drum and freeze dryers) (Barta 2006).

Natural open-air sun-drying is widely used in tropical and semitropical countries due to its low cost, as it uses a free renewable energy source. McBean (1959) reported that Australian pears were mainly dried in the sun as halves, which were unpeeled und uncored, while Somogyi and Luh (1975) informed that sun-drying of ripe pear fruit has traditionally been done by exposing the peeled, cored, and halved fruit to the fumes of burning sulfur.

Although world pear production has increased rapidly over the last decade, the most available data about pear drying are related to Portuguese pears. In Portugal, dried pears are produced in the summer by a traditional open-air solar drying process, that involves the following steps: (1) peeling, in which the skin is removed; (2) first drying stage, in which the pears are exposed to the sun for 5–8 days; (3) barrelling, in which the pears are covered and left in the shadow to increase their elasticity; (4) pressing, in which the pears change their spheroid shape to a flattened shape; and (5) second drying stage, in which the pears are left in the sun for 2–3 more days. However, sun-drying has some unquestionable and important disadvantages, namely the high dependence on weather conditions and the large areas required for product exposure, making it too expensive to be economically attractive (Guine and Castro 2002).

Because of the well-recognized importance of preservation of this traditional product, most appreciated for its distinctive characteristics, it is evidently necessary to adapt the traditional drying process to an industrial scale, making it a profitable and competitive production method, offering consumer products of unquestionable quality. Therefore, many alternative methods are used to dry foods but the most popular being the convective drying. Guine (2006) compared solar drying of uncut pears with convective drying of pear slices at 30°C, and from the obtained results, it was possible to conclude that both tested drying methods could be considered

equivalent with respect to the pear structure developed, despite being considerably different. Furthermore, it was possible to conclude that the pears developed relatively low porosities during drying (ranging from 2% to 12%), as a consequence of the slow drying rates used and the high degree of shrinkage observed. Presently, the open-air drying method is being substituted by drying in solar stoves, taking advantage of the lack of cost of solar energy and, at the same time, allowing the production of dried fruits of better quality, since the problems of contamination and infestation are minimized.

Some efforts are also being made to develop industrial or semi-industrial dryers, where the drying time is reduced. Because of the seasonal production of many food products, the operation period of the dryer is reduced to a few weeks per year, and therefore, any improvement can only be validated in the following year (Guine et al. 2007a).

Tunnel and conveyor dryers both shorten the drying time and enable closer control of moisture content (Wrolstad et al. 1991).

Spray-drying is suitable for juice concentrates, and vacuum dehydration processes are useful for low moisture/high sugar fruits like peaches, pears, and apricots (Barta 2006).

Freeze-drying has been used for a wide variety of products and is a commonly used drying method for high-value products. In regard to the low processing temperatures applied in freeze-drying, thermal degradation reactions are excluded, and high aroma retention is attainable in a porous product with excellent rehydration properties (Beaudry et al. 2004; Coumans et al. 1994; Lovric and Pozderovic 1986; Sabarez et al. 2000).

Cohen and others (1992) reported that the combination of microwaves with freeze-drying significantly increased the drying rates of pears and enhanced the rehydration capacity of the dried product.

Modeling of Pear Drying Processes

The modeling of food drying processes is far from simple, since these products have structures that are highly affected by water removal (Saravia and Passamai 1997). For some type of dryer, the drying rate at some specific temperature depends on pretreatments (blanching), type of product (fresh or frozen) with or without skin, composition, size and geometry (sliced, diced, etc.), and drying load or feed rate. Among the various theoretical models proposed in literature for the drying of foods, the diffusion models that describe the nonsteady-state transfer according to Fick's second law are the most common (Bonazzi et al. 1997; Yang et al. 2001).

In the study of Guine and others (2007b), diffusion-based model was adopted to represent the drying behavior of pears in a continuous convective dryer, considering the variation of the properties of the pears during the drying process. From all the drying conditions tested, a selection was made taking into account only processes finished in about 5 or 6 days. This period was chosen because it represents a compromise between the time to achieve the characteristics of the traditional solar dried pears (e.g., fruit oxidation) and the economic viability of the process. Furthermore, from the cases studied and considering the specifications of the product (as close as possible to the solar-dried pears), it was concluded that the best drying conditions correspond to a drying temperature of 40°C, with the drying air

at 1.5 m/s and containing 60% relative humidity (RH). The reasons that support this choice are the following: (1) low temperatures minimize the eventual degradation of sugars and proteins; (2) high air velocities favor the oxidation and the darkening of the surface of the pears (thus compensating for the shortening of the process when compared with the solar drying); and (3) this situation is characterized by a higher moisture loss at the initial stages and a tendency for stabilization at the final stage, similar to the behavior experimentally observed.

In the case of fruits, particularly pears, the sugar concentrations are relatively high, and their increase is also significant because of the water evaporation. Thus, it is very important to study the evolution of this property along the drying process. Moreover, for higher temperatures, the changes in the water content are sharper, because the evaporation process is highly accelerated. The natural shrinking and the eventual migratory movements of the sugars from the inner to the outer parts of the pear in addition to the normal diffusion process explain the increasing radial profile of sugar concentration observed in the final periods of the process. In the study of Guine and Castro (2002), the drying rates were determined for three different temperatures (30, 40, and 50°C), and this enabled to identify the strong influence of the operating temperature over the global rate of the process. At a drying temperature of 30°C, the process is smooth and slow, whereas at temperatures above 40 or 50°C, it becomes very fast, which might give rise to problems in respect to morphological and other aspect quality measures.

Pretreatment of Pear before Drying

Many researchers have shown the benefits of different pretreatments of pear before drying. One of the most useful pretreatments before drying of fruit is osmotic dehydration (Beaudry 2001; Torreggiani et al. 2000).

Osmotic dehydration is based on the immersion of fruits in a hypertonic solution of sugar, which presents a higher osmotic pressure and a lower water activity. The water activity decrease reduces the growth of microorganisms and reduces the biochemical reactions (Adambounou et al. 1983). To achieve greater water loss associated with greater solids' gain, the process must use a solution of a high concentration at a high temperature (Park et al. 2002b). Food, such as fruits, has its weight reduced by approximately 50% of the original weight due to osmotic dehydration.

Lerici and others (1988) reported that the osmotically dehydrated products had very good texture and good retention of aroma and the color; the a_w was reduced sufficiently to improve shelf life, but further processing (e.g., freezing, drying, and pasteurization) was necessary to ensure shelf-stable products.

The main advantage of osmotic dehydration is its influence on the principal drying method, shortening of the drying process, resulting in lower energy requirements. Considering that heat is not applied in this stage, this process offers higher retention of initial food characteristics, such as color, aroma, and nutritional constituents (Beaudry 2001).

For most kinds of fruits, pretreatment processing includes blanching in order to inactivate the enzymes responsible for quality deterioration of fruits. This inactivation is normally achieved by exposing fruits to an elevated temperature (70–100°C) for a short time (1–10 min).

Blanching has generally been done by utilizing hot water or steam. This requires a large amount of energy and can cause significant loss of nutrients, phytochemicals, and/or flavors.

Recently, some researchers have tried to use microwave energy and infrared technologies as attractive alternatives to traditional techniques of fruit blanching. Microwave treatment saves energy and time, but it can also cause significant loses of nutrients and phytochemicals, as well as quality deterioration due to uneven heating inside food products (Schirack et al. 2006).

The use of infrared technology in food processing, including the separate processes of blanching and dehydration, has not been widely implemented because of problems involving lack of effective protocols as well as general reliability (Pan 2004).

Beside previously mentioned pretreatments, sulfite and its derivatives have been used for many years to inhibit nonenzymatic and enzymatic browning reactions during fruit drying and subsequent storage of dehydrated fruit (Kadam et al. 1995; Sapers 1993).

A major producer of dried fruit in South Africa stipulates sulfur dioxide levels of 800–2500 mg/kg for dried pears on receipt at their depots, where the fruit are bulk stored at 6–10°C before processing (washing and addition of further SO_2) and packaging.

Mahmutoglu and others (1996) showed that the sulfur dioxide content of unpacked dried apricots, even when stored at the relatively low temperatures of 5 and 13°C, declined considerably. Increases in temperature speed up the loss of sulfur dioxide and its degree of binding, which makes it ineffective in retarding product deterioration (Bolin and Jackson 1985).

McBean (1959) experimented with a number of predrying modifications of pears including steam peeling, steam blanching, infusion of citric acid, and flavoring with passion fruit juice and ginger essence. In order to decrease or eliminate SO_2 content of the product, dehydrated pears have been prepared by dipping in a solution of 1% ascorbic acid and 0.25% malic acid for 3 min as an alternative to bisulfite treatment.

While the color of the dehydrated product was not equal to that of the sulfured product, the flavor was judged to be superior (Wrolstad et al. 1991).

DRIED PEAR FLAVOR COMPOSITION

Drying is a major conservation technique for foods and food materials, but like many other evaporative concentration processes, it is often linked with reduced product quality resulting from large losses of volatile flavor and aroma components. The changes in color and specific flavors of dried pears occurring during the processing are associated with the presence of phenolic and volatile compounds.

Ferreira and others (2002) studied the effect of the traditional sun-drying process on the phenolic profile of pears. For the sun-dried pear, the total amount of phenolics quantified by high-performance liquid chromatography (HPLC), after thiolysis, was 8 g/kg, on a dry pulp basis. This value was 32% of the amount present in the pulp of the fresh pears. Comparing the phenolic composition of dried and fresh

pears, the results showed an overall decrease in concentration for all phenolic compounds in sun-dried fruit, except for arbutin. There was an overall decrease of 64%, on a dry pulp basis, in the total concentration of native phenolic compounds after sun-drying. The most affected compounds were the hydroxycinnamic acids and the procyanidins. The results of this study gave evidence that the traditional processing of "San Bartolomeu" pear caused a modification of its phenolic compounds that could result from oxidation and from reaction with other components such that these polyphenols became irreversibly bound.

Food aroma is a primary factor that distinguishes the flavor of one food from that of another, and aroma quality often determines the acceptability of a food.

Quamme and Marriage (1977) studied the relationship of aroma volatile compounds to canned fruit flavor among several pear cultivars, finding high correlations between aroma and flavor, indicating the importance of volatiles in canned pear flavors.

Changes in aroma can occur during concentration and drying. These changes usually decrease the quality of the product. Aroma components are often more than 100 or even 10,000 times more volatile than water and therefore are expected to be removed almost completely in a process designed to remove water by evaporation (Bruin 2000).

The retention of some original compounds after drying may be due to their lower volatility. In addition, since the flavor volatiles are generally larger than water molecules, they may not readily diffuse (Rulkens 1973) or are trapped (Flink and Karel 1970) within the fruit matrix during drying.

Basically, there are three possible solutions to the problems of maintaining the characteristic aroma of food during processing: (1) the aroma can be separated from the food before actual concentration or drying and added back later, (2) the process can be chosen and operated in a way that most of the aroma is retained in the food during water removal, and (3) the concentrated or dried product can be flavored with synthetic formulation of the original aroma (Bomben et al. 1973).

Leonard and others (1976) described the effect of the capture and adding back of pear volatiles lost during the process of pureeing with preparation in open systems. Use of a closed system resulted in a pear puree with higher flavor intensity. However, the most acceptable product was the puree with only 50% of the essence added back.

Strandjev (1982) studied the influence of sterilization temperature on the aroma compounds of "Beurre Williams" and "Passe-Crassane" compotes. Low-temperature sterilization (98°C) and short time of sterilization (20 min) of pears provided the best conditions to avoid excessive loss of aromatic volatile compounds from pear compotes. Increased temperature (>105°C) and prolonged sterilization time leads to quality decrease. Processed product contained the typical pear aroma, likely due to the presence of the high boiling point esters, which are typical of pear aroma. The methyl and ethyl esters of (*E*,*Z*)-2,4-decadienoic acid have high boiling points and are retained in preserved pears.

It is interesting to point out the study of Komes and others (2007) who investigated the influence of the addition of different sugars (sucrose and trehalose) and different dehydration processes (freeze-drying and foam-mat drying) on the retention of aroma compounds of pear (*Pyrus communis* L. var. "Packham's Triumph") purees and cubes (10% solids).

Trehalose is a nonreducing, bland, nontoxic, dietary disaccharide, which does not significantly change the flavor of food to which it is added. When sucrose and its solutions were compared with trehalose, the former demonstrated higher water diffusion coefficients, lower T_g, lower densities and higher intramolecular hydrogen bonding, and lower hydration capacity. All these characteristics play an important role in the preservation process (Colaco and Roser 1994; Komes et al. 2003, 2005). Trehalose has also been introduced commercially to the United States as a food ingredient by Cargill Health & Food Technologies and also recognized by the Food and Drug Administration as generally recognized as safe (GRAS).

In order to compare the influence of different sugars on flavor retention, pear cubes were immersed in 25°Brix sugar (trehalose, sucrose) solution at room temperature for 1 h, while the pear purees were prepared with and without previously mentioned sugars.

The amount of added sugar (trehalose or sucrose) in freeze-dried purees was 8% (wet basis), but because of the difficulties in the foaming step, sugar addition to foam-mat-dried products was lower (4%). Foam-mat drying of purees and dehydration of cubes were performed in a cabinet dryer (adapted for foam-mat drying). Dehydrated pear cubes reached approximately 7.7% of moisture content. The moisture loss was monitored by periodically weighing the tray. Purees were foam-mat dried to approximately 7.3% moisture content and were freeze-dried until they reached 2% of moisture content.

Among the samples of the dehydrated pear cubes, the best total aroma retention was obtained in the sample previously dipped in trehalose solution, followed by pear cubes dipped in sucrose solution, and the lowest retention was determined in pear cubes without previous dipping in sugar solution (Fig. 31.1).

The retention of total aroma in dehydrated pear purees was in the range from 29% in foam-mat-dried pear puree with sucrose addition to 92% in freeze-dried puree with trehalose addition (Fig. 31.2).

Nineteen compounds were identified by SPME sampling of "Packham's Triumph" pear purees and cubes (Table 31.1), which are known to have a very similar flavor to "Bartlett" pears, the most aromatic and favorite pears. Most of the identified compounds of "Packham's Triumph" pears were esters and alcohols. According to literature data (Chervin et al. 2000; Suwanagul and Richardson 1998), hexyl acetate

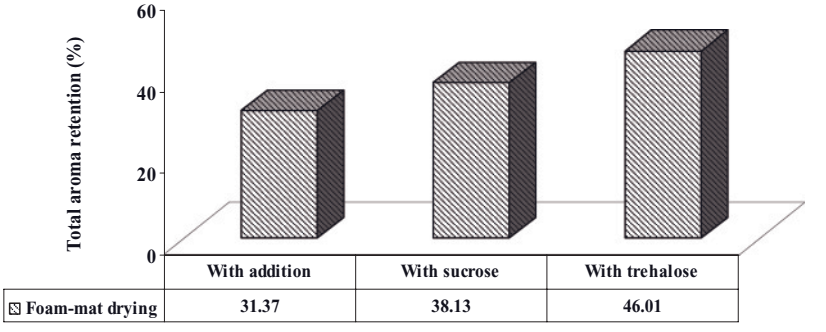
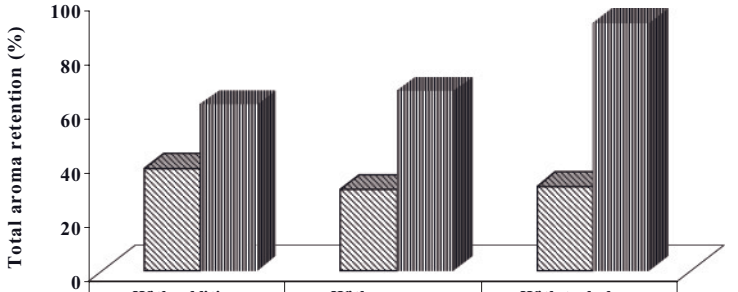


Figure 31.1. Retention of total aroma in dehydrated pear cubes (pear cubes before drying = 100%).



	With addition	With sucrose	With trehalose
Foam-mat drying	37.67	29.94	30.99
Freeze-drying	61.45	66.49	91.65

Figure 31.2. Retention of total aroma in dehydrated pear purees (pear puree before drying = 100%).

TABLE 31.1. Volatile Compounds and Their Odor Description Identified in Dried Pear Purees and Pear Cubes (Komes et al. 2007)

Volatile Compounds	Odor Description
Esters	
Methyl butanoate	Fruity
Propyl acetate	Fruity
Ethyl butanoate	Fruity
Butyl acetate	Ethereal
n-Amyl acetate	Banana or pear like
Butyl butanoate	Fruity
Ethyl hexanoate	Fruity-apple peel
Hexyl acetate	Fruity
Ethyl octanoate	Fruity
Ethyl decanoate	Waxy, fruity
2-Phenylethyl acetate	Floral-rose
Carbonyl and alcohols	
Hexanal	Green, leafy
2-Hexenal	Grassy
1-Octanol	Sharp fatty-citrus
2-Phenylethanol	Floral-rose
1-Hexanol	Grassy
Terpenes	
Linalool	Floral-spiciness
α-Terpineol	Floral-lilac like
Citronellol	Floral-rose

and butyl acetate were the two major esters, which accounted for more than 80% of the pear profile.

The results obtained by Komes and others (2007) showed that the best retention of volatile compounds in dehydrated pear puree was obtained by trehalose addition, regardless of the dehydration process applied (freeze-drying or foam-mat drying), although much higher retention of flavor volatiles was obtained in freeze-dried

purees. In dehydrated pear cubes, previously dipped in trehalose solution, the highest aroma retention was also determined. The aforementioned products are free of preservatives, maintain their natural flavor and color, and have an agreeable texture and good rehydratability properties.

According to Saravacos (1986), carbohydrates are known to lock in volatile flavors. This shows that some original flavor components could be retained within the dried solid, which are responsible for the natural aroma of the product. In order to provide a detailed explanation of the mechanism of trehalose action, three theories were suggested: water replacement hypothesis (Crowe et al. 1994), glass transformation (Ekdawi-Sever et al. 2001), and chemical stability hypotheses (Librizzi et al. 1999).

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REFERENCES

Adambounou TL, Castaigne F, Dillon JC. 1983. Abaissement de l'activite de l'eau de legumes tropicaux pear deshydration osmotique partielle. *Sci Aliments* 3:551–67.

Ashoor SH, Kanox JM. 1982. Determination of organic acids in foods by high-performance liquid chromatography. *J Chromatogr* 299(2):288–92.

Ayaz FA, Kadioglu A, Reunanen M. 1997. Changes in phenolic acid contents of *Diospyros lotus* L. during fruit development. *J Agric Food Chem* 45(7):2539–41.

Barta J. 2006. Fruit drying principles. In Hui YH (ed.), *Handbook of Fruits and Fruit Processing*. Ames, IA: Blackwell Publishing, pp. 81–94.

Beaudry C. 2001. *Evaluation of drying methods on osmotically dehydrated cranberries. Unpublished MSc thesis, Department of Agricultural and Biosystems Engineering*, McGill University, Montreal.

Beaudry C, Raghavan GS, Ratti C, Rennie TJ. 2004. Effect of four drying methods on the quality of osmotically dehydrated cranberries. *Drying Technol* 22(3):521–39.

Bell RL, Quamme HA, Layne REC, Skirvin RM. 1996. Pears. In Janick J, Moore JN (eds.), *Fruit Breeding*. New York: Wiley, pp. 441–514.

Berger RG. 1991. Fruits I. In Maarse H (ed.), *Volatile Compounds in Foods and Beverages*. New York: Marcel Dekker, pp. 287–304.

Bolin HR, Jackson R. 1985. Factors affecting sulphur dioxide binding in dried apples and apricots. *J Food Process Preserv* 9:25–34.

Bomben JL, Briun S, Thijssen HAC, Merson RL. 1973. Aroma recovery and retention in concentration and drying foods. In Chichester CO (ed.), *Advances in Food Research*. London: Academic Press, pp. 1–105.

Bonazzi C, Ripoche A, Michon C. 1997. Moisture diffusion in gelatin slabs by modelling drying kinetics. *Drying Technol* 15(6-8):2045–59.

Bruin S. 2000. Flavor retention in dehydration processes. In Singh RP, Wirakartakusumah E (eds.), *Advances in Food Engineering*. Boca Raton, FL: CRC Press, pp. 15–32.

Chen J, Wang Z, Wu J, Wang Q, Hu X. 2007. Chemical compositional characterization of eight pear cultivars grown in China. *Food Chem* 104(1):268–75.

Chervin C, Speirs J, Loveys B, Patterson BD. 2000. Influence of low oxygen storage on aroma compounds of whole pears and crushed pear flesh. *Postharvest Biol Technol* 19(3): 279–85.

Cohen JS, Ayoub JA, Yang TC. 1992. A comparison of conventional and microwave augmented freeze-drying of peas. In *Mujumdar AS* (ed.), *Drying*. San Diego, CA: Elsevier Science Publishers, pp. 585–94.

Colaco CAL, Roser B. 1994. Trehalose—A multifunctional additive for food preservation. In Heis R (ed.), *Food Packaging and Preservation*. London: Blackie Academic and Professional Press, pp. 123–40.

Coumans WJ, Kerkhof PJA, Bruin S. 1994. Theoretical and practical aspects of aroma retention in spray drying and freeze drying. *Drying Technol* 12(1–2):99–149.

Creveling RK, Jennings WG. 1970. Volatile components of Bartlett pear: Higher boiling esters. *J Agric Food Chem* 18(1):19–24.

Crowe JH, Leslie SB, Crowe LM. 1994. Is vitrification sufficient to preserve liposomes during freeze drying? *Cryobiology* 31:355–66.

Ekdawi-Sever N, Conrad PB, de Pablo JJ. 2001. Molecular simulations of sucrose solutions near the glass transition temperature. *J Phys Chem* 105:734–42.

Ferreira D, Guyot S, Marnet N, Delgadillo I, Renard CMGC, Coimbra MA. 2002. Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. S. Bartolomeu) and changes after sun-drying. *J Agric Food Chem* 50(16):4537–44.

Flink J, Karel M. 1970. Retention of organic volatiles in freeze-dried solutions of carbohydrates. *J Agric Food Chem* 18(2):295–9.

Guadagni DG, Bomben JL, Harris JG. 1971. Recovery and evaluation of aroma development in apple peels. *J Sci Food Agric* 22(3):115–9.

Guiao EP. 1964. *A cultura da Pereira*, 2nd Ed. Sao Paulo, SP, Brazil: ABC do Lavrador Pratico.

Guine RPF. 2006. Influence of drying method on density and porosity of pears. *Food Bioprod Process* 84(3):179–85.

Guine RPF, Castro JAAM. 2002. Pear drying process analysis: Drying rates and evolution of water and sugar concentrations in space and time. *Drying Technol* 20(7):1515–26.

Guine RPF, Ferreira DMS, Barroca MJ, Goncalves FM. 2007a. Study of the drying kinetics of solar-dried pears. *Biosyst Eng* 98(4):422–9.

Guine RPF, Rodrigues AE, Figueiredo MM. 2007b. Modelling and simulation of pear drying. *Appl Math Comput* 192(1):69–7.

Hanna P, Naim M, Rousel RL, Zehavi U. 1991. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J Sci Food Agric* 57(3):417–26.

Harley CP, Fisher DF. 1927. The occurrence of acetaldehyde in "Bartlett" pears and its relation to pear scald and breakdown. *J Agric Res* 35:983–93.

Heath HB, Reineccius G. 1986. Flavor potentiators. In Heath HB, Reineccius G (eds.), *Flavor Chemistry and Technology*. Westport, CT: AVI Pub, pp. 318–31.

Heinz DE, Jennings WG. 1966. Volatile components of Bartlett pear. *J Food Sci* 31(1):69–80.

Heinz DE, Pangborn RM, Jennings WG. 1964. Pear aroma: Relation of instrumental and sensory techniques. *J Food Sci* 29(6):756–67.

Jaeger SR, Lund CM, Lau K, Harker ER. 2003. In Search of the "ideal" pear (*Pyrus* spp.): Results of a multidisciplinary exploration. *J Food Sci* 68(3):1108–17.

Jennings WG. 1961. Volatile esters of Bartlett pears. *J Food Sci* 26:564–8.

Jennings WG, Creveling RK. 1963. Volatile esters of Bartlett pear II. *J Food Sci* 28:91–4.

Jennings WG, Creveling RK, Heinz DE. 1964. Volatile esters of Bartlett pear IV. trans:2-cis:4-decadienoic acid. *J Food Sci* 29(6):736–44.

Jennings WG, Leonard S, Pangborn RM. 1960. Volatiles contributing to the flavor of Bartlett pears. *Food Technol* 14:587–94.

Jennings WG, Sevenants MR. 1964. Volatile esters of Bartlett pear III. *J Food Sci* 29(2):158–63.

Jennings WG, Tressl R. 1974. Production of volatile compounds in the ripening Bartlett pear. *Chem Mikrobiol Technol Lebensm* 3:52–5.

Kadam PY, Dhumal SA, Shinde NN. 1995. Pears. In Salunkhe DK, Kadam SS (eds.), *Handbook of Fruit Science and Technology: Production, Composition, Storage and Processing*. New York: Marcel Dekker, pp. 183–203.

Kays SJ, Wang Y. 2000. Thermally induced flavor compounds. *HortScience* 35:1002–12.

Kolesnik AA, Golubev VN, Farzaliev EB. 1989. Lipids of the fruit of *Pyrus communis*. *Chem Nat Compounds* 25(2):148–52.

Komes D, Lovric T, Kovacevic Ganic K. 2007. Aroma of dehydrated pear products. *LWT-Food Sci Technol* 40(9):1578–86.

Komes D, Lovric T, Kovacevic Ganic K, Gajdos Kljusuric J, Banovic M. 2005. Trehalose improves flavor retention in dehydrated apricot puree. *Int J Food Sci Technol* 40(4):425–35.

Komes D, Lovric T, Kovacevic Ganic K, Gracin L. 2003. Study of trehalose addition on aroma retention in dehydrated strawberry puree. *Food Technol Biotechnol* 41(2):111–9.

Lee HS, Nagy S. 1990. Formation of 4-vinyl guaiacol in adversely stored orange juice as measured by an improved HPLC method. *J Food Sci* 55:162–73.

Leonard S, Pangborn RM, Tombropoulos D. 1976. Influence of processing variables on flavor stability of pear puree. *J Food Sci* 41:840–4.

Lerici CR, Mastrocola D, Nicoli MC. 1988. Use of direct osmosis as fruit and vegetables dehydration. *Acta Aliment Pol* 14(1):35–40.

Librizzi F, Vitrano E, Cordone L. 1999. Dehydration and crystalilization of trehalose and sucrose glasses containing carbonmonoxy-myoglobin. *Biophys J* 76:2727–34.

Lovric T, Pozderovic A. 1986. Study on retention phenomena of fruit flavor components during freeze-drying on model system. *Food Technol Biotechnol* 22:107–15.

Maga J. 1978. Simple phenol and phenolic compounds in food flavor. *Crit Rev Food Sci Nutr* 10(4):323–72.

Mahmutoglu T, Birol Saygi Y, Borcakli M, Ozay G. 1996. Effects of pretreatment-drying method combinations on the drying rates, quality and storage stability of apricots. *LWT-Food Sci Technol* 29(5–6):418–24.

Makkar HPS, Dawra RK, Singh B. 1991. Tannin levels in leaves of some oak species at different stages of maturity. *J Sci Food Agric* 54:513–7.

McBean DMG. 1959. Improved dried pears. *CSIRO Food Preserv Q* 29(3):4–8.

Paillard NMM. 1990. The flavor of apples, pears and quinces. In Morton ID, Macleod AJ (eds.), *Food Flavors, Part C. The Flavor of Fruits*. Amsterdam: Elsevier, pp. 23–4.

Pan Z. 2004. Applications of infrared technology for processing foods and agricultural products. Proceedings of 2004 CIGR International Conference, October 11–14, Beijing, China.

Park KJ, Bin A, Reis Brod FP, Brandini Park THK. 2002a. Osmotic dehydration kinetics of pear D'anjou (*Pyrus communis* L.). *J Food Eng* 52(3):293–308.

Park KJ, Bin A, Reis Brod FP. 2002b. Drying of pear d'Anjou with and without osmotic dehydration. *J Food Eng* 56(1):97–103.

Phoungchandang S, Woods JL. 2000. Solar drying of bananas: Mathematical model, laboratory simulation and field data compared. *J Food Sci* 65(6):990–6.

Quamme HA, Marriage BH. 1977. Relationship of aroma compounds to canned fruit flavor among several pear cultivars. *Acta Hortic* 69:301–6.

Rapparini F, Gatti E, Predieri S. 2005. Volatile constituents and pear aroma studied by dynamic headspace technique. *Acta Hortic* 671:393–6.

Rapparini F, Predieri S. 2003. Pear fruit volatiles. In Junick J (ed.), *Horticultural Reviews*, Vol. 28. San Francisco, CA: John Wiley and Sons, pp. 237–324.

Riu-Aumatell M, Lopez-Tamames E, Buxaderas S. 2005. Assessment of the volatile composition of juices of apricot, peach, and pear according to two pectolytic treatments. *J Agric Food Chem* 53(20):7837–43.

Rizzolo A, Lombardi P, Vanoli M, Polesello S. 1995. Use of capillary gas chromatography/sensory analysis as an additional tool for sampling technique comparison in peach aroma analysis. *J High Resolut Chromatogr* 18(5):309–14.

Romani RJ, Ku L. 1966. Direct gas chromatographic analysis of volatiles produced by ripening pears. *J Food Sci* 31(4):558–60.

Rulkens WH. 1973. Retention of volatile trace components in drying aqueous carbohydrate solutions. Dissertation, Technical University, Eindhoven, The Netherlands.

Russell LF, Quamme HA, Gray JI. 1981. Qualitative aspects of pear flavor. *J Food Sci* 46(4):1152–8.

Sabarez HT, Price WE, Korth J. 2000. Volatile changes during dehydration of d'Agen prunes. *J Agric Food Chem* 48(5):1838–42.

Sapers GM. 1993. Browning of foods: Control by sulfites, antioxidants and other means. *Food Technol* 47(10):75–84.

Saravacos GD. 1986. Mass transfer properties of foods. In Rao MA, Risvi SSH (eds.), *Engineering Properties of Foods*. New York: Marcel Dekker, pp. 89–132.

Saravia L, Passamai V. 1997. Relationship between a solar drying model of red pepper and the kinetics of pure water evaporation (I). *Drying Technol* 15(5):1419–32.

Schirack A, Andriana V, Drake M, Sanders TH, Sandeep KP. 2006. Impact of microwave blanching on the flavor of roasted peanuts. *J Sens Stud* 13:428–40.

Somogyi LP, Luh BS. 1975. Dehydration of fruits. In Woodroof JG, Luh BS (eds.), *Commercial Fruit Processing*. Westport, CT: AVI Publishing, pp. 374–98.

Song J, Bangerth F. 2003. Fatty acids as precursors for aroma volatile biosynthesis in preclimacteric and climacteric apple fruit. *Postharvest Biol Technol* 30(2):113–21.

Strandjev A. 1982. Influence du temps et de la temperature de sterilisation sur les substances aromatiques des poires. *C R Acad Bulg Sci* 35(8):1129–32.

Suwanagul A. 1996. Ripening pear flavor volatiles: Identification, biosynthesis and sensory perception. Dissertation, Oregon State University, Corvallis.

Suwanagul A, Richardson DG. 1998. Identification of headspace volatile compounds from different pear (*Pyrus communis* L.). *Acta Hortic* 475:605–23.

Takeoka GR, Buttery RG, Flath RA. 1992. Volatile constituents of Asian pear (*Pyrus serotina*). *J Agric Food Chem* 40(10):1925–9.

Takeoka GR, Full GH. 1997. Analysis of volatile constituents of fruits. In Linskens HF, Jackson JF (eds.), *Modern Methods of Plant Analysis, Vol. 19. Plant Volatile Analysis*. Berlin, Germany: Springer-Verlag, pp. 23–45.

Togrul IT, Pehlivan D. 2003. Modeling of drying kinetics of single apricot. *J Food Eng* 58(1):23–32.

Torreggiani D, Lucas T, Raoult-Wack AL. 2000. The pre-treatments of fruits and vegetables. In Kennedy CJ (ed.), *Managing Frozen Foods*. Cambridge, UK: CRC Press Woodhead Publishing, pp. 57–80.

Tucker GA. 1993. Introduction. In Seymour GB, Taylor JE, Tucker GA (eds.), *Biochemistry of Fruit Ripening*. London: Chapman & Hall, pp. 1–51.

Wrolstad RE, Lombard PB, Richardson DG. 1991. The pear. In Eskin NA (ed.), *Quality and Preservation of Fruits*. Boca Raton, FL: CRC Press, pp. 67–95.

Yang H, Sakai N, Watanabe M. 2001. Drying model with non-isotropic shrinkage deformation undergoing simultaneous heat and mass transfer. *Drying Technol* 19(7):1441–60.

SECTION B: VEGETABLE FLAVORS

PART VI

VEGETABLE FLAVORS: BIOLOGY, CHEMISTRY, PHYSIOCHEMISTRY, AND BIOTECHNOLOGY

CHAPTER 32

Chemistry and Biochemistry of Some Vegetable Flavors

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CAROTENOID CHEMISTRY, BIOCHEMISTRY, BIOAVAILABILITY, AND THEIR GENETIC MANIPULATION

Introduction

The word carotenoid is obtained from its availability as a major pigment in the carrot root, *Daucus carota*. Carotenoids are C 40 isoprenoid polyene compounds that include lipid-soluble yellow, orange, and red pigments (Sandmann 2001; Zaripheh and Erdman 2002). An attempt has been made to review and discuss about the current aspects of carotenoid chemistry, biochemistry, and health benefits. Plants are most important sources of carotenoids for humans, where often the brilliant colors of carotenoids are masked by the green chlorophyllic pigments, that is, in green vegetables and in leaves. Carotenoids are secondary plant metabolites, which are responsible for the color of many plant products. These are lipid-soluble pigments found in many vegetables and have been classified into two categories on the basis of their structures: xanthophylls such as lutein, zeaxanthin, and violaxanthin, and hydrocarbon carotenes such as β-carotene, α-carotene, and lycopene (Zaripheh and Erdman 2002). Most xanthophylls are present in green leafy vegetables, and nearly all carotenes are found in yellow vegetables. The most important carotenoids are α-carotene, β-carotene, and β-cryptoxanthin, lutein, violaxanthin, neoxanthin, and lycopene. Carotenoid carbon positions and structures of zeaxanthin, antheraxanthin, violaxanthin, lutein, and 5,6-epoxylutein are demonstrated in Figure 32.1.

Terpenes are a diverse group of plant secondary metabolites formed via acetyl-CoA (mevalonic acid pathway) or from glycolytic intermediates (methyl erythritol phosphate pathway). The basic structural element of all terpenes is the 5-carbon branched isopentane. Terpenes are classified on the basis of the number of isoprene units present. Carotenoids are composed of 40 carbons and are thus called as tetraterpenes. α-Carotene, β-carotene, and β-cryptoxanthin are carotenes that are

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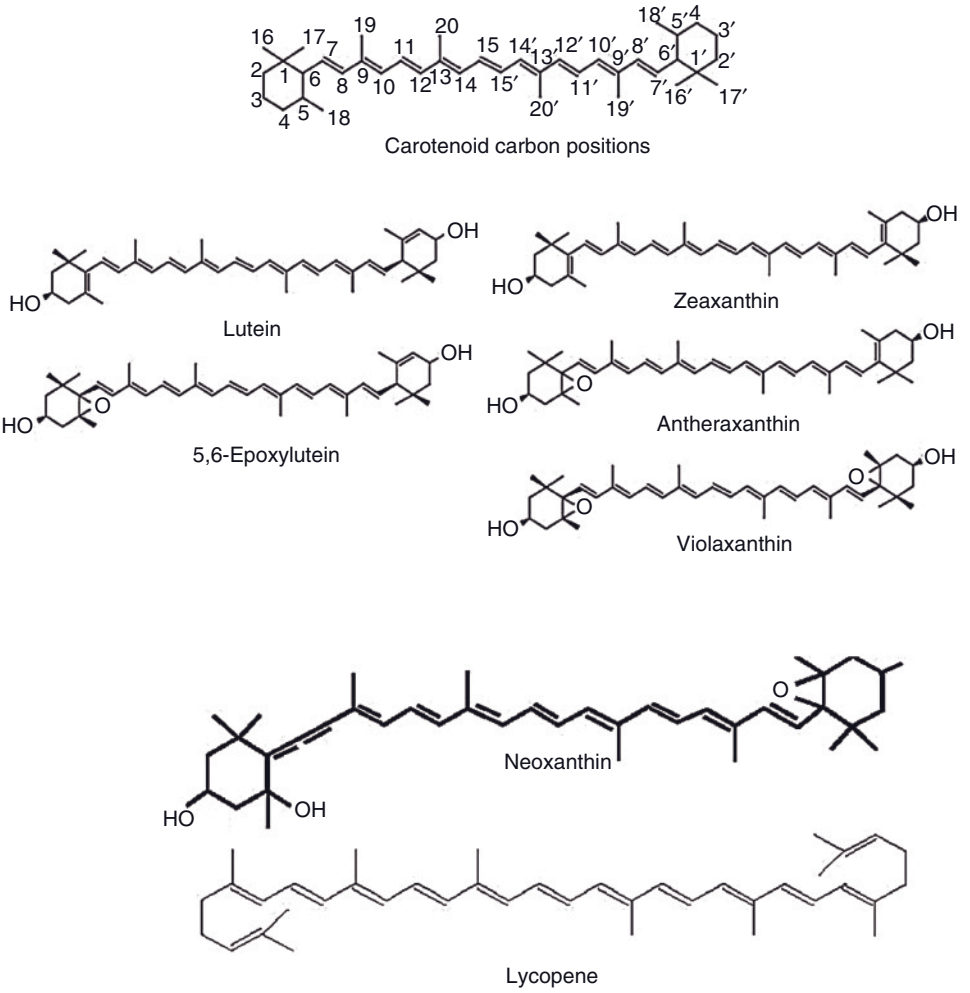

Figure 32.1. Structure of selected carotenoids.

converted into vitamin A or retinol in the body. β-Carotenes are the most widely studied carotenoids. Lutein and zeaxanthin are both stored in the retina of the eye, but they cannot be converted into vitamin A. Both are powerful antioxidants and may be very important for healthy eyes. Lutein is also present in green vegetables, such as broccoli, cabbage, and kale. Lycopene is responsible for the red color in vegetables, including tomatoes and carrots. Therefore, the present study is aimed to collect recent information on the important carotenoid contents from various sources.

Carotenoids are present in a significant amount in a large number of vegetables such as carrot, corn, kale, lettuce, potato, soybean, and tomato. There are more than 600 carotenoids found in nature and around 40 carotenoids regularly consumed in the human diet (Bendich 1993; Shi and Le Maguer 2000). Provitamin A activity is the classical biological function of β-carotene, α-carotene, and cryptoxanthins in mammalian systems. Health benefits attributed to carotenoids include prevention

of certain cancers (Finley 2005; Seifried et al. 2003; Tang et al. 2005), cardiovascular diseases (Granado et al. 2003), eye diseases (Johnson et al. 2000; Sommerburg et al. 1998), and function in the enhancement of the immune system (Garcia et al. 2003; Hughes 1999; Shi and Le Maguer 2000).

Carotenoid Biosynthetic Pathway

The pathway for the biosynthesis of carotenoids was discovered in the mid-1960s as given in Figure 32.2 (Fraser and Bramley 2004). Genes and cDNAs for the major enzymes functioning in carotenoid biosynthesis have been cloned from various plant sources (Cunningham and Gantt 1998; Kopsell and Kopsell 2006). Carotenoids are synthesized in the plastids and are derived from isopentenyl diphosphate (IPP). The first step in the biosynthesis of carotenoid is the isomerization of IPP to dimethylallyl diphosphate (DMAPP). DMAPP is used as the substrate for the C 20 geranylgeranyl diphosphate (GGPP) biosynthesis (Bramley 2002). The formation of GGPP from IPP and DMAPP is catalyzed by GGPP synthase (Cunningham and Gantt 1998). However, the first step in the carotenoid synthesis is unique because phytoene synthase catalyzes the condensation of two molecules of GGPP to form the first C 40 carotenoid, phytoene (Gross 1991). Further, the conversion of phytoene to lycopene is catalyzed by phytoene desaturase and j-carotene desaturase (Bramley 2002; Cunningham and Gantt 1998; DellaPenna 1999). These desaturases produce chromophores, which are found in the carotenoid pigments, and change the less colored phytoene into a colored lycopene. The carotenoid pathway branches at the cyclization reactions of lycopene to produce carotenoids with either two b-rings, for example, β-carotene, zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin or carotenoids with one b-ring and one e-ring, for example, α-carotene and lutein (Cunningham 2002; Cunningham and Gantt 1998; Kopsell and Kopsell 2006). The pathway advances with the additions of oxygen moieties, which convert the hydrocarbon α- and β-carotenes into the subgroup referred to as the xanthophylls. Further steps in xanthophyll synthesis include epoxidation reactions (Fig. 32.2). The reversible epoxidation–de-epoxidation reaction converts violaxanthin back to zeaxanthin via the intermediate antheraxanthins collectively called the violaxanthin cycle and is important for energy dissipation from incoming solar radiation (Bramley 2002; Fraser and Bramley 2004). Some vegetables have a modified carotenoid biosynthetic capacity and produce compounds exclusively associated with the irrespective genus, or even species. Peppers (*Capsicum* species) contain capsanthin, capsorubin, and cryptocapsin, which are responsible for the vibrant fruit coloration within the genus (Gross 1991). Lettuce (*Lactuca* species) contains lactuca xanthin, along with other chloroplast carotenoids. Zeinoxanthin is found in yellow corn (*Zea mays*) grains.

Xanthophylls might also be involved in the structural stabilization of light-harvesting complexes (LHCs) and in the reduction of lipid peroxidation (Frank and Cogdell 1996). Within the thylakoid membranes of chloroplast organelles, carotenoids are attached to some specific protein complexes of photosystem I and photosystem II (PSII). Carotenoids help harvest to light energy mostly in the blue-green wavelength range, which is transferred to the photosynthetic reaction centers (RCs). In the PSII complex, β-carotene is highly concentrated close to the RC, and lutein is present in several light-harvesting antennae components (Niyogi et al. 1997).

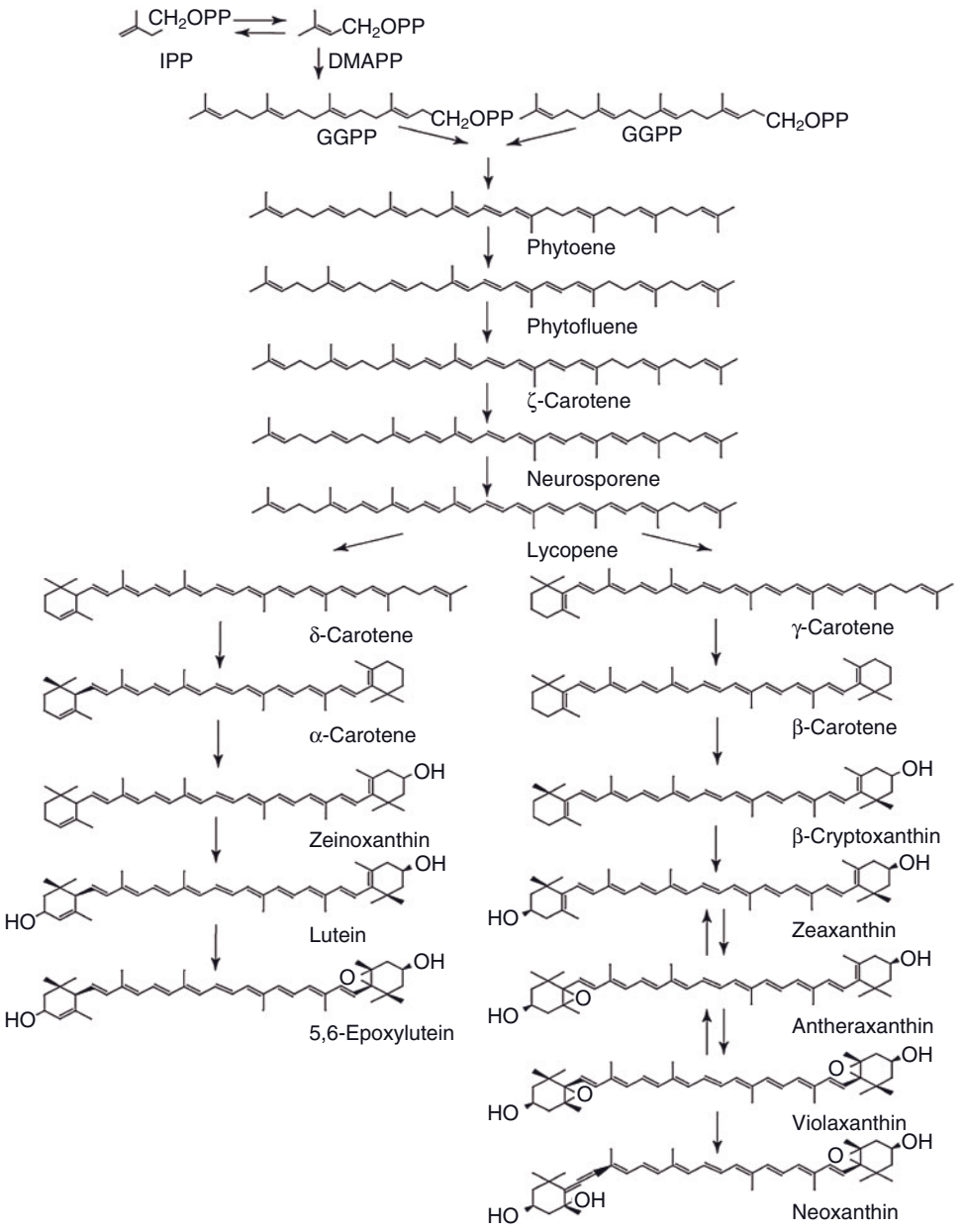

Figure 32.2. Precursor compounds and major carotenes and xanthophylls in the carotenoid biosynthetic pathway in plants (Kopsell and Kopsell 2006).

Carotenoids and Human Health

More recently, the protective effects of carotenoids against serious disorders such as cancer, heart disease, and degenerative eye disease have been recognized and have stimulated intensive research with regard to the role of carotenoids as

antioxidants and as regulators of the immune response system. Carotenoids are an important factor in human health and are essential for vision (Zeb and Mehmood 2004). One of the most important physiological functions of carotenoids in human nutrition is to act as vitamin A precursors. The role of β-carotene and other carotenoids as the main dietary source of vitamin A has been known for a long time. Provitamin A carotenoid compounds are highly useful for the maintenance of healthy epithelial cell differentiation, normal reproductive performance, and visual functions (Combs 1998). Both provitamin A (β-carotene, α-carotene, and cryptoxanthins) and non-provitamin A (lutein, zeaxanthin, and lycopene) carotenoids act as free radical scavengers. They enhance the immune response, suppress cancer development, and protect against several degenerative diseases (Yeum and Russell 2002; Zeb and Mehmood 2004). Human beings cannot synthesize carotenoids and thus depend on dietary sources to provide with the required amount of carotenoids. Vegetables and fruits are primary sources of carotenoids in the human diet, and their consumption has been associated with numerous health benefits (Grusak and DellaPenna 1999; Mortensen et al. 2001).

Lycopene, a carotenoid found in tomato products, prevents oxidation of low-density lipoprotein (LDL) cholesterol and minimizes the problems of developing atherosclerosis and coronary heart disease (Rao and Ali 2007). It has been demonstrated that daily intake of tomato products providing at least 40 mg of lycopene is enough for substantially reducing LDL oxidation. High LDL oxidation is associated with increased risk of atherosclerosis and coronary heart disease. However, such lycopene level can be obtained by drinking just two glasses of tomato juice per day. Further findings suggested that lycopene in tomatoes can be absorbed more efficiently by the body if processed into tomato juice, sauce, paste, and ketchup (Shi and Le Maguer 2000; Zeb and Mehmood 2004)). The bound chemical form of lycopene present in tomatoes is converted by temperature change involved in processing to make it more easily absorbed by the human body and has been suggested to overcome the problems of prostate cancer and cancers of the lung, bladder, cervix, and skin (Kopsell and Kopsell 2006).

It has been reported that carotenoids can minimize the risk of cardiovascular disease through reductions in LDL oxidation and oxidative stress at locations of plaque formations. Recent studies have developed a link between increased intake of fruits and vegetables that are rich in carotenoid contents with reduced incidences of mortality related to cardiovascular disease (Buijsse et al. 2005; Ito et al. 2006; Kabagambe et al. 2005; Tavani et al. 2006). Cardiovascular disease reductions are mostly associated with increased intake of α-carotene, β-carotene, and β-cryptoxanthin.

Carotenoids can inhibit cell proliferations and cell transformations and can modulate the expression of gene determinants in the prevention of certain types of cancers. A great deal of research has been done on the anticarcinogenic effects of individual carotenoids on specific types of cancers. Touvier and others (2005) reported protective effects from β-carotene intakes against the risk of tobacco-related cancers in nonsmokers; however, β-carotene can increase cancer risks among smokers. The protective effects of carotenoids against lung cancers differ between the sexes; men benefit more from increased carotenoid intakes as compared with females (Ito et al. 2005). Higher carotenoid intakes can also lower the risk of certain lymphomas (Kelemen et al. 2006). Holick and others (2005) revealed that

increased levels of dietary carotenoids have no preventative effects on incidences of bladder cancers.

Epidemiological evidence has been inconsistent regarding the potential relationships between diet, genetics and environment, and the development of age-related macular degeneration. A direct correlation between macular pigment (MP) levels and development of macular disease has not been confirmed (Landrum and Bone 2001; Mares-Perlman and Klein 1999), although strong associative relationships are reported. The investigations show that a high intake of a variety of vegetables, providing a mixture of carotenoids, is more strongly related to the decreased level of eye disease risk than intake of individual carotenoid supplements (Johnson et al. 2000; Zeb and Mehmood 2004).

Several investigators have found that in the human body, various oxidants produced during normal metabolism and immune defense against infectious and chemical agents are responsible for damage to DNA, proteins and cellular tissues (Ames et al. 1993; Mortensen et al. 2001). This harmful oxidative damage is considered the major cause of aging and degenerative diseases such as cancer, cardiovascular disease, immune system decline, and cataracts. Ames and others (1993) reported that compounds such as ascorbate, α-tocopherol, and carotenoids are examples of antioxidants that have the ability to quench reactive oxygen species. The physical properties of carotenoid molecules, particularly the conjugated carbon–carbon double-bond system, permit the quenching of $^1O_2$. *In vivo* antioxidant activity is determined by carotenoid structure and concentration, as well as by the nature and concentration of the reactive oxygen species. The localization of carotenoid molecules in biological tissues also influences their ability to encounter and scavenge free radicals. Carotenoids have been proven as the most potential biological quenchers of singlet oxygen (DiMascio et al. 1989).

Mortensen and others (2001) have described the antioxidant activity of cyclic carotenoids which comes from the susceptibility of 5, 6 and 50, 60 double bonds in their cyclic end groups to undergo epoxidation with $^1O_2$. Acyclic carotenoids, such as lycopene, derive potent antioxidant activity from their high number of conjugated dienes (Young and Lowe 2001). Epoxide forms of carotenoids, with an oxygen bound to the 5, 6 or 50, 60 positions, are present in vegetable tissues. The ratios of lutein epoxide : all-*trans*-lutein is 1:2 in broccoli (*Brassica oleracea* var. botrytis), 1:6 in spinach (*Spinacia oleracea*), 1.0:1.5 in cabbage (*B. oleracea* var. capitata), and 1:23 in kale (*B. oleracea* var. acephala) (Khachik et al. 1986). However, the low doses of lycopene from tomato (*Lycopersicon esculentum*) products remarkably increased serum lycopene levels and reduced lipid peroxidation *in vivo* (Rao and Shen 2002). The complexity of measuring *in vivo* antioxidant behavior, the variability associated with the carotenoid content of vegetables, and the nutritional status of subjects used in human dietary intervention studies all affect the interpretation of the results (Faulks and Southon 2005; Young and Lowe 2001). Based on their chemistry, epoxide isomers are predicted to lack antioxidant activity because they are unable to bind O_2, and some might even have pro-oxidant activity.

Table 32.1 summarizes all carotenoids present in vegetables and those exhibiting cis–trans isomerization. The structures of all isomers of carotenoids are mentioned in Figure 32.3. However, phytoene exists predominately as the 15-*cis* isomer, the predominant isomer of lycopene among the all-*trans* geometric form. All-*trans* carotenoids in plants are susceptible to photo, thermal, and chemical isomerization,

TABLE 32.1. Carotenoids Identified and Quantified in Major Vegetables (Kopsell and Kopsell 2006)

Commodity	Carotenoids Identified[a]
Beans, green	All-*trans* β-carotene, **all-*trans* lutein**, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* lutein epoxide, 9′-*cis* neoxanthin, neolutein, all-*trans* violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, and 13-*cis* zeaxanthin
Broccoli	All-*trans* β-carotene, **all-*trans* lutein**, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, and 13-*cis* zeaxanthin
Carrot	All-*trans* α-carotene, **all-*trans* β-carotene**, lutein, and lycopene
Corn	α-Carotene, β-carotene, β-cryptoxanthin, all-*trans* lutein, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, **all-*trans* zeaxanthin**, and 9-*cis* zeaxanthin
Kale/collards	All-*trans* β-carotene, **all-*trans* lutein**, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, and 13-*cis* zeaxanthin
Lettuce	**All-*trans* β-carotene, lactucaxanthin, all-*trans* lutein**, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, and 13-*cis* zeaxanthin
Pepper (bell)	**α-Carotene**, β-carotene, β-cryptoxanthin, capsanthin, lutein, and zeaxanthin
Spinach	All-*trans* β-carotene, **all-*trans* lutein**, 9-*cis* lutein, 9′-*cis* lutein, 13-*cis* lutein, all-*trans* and *cis* lutein epoxide, neolutein, all-*trans* neoxanthin, 9′-*cis* neoxanthin, violaxanthin, all-*trans* zeaxanthin, 9-*cis* zeaxanthin, and 13-*cis* zeaxanthin
Tomato (raw)	All-*trans* β-carotene, all-*trans* γ-carotene, all-*trans* δ-carotene, ξ-carotene, all-*trans* lutein, **all-*trans* lycopene**, neurosporene, phytoene, phytofluene, and lycopene-5,6 diol

[a]Carotenoids in bold text identify those reported in highest concentrations.

and cis–trans isomers differ in their intestinal absorption in humans (Koyama and Fujii 1999). Human blood plasma contains mostly all-*trans* carotenoids, but levels of the cis form of some carotenoids are as high as 50% (Khachik et al. 2002). It has already been described that the majority of lycopene found in fresh and processed tomatoes exists in the all-*trans* form (Clinton et al. 1996; Gartner et al. 1997; Humphries and Khachik 2003). However, lycopene in human and animal tissues exits predominately as cis-isomers, indicating a preference for *cis*-lycopene in intestinal absorption (Boileau et al. 2002; Wu et al. 2003). By contrast, greater excretions of *cis*-β-carotene and lower excretions of *trans*-β-carotene were measured in human subjects after ingestion of both raw and processed carrots (*D. carota* var. sativa), indicating an absorption preference for all-*trans* β-carotene (Emenhiser et al. 1996; Livny et al. 2003; Updike and Schwartz 2003).

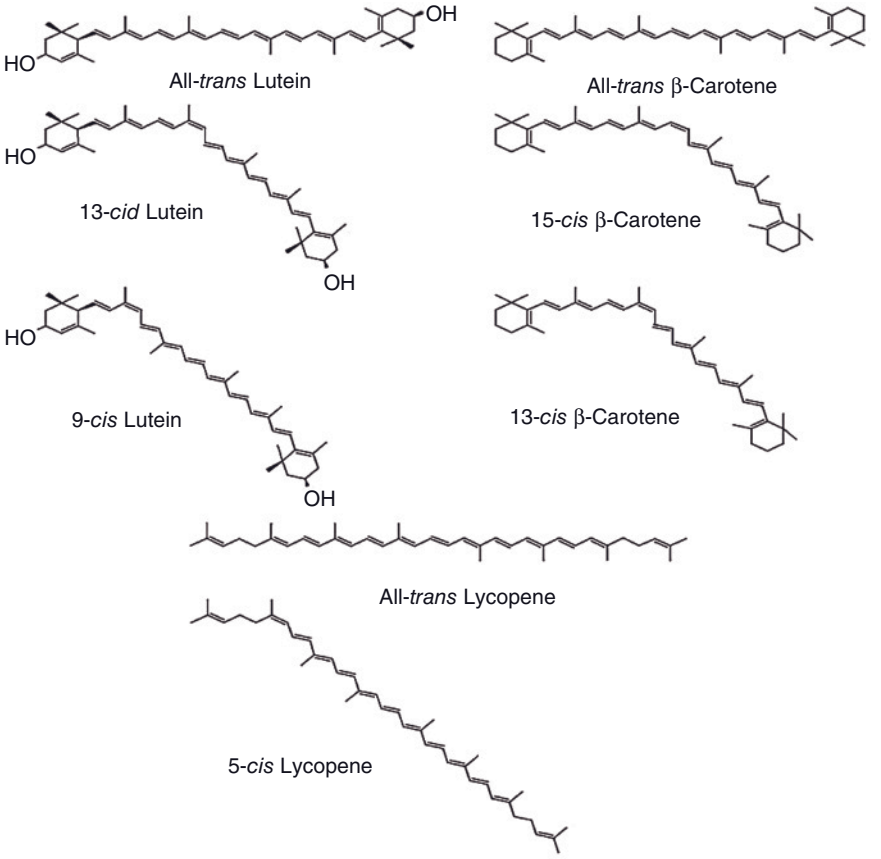

Figure 32.3. Structures of some common all-*trans* and *cis*-carotenoid isomers found in vegetables (Kopsell and Kopsell 2006).

Factors Responsible for Carotenoid Accumulation and Bioavailability

Recent methods to evaluate bioavailability include serum measurements and *in vitro* digestion models. Release of carotenoid from the membranes of plant tissues facilitates intestinal absorption; however, changes in carotenoid chemistry by various factors can influence bioavailability. Programs to improve carotenoid levels in vegetable crops must successfully link plant physiology with accurate bioavailability assessments in human subjects. Carotenoid accumulation in plant tissue appears to be shaped by the physiological, genetic, and biochemical attributes of a plant species, as well as by environmental growth factors such as light, temperature, and fertility (Goldman et al. 1999; Kopsell et al. 2003, 2004; Kurilich et al. 1999). Significant differences in carotenoid accumulation among different vegetable crop species have been reported (Kimura and Rodriguez-Amaya 2003), and significant genetic variation within species has been found for carrot (Nicolle et al. 2004), corn (Kurlich and Juvik 1999), kale (Kopsell et al. 2004; Kurilich et al. 1999), lettuce (Mou 2005), potato (*Solanum tuberosum* ssp. *tuberosum*) (Nesterenko and Sink 2003), pepper (Howard et al. 2000; Simonne et al. 1997), and soybean (*Glycine max*) (Simonne et al. 2000).

The genetic variation for carotenoid concentrations within species might be advantageous to vegetable improvement programs.

The accumulation of carotenoid in plant foods is also influenced by environmental growing conditions. Carotenoid accumulation has been shown to increase and decrease in response to environmental manipulations, with varying results for various plant species. The factors such as changes in the growing air temperature (Lefsrud et al. 2005), irradiance level (Lefsrud et al. 2006a), irradiance photoperiod (Lefsrud et al. 2006b), and nutritional fertility (Chenard et al. 2005; Hochmuth et al. 1999) all affect plant carotenoid accumulation. The increased coloration in vegetable and fruit tissues associated with maturity is often indicative of an increase in carotenoid concentrations (Howard et al. 2000; Russo and Howard 2002; Simonne et al. 1997). Carotenoid concentrations increase in leaf tissues with maturity (de Azevedo and Rodriguez-Amaya 2005a,b) but decrease during senescence (Gross 1991). Manipulation of cultural growing conditions and time of harvest would therefore affect the carotenoid concentrations in vegetable crops. The bioavailability of carotenoids from plant foods is highly variable and is also influenced by the species and structures of carotenoids present in the food, the composition and release of carotenoids from the food matrix, the amount consumed and absorption in the intestinal track, the transportation within the lipoprotein fractions, the biochemical conversions and tissue-specific depositions, as well as by the nutritional status of the ingesting host (Castenmiller et al. 1999; Faulks and Southon 2005). Carotenes are entirely lipophilic molecules located in the hydrophobic cores of plant membranes. Similarly, xanthophylls are largely hydrophobic molecules with their polar groups at opposite ends of a nonpolar carbon skeleton (Gruszecki 1999). Due to their lipophilic nature, biotic or abiotic activities that expose carotenoid molecules to potential oxidation, degradation, or isomerization will ultimately have an influence on carotenoid biochemistry and bioavailability.

During the bioavailability of carotenoids, the first step is their release from the food matrix. Food processing activities, such as thermal processing, mincing, or liquefying, result in chemical changes to the carotenoid, probably through isomerization or oxidation reactions (Castenmiller 1999; Livny et al. 2003; Rodriguez-Amaya 1999, Updike and Schwartz 2003). However, freezing or low-temperature storage generally preserves carotenoid concentrations by reducing potential enzymatic oxidation (Rodriguez-Amaya 1999). Processing activities usually increase bioavailability through increased release of bound carotenoids from the food matrix. However, thermal degradations in carotenoid chemistry might adversely affect bioavailability in some food crops. Absorption of carotenoids in humans is passive and follows digestive pathways similar to those of lipids. Protein- or membrane-bound carotenoids must first be released from tissues and dissolved in a hydrophobic domain: oils, fats or bulk lipid emulsions (Faulks and Southon 2005). Due to their hydrophobic nature, carotenoids in the aqueous environment of plant foods are present as bulk lipids or intestinal micelles in the digesta (Faulks and Southon 2005). Carotenoid absorption requires the presence of dietary fat in the small intestine, which stimulates the release of emulsifying bile acids by the gallbladder. Recent studies have shown that absorption of carotenoids increases when they are ingested with dietary lipids (Brown et al. 2004; Unlu et al. 2005). After release from the food matrix, carotenoids are assimilated and oriented into lipid micelles before uptake by intestinal mucosal cells. After release to the enterocyte, carotenoids are incorporated

into chylomicrons, which are eventually delivered to the blood stream and ultimately to the liver. Carotenoid compounds can remain in the liver or can be transferred to LDLs or high-density lipoproteins before eventual tissue-specific deposition (Faulks and Southon 2005).

Carotenoid bioavailability is usually assessed in blood serum after ingestion in dietary trials. The relatively simple analysis quantifies carotenoid changes in serum at various time intervals following ingestion of whole foods or supplements. Some caveats to interpreting serum carotenoid bioavailability include the following: (1) serum responses to single oral doses of carotenoids are highly variable; (2) carotenoids measured in serum signify an equilibrium between intestinal absorption, breakdowns, tissue uptake, and tissue release; and (3) high concentrations of endogenous carotenoids (i.e., α-carotene, β-carotene, lycopene, and lutein) are already present in serum (Yeum and Russell 2002). Current studies have also used *in vitro* Caco-2 human intestinal cell lines to assess carotenoid bioavailability (Chitchumroonchokchai et al. 2004; Liu et al. 2004; Reboul et al. 2005). In these studies, pure carotenoid compounds and whole food samples are brought through an *in vitro* digestion and reacted with Caco-2 human intestinal cells. Absorption potential is measured using standard high-performance liquid chromatography (HPLC) carotenoid analysis. These findings demonstrate that *in vitro* Caco-2 cells can be used to predict carotenoid bioavailability from supplements and whole foods.

Carotenoid concentrations were increased in serum after ingestion of carotenoids from whole food or mono-molecular supplements (Chitchumroonchokchai et al. 2004; Livny et al. 2003; Rao and Shen 2002; Reboul et al. 2005; Unlu et al. 2005; Yeum and Russell 2002). Kopsell and Kopsell (2006) has revealed differences in serum carotenoids and responses to macular pigment optical density (MPOD) evaluations in human subjects administered different doses of lutein from mono-molecular supplements and whole food sources. Serum carotenoid levels were higher than the baseline level after the ingestion of 10- or 30-mg lutein supplements or spinach fortified with 8- or12-mg lutein per 100-g fresh weight. Increases in MPOD from the baseline to the end of a 12-week intervention occurred in subjects administered with 30-mg lutein supplements and spinach with 12-mg lutein per 100-g fresh weight. *In vitro* and *in vivo* studies show that carotenoid bioavailability is influenced by source (whole food vs. supplement); degree of processing; interactions with other carotenoid compounds; the degree of isomerization before, during, and after absorption; transit time in the intestine; and the nutritional status of the human subjects (Faulks and Southon 2005).

Recent Efforts to Increase Vegetable Carotenoid Concentrations

By using both traditional breeding and molecular approaches, some efforts have been made to produce plant products with enhanced carotenoid concentration. Assessment of genetic variation in several crop species has been assumed to be the beginning of improvement programs using traditional breeding methods. However, improvements in carotenoid concentrations using traditional breeding efforts are limited because of the high costs associated with analytical laboratory measurements.

Numerous workers have chronicled molecular advances in carotenoid pathway manipulation to improve biosynthesis and partitioning (Fraser and Bramley 2004;

Nail et al. 2003; Sandmann 2001). Successful approaches have focused on the modification of the biosynthetic pathway to change the flux and end products, increasing preexisting carotenoids and engineering carotenogenic behavior in tissues completely devoid of carotenoid activity (Sandmann 2001). The progress in the identification and cloning of genes and cDNAs of the carotenoid pathway has facilitated genetic manipulations. It has been demonstrated that phytoene synthase has the greatest control over fluxes in the carotenoid pathway; however, success has also been obtained over expressing phytoene desaturase enzymes (Fraser and Bramley 2004). However, genetic manipulations to the carotenoid biosynthetic pathway have resulted in abnormal growth and dwarfing in tomato crop (Fray et al. 1995). These results were attributed to the metabolic precursor competition between carotenoid and gibberellin (GA), both stemming from the common precursor, GGPP. Fluxes of metabolites away from GA resulted in stunted tomato crop with abnormal pigmentation. A two- to threefold increase in tomato fruit-specific carotenoid accumulation has been observed by using a bacterial phytoene synthase (crtB) and a tissue-specific promoter (Fraser et al. 2002). Genetic strategies have also increased β-carotene production in canola (*Brassica napus*) using a seed-specific promoter (Shewmaker et al. 1999) and increased xanthophyll carotenoid production in potato using a tuber-specific promoter (epoxidation reactions were suppressed) (Römer et al. 2002). Burkhardt and others (1997) have reported that xanthophyll and carotene production in rice (*Oryza sativa*) endosperm ("GoldenRice") has been increased using exogenous cyclase and desaturase enzymes. The example of tomato helps in understanding how genetic engineering influences carotenoid precursor pools, enzyme activities, and locations of expression, as well as other isoprenoid pathways and metabolic regulations. The advantages of genetic engineering techniques must be accompanied by a clear understanding of how these manipulations will ultimately affect plant physiology. The development in the field of carotenoid biosynthesis has facilitated the use of the molecular approach.

On the basis of extensive epidemiological observations, it has been understood that vegetables rich in carotenoids provide health benefits by decreasing the risk of various diseases, particularly certain cancers and eye diseases. The most studied carotenoids in this regard are β-carotene, lycopene, lutein, and zeaxanthin (Zeb and Mehmood 2004). It is considered that the beneficial effects of carotenoids may be due to their role as antioxidants. β-Carotene has added its benefits due to its ability to convert into vitamin A. In addition, lutein and zeaxanthin may be protective in eye disease because they absorb damaging blue light that enters the eye. The primary sources of lycopene are tomato and tomato products (Ishida et al. 2007; Zeb and Mehmood 2004). However, intervention trials with large doses of β-carotene found an adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos. Krinsky and Johnson (2005) have suggested the intake of a diet rich in vegetables containing high carotenoid content.

Earlier workers have developed a transgenic strategy to alter the expression of carotenoid biosynthetic genes that have enhanced carotenoid level in some vegetables for nutritional purpose (Botella-Pavia and Rodriguez-Concepcion 2006; Fraser and Bramley 2004; Sandmann et al. 2006; Taylor and Ramsay 2005). Recently, an approach has been employed for the successful production of "golden" potato tubers (Diretto et al. 2007). Moreover, metabolic engineering of potato and tomato

has provided a new direction for the production of more β-carotene, lycopene, and zeaxanthin (Ducreux et al. 2005; Fraser et al. 2002; Romer et al. 2000; Zeb and Mehmood 2004), and for the accumulation of astaxanthin, a new and high-economic-value carotenoid in potato tubers (Gerjets and Sandmann 2006).

Some investigators have shown that carotenoids in plants are synthesized in the membranes of nearly all types of plastids and accumulate in high levels in chromoplasts of many plant parts (Howitt and Pogson 2006; Kopsell and Kopsell 2006). The novel carotenoid–lipoprotein substructures inside chromoplasts are known as carotenoid-sequestering structures (Bartley and Scolnik 1995; Vishnevetsky et al. 1999). These structures serve as deposition sinks to sequester excess carotenoids and may also prevent the end products of the carotenoid biosynthetic pathway from overloading the site of carotenoid biosynthesis in chromoplast membranes (Al Babili et al. 1999; Deruere et al. 1994). Moreover, the control in the formation of a metabolic sink can provide a novel and complement approach to mediate carotenoid accumulation in vegetables.

Cauliflower Orange (Or) Gene and Carotenoid Accumulation

Lu and others (2006) have carried work on the isolation and functional characterization of a novel carotenoid gene mutation in cauliflower. This Or gene, which encodes a DnaJ cysteine-rich domain-containing protein, confers orange curd with high levels of β-carotene accumulation (Fig. 32.4A). The Or gene appears to mediate the differentiation of proplastids and/or noncolored plastids in apical shoot and inflorescence meristematic tissues of curd into chromoplasts for the associated carotenoid accumulation (Li et al. 2001; Lu et al. 2006). Transformation of the Or gene into a wild-type cauliflower converts the white color of curd tissue into a different orange color with an enhanced concentration of β-carotene. The monitoring of the cytological effects of the Or transgene exhibited that expression of the Or transgene leads to the formation of large membranous chromoplasts in the cauliflower curd cells of the Or transformants (Lu et al. 2006). The Or gene under the control of a

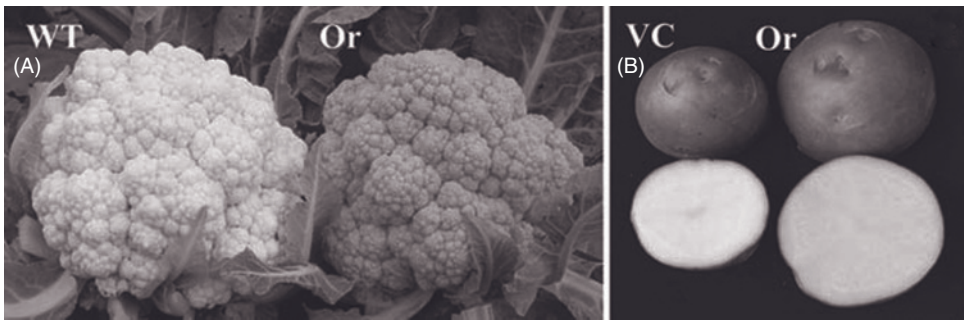

Figure 32.4. Phenotype of cauliflower curds and potato tubers in the presence and absence of the Or gene. (A) Curd of wild-type (WT) and commercial hybrid orange cauliflower (Or). (B) Transgenic potato tubers of vector-only control (VC) and Or transformant (Or) (Li and Van Eck 2007).

potato granule-bound starch synthase (GBSS) promoter was introduced into a major staple crop, potato; expression of the Or gene in the transgenic potato tubers resulted in the production of orange-yellow flesh tubers (Fig. 32.4B). The total carotenoid levels in the Or transgenic lines were up to sixfold higher than the nontransformed and vector-only controls. Further, the cellular contents of these transgenic tubers were examined by light microscopy, which showed that the tubers in the nontransformed and vector-only controls contained exclusively various sizes of starch grains in amyloplasts; the Or transgenic tubers have additional orange bodies. In comparison, examination of potato tubers from a high carotenoid breeding line, 91E22 (Brown et al. 2005), showed that increased levels of carotenoid accumulation per se would not lead to the formation of carotenoid-sequestering structures in the tubers. It was suggested that the Or gene-associated carotenoid accumulation in these transgenic tubers is most likely due to the formation of carotenoid-sequestering structures in chromoplasts, which produced a metabolic sink to facilitate the accumulation of carotenoids.

Carotenoid-sequestering structures are composed of carotenoids, lipids, and proteins. A difference in these components attributes to different types of carotenoid accumulation structures in chromoplasts (Camara et al. 1995; Vishnevetsky et al. 1999). The biosynthesis of components of carotenoid-sequestering structures has shown that it plays a fundamental role in carotenoid sequestration and accumulation. For example, in red pepper and cucumber flowers, carotenoids accumulate in specific lipoprotein fibrils in chromoplasts, and the massive synthesis of carotenoids during flower development and fruit ripening is parallel to the accumulation of a carotenoid-associated protein, fibrillin, or chromoplast-specific protein from cucumber corollas (CHRC) (Vishnevetsky et al. 1996). Further, carotenoid accumulation in the tomato high pigment-1 mutant is associated with an increased plastid number and size for deposition (Cookson et al. 2003; Liu et al. 2004). These studies clearly demonstrate that the formation of carotenoid-sequestering structures for deposition plays an important role in regulating carotenoid accumulation. Manipulation of the formation of deposition sinks offers a new strategy for metabolic engineering of carotenoid content in storage tissues of food crops. In many white and low-pigmented tissues of roots and seeds, low levels of carotenoids accumulate in plastids such as in amyloplasts of starch-storing seeds of wheat, rice, barley, and maize, and in elaioplasts of lipid-storing seeds of canola, sunflower, and pumpkin (Howitt and Pogson 2006). In spite of low amounts of carotenoid accumulation, many carotenoid genes involved in carotenoid biosynthesis are expressed in these tissues (Diretto et al. 2006; Schaub et al. 2005). The presence of the likely gain-of-function mutation of the Or gene induces the formation of sequestering structures in chromoplasts, resulting in the dramatic accumulation of β-carotene without the alteration of the expression of carotenoid biosynthetic genes. This demonstrates that creating a metabolic sink has a significant effect on carotenoid accumulation in the low-pigmented tissues of food crops.

However, it should be noted that enhancing sink capacity for associated carotenoid accumulation in storage tissues of crops requires the presence of all functional genes and enzymes in the biosynthetic pathway. The extent of carotenoid enhancement depends on the maximal potential catalytic activity of this pathway in particular tissues of crops. The specific carotenoid accumulation depends on the endogenous rate-limiting steps of the pathway in the tissues of crops. When the enhanced sink

capacity provides a pulling force to draw the metabolic flux through the carotenoid biosynthetic pathway, the limiting catalytic activities of rate-limiting steps will result in the accumulation of the immediate precursors in specific tissues of crops.

However, it has been demonstrated that modification of sink capacity provides a new strategy to enhance carotenoids in storage tissues of food crops. The concomitant manipulation of catalytic activity with the capacity of sequestering carotenoids may be a more effective strategy to modify carotenoids. Some earlier investigators have shown that overexpression of genes in the carotenoid biosynthetic pathway results in the production of food crops with increased levels of carotenoids (Fraser and Bramley 2004; Taylor and Ramsay 2005). This strategy specifically is more effective with upregulation of the potential rate-limiting steps (Shewmaker et al. 1999), using the genes encoding enzymes with significant enzymatic activities (Paine et al. 2005), and a combination of expressing multiple genes in the pathway (Diretto et al. 2007). It is possible to further increase carotenoid content in many food crops by providing deposition sinks to effectively sequester carotenoids, to meet the requirement for optimal human nutrition and health. The successful demonstration of increased carotenoid accumulation in association with the formation of sink structures in transgenic crops opens new avenues to increase carotenoid content. Manipulation of the formation of metabolic sink along with the catalytic activity of the pathway may represent a promising strategy for maximally improving the nutritional quality of vegetables (Li and Van Eck 2007).

CHEMISTRY AND BIOCHEMISTRY (PATHWAYS) OF PHENOLIC COMPOUNDS IN VEGETABLES

Introduction

Plants synthesize primary compounds such as carbohydrates, lipids, and proteins. Secondary plant compounds are synthesized from lipid precursors and aromatic amino acids. Among the secondary metabolites, phenolic compounds are occupying a prime position. Lignin, a complex polymer of phenylpropane units, is the most important phenolic compound present in plants (Ferrer et al. 2008; Heldt 1997). It is a principal structural compound of cell walls in higher plants and, after cellulose, the second most abundant plant polymer. Lignin is the phenylpropanol units (monolignols) oxidatively coupled through ether and carbon–carbon linkage. Other abundant phenolic compound classes are flavonoids, stilbenes, coumarins, and polyflavonoids (condensed tannins). Phenols in plants exhibit varying functions such as stabilization of the structure, protection from herbivory, protection from ultraviolet (UV) light, exchange of information with symbionts, coloration of blossoms, and biocidal effects against bacteria and fungi (Daniel et al. 1999; Heldt 1997). After the death of plants, phenolics may persist for weeks or months and may affect decomposer organisms and decomposition processes in soils (Horner et al. 1988). Therefore, their effects are not restricted to single plants but may extend to the functioning of whole ecosystems.

Phenolic compounds may have both beneficial (Bitsch 1996) and toxic (Schlatter and Luethy 1986) effects on human health. Among thousands phenolic compounds, only few of them have been evaluated for their beneficial and health-damaging

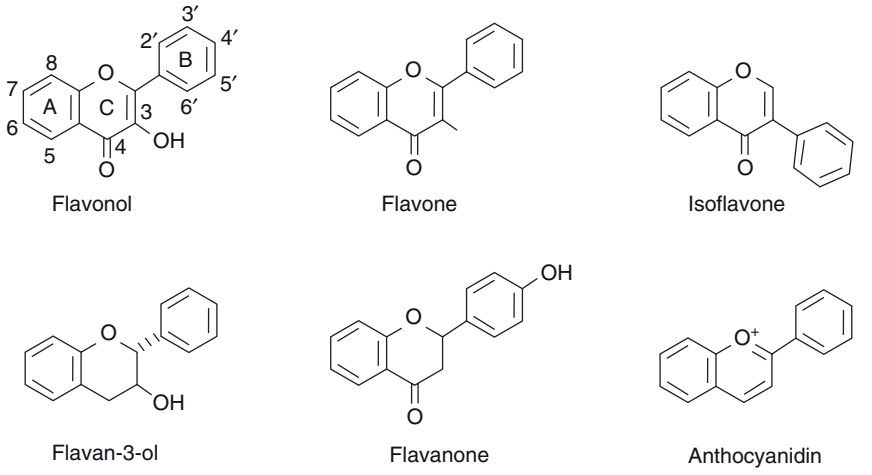

Figure 32.5. Structures of the main classes of flavonoids.

effects. Flavonoids are polyphenolic secondary metabolites widely distributed throughout the plant kingdom and are present in good amounts in commonly consumed vegetables (Ferreres et al. 2005; Young et al. 2005). The flavonoid family has been divided into several subgroups; the six main classes are flavonols, flavones, flavan-3-ols, isoflavones, flavanones, and anthocyanidins (Fig. 32.5). Rusznyák and Szent-Gryörgi (1936) proposed to name flavonoids as vitamin P or vitamin C_2. However, by the 1950s, the vitamin claim for flavonoids had been rejected due to a lack of sufficient support as vitamin property.

In the 1990s, phenolic compounds again have attracted the attention of biochemists due to a combination of increased interest surrounding high levels of coronary heart disease, cancer, and diabetes and an increased interest in its prevention by dietary components. The protection offered by such compounds has been considered due to their antioxidant property. The aromatic ring structures of the flavonoid molecules allow the donation and acceptance of electrons from free radical species (Kanner et al. 1994). In addition to quenching free radicals, flavonoids are able to regenerate the traditional antioxidant vitamins, vitamin C and vitamin E (Martínez-Sánchez et al. 2008; Vinson et al. 1995).

Biosynthetic Pathways for the Phenolic Compounds

Figure 32.6 demonstrates the biosynthetic pathways for the synthesis of various phenolic compounds in plants. Phenylalanine synthesized via the shikimate pathway is used a starting point for the synthesis of plant phenols (Heldt 1997). Phenylalanine ammonia lyase (PAL) catalyzes the deamination of phenylalanine to cinnamate. Coumaric acid is synthesized by introducing a hydroxy group in the phenyl ring of the cinnamic acid, and this step is catalyzed by cinnamate-4-hydroxylase (a P 450-monooxygenase). This phenylpropanoid is a precursor for the synthesis of stilbenes, flavonoids, and furanocoumarins. Stilbenes are obtained from coumaroyl-CoA and three molecules of malonyl-CoA under cleavage of four molecules of carbon dioxide (Daniel et al. 1999; Heldt 1997). The formation of chalcone is

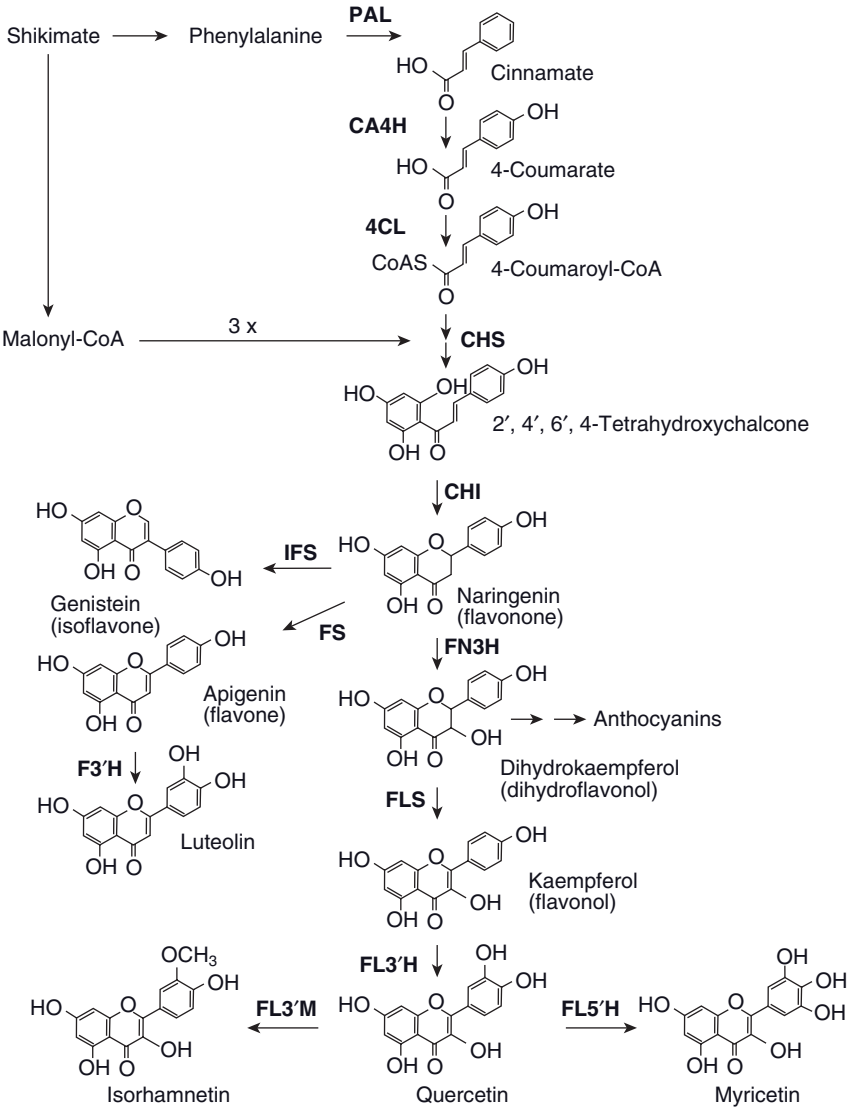

Figure 32.6. The phenylpropanoid pathway by which plants synthesize a wide range of secondary metabolites. Chalcone synthase (CHS) is the first step in the branch of the pathway that produces the flavonoids including isoflavones, flavones, flavonols, and anthocyanins. PAL, phenylalanine ammonia lyase; CA4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; IFS, isoflavone synthase; FN3H, flavanone 3-hydroxylase; FS, flavone synthase; F3′H, flavone 3′-hydroxylase; FLS, flavonol synthase; FL3′H, flavonol 3′-hydroxylase; FL3′M, flavonol 3′-methylase; FL5′H, flavonol 5′-hydroxylase (Daniel et al. 1999).

catalyzed by chalcone synthase from coumaroyl-CoA and three molecules of malonyl-CoA under cleavage of three molecules of carbon dioxide. Chalcone is transformed into flavonon by the enzyme chalcone isomerase. Flavanone is the main precursor for the synthesis of a variety of flavonoids: flavones, flavonoles, anthocyanidins, and isoflavones. Coumaric acid is considered as the precursor for the

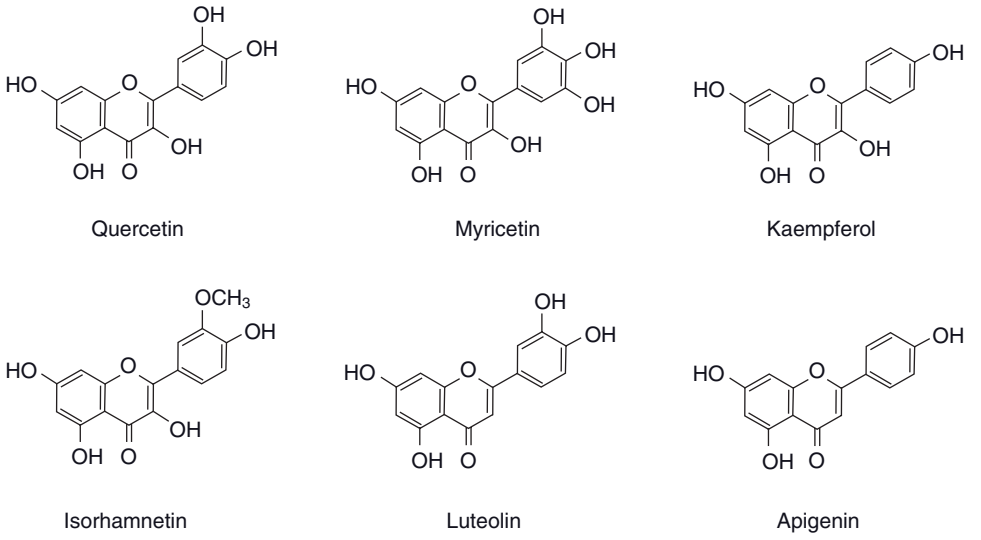

Figure 32.7. Structures of the main dietary flavonols and flavones.

synthesis of furocoumarins. Psoralen is synthesized by the introduction of a C-2 group in hydroxycoumarin, which is formed by hydroxylation and by building an inner ester of cumaric acid.

The major flavonol aglycones found in vegetable products are quercetin, myricetin, kaempferol, and isorhamnetin, while a more limited number of vegetables contain the structurally related flavones, apigenin and luteolin (Fig. 32.7). However, in plants, several flavonols and flavones are present in conjugated form with sugars, primarily glucose, rhamnose, and rutinose (Herrmann 1988; Lanzotti 2006; Slimestad et al. 2007). Most conjugation is present at the 3 position of the B ring, although it can also occur frequently at the 7 and 4′ positions.

Quantitative Analysis of Phenolic Compounds

Flavonoids are polyphenolic compounds occurring in a variety of food and beverages of plant origin. Over 6500 flavonoids have been described so far and have been classified into several subclasses (Daniel et al. 1999). They play an important role in plant development and in the protection of plants against UV radiation, pathogens, and herbivores (Harborn and Williams 2000; Ross and Kasum 2002). Flavonoids are secondary plant metabolites contributing substantially to the energetic part of the human diet (Noteborn et al. 1997). The flavonoids of dietary significance can be divided into six main classes: flavones, flavonols, flavanones, isoflavones, flavanols (including catechins and tannins), and anthocyanins (Fig. 32.5). Flavan-3,4 diols are also referred to as lecoanthocyanidins. Polymeric structures based on flavan-3,4-diol and flavan-3-ol make up the procyanidins or condensed tannin (Harborne and Williams 2000; Spanos and Wrolstad 1992).

There is an increased awareness of the role of flavonoids as epidemiological studies suggested that the composition of a flavonol- and isoflavone-rich diet might decrease the risk of the development of coronary heart disease and certain cancers (Arai et al. 2000; Formica and Regelson 1995; Young et al. 2005). Several studies

showed a negative correlation between flavonol intake and the development of cardiovascular disease, and the protective effect of flavanols seems to be a systemic action (Walle 2004; Young et al. 2005).

There are a large number of flavonol and flavone conjugates, and, as reference compounds are rarely available, this makes it difficult to determine their concentrations in plant products particularly in vegetables. Hertog and others (1992b) solved this problem by treating such compounds by acid, which removed sugar moieties from flavonols and flavones as aglycones. The acid-hydrolyzed compounds could be readily analyzed by reversed-phase HPLC. However, this procedure was employed to monitor the flavonol/flavone content of a range of common plant products (Hertog et al. 1992a, 1993a,b). This quasi-quantitative method conveniently avoided the problems associated with the analysis of the wide variety of flavonol and flavone conjugates that exist in nature. Some investigators employed HPLC-based methods for the analysis of flavonols and flavones in fruits and vegetables (Crozier et al. 1997a,b; Nemeth and Piskula 2007).

Measurement of the identified compounds was obtained by HPLC–diode array detection (DAD) and was carried out in samples cultivated under conventional or organic practices and collected at different times. Samples from organic production exhibited higher total phenolic content than those from conventional practices collected in the same period (Ferreres et al. 2004, 2005; Llorach et al. 2003; Vallejo et al. 2004).

Red leaf lettuce (*Lollo rosso*) was grown under three types of plastic films that varied in transparency to UV. Flavonoid composition was determined by HPLC, total phenolics by the Folin–Ciocalteu assay, and antioxidant capacity by the oxygen radical absorbance capacity (ORAC) assay. Exposure to increased levels of UV radiation during cultivation caused the leaves to redden and increased concentrations of total phenols and the main flavonoids, quercetin and cyanidin glycosides, as well as luteolin conjugates and phenolic acids. Higher concentrations of the flavonoid glycosides were observed with increased exposure to UV radiation, as demonstrated by the concentrations of aglycones after hydrolysis. These concentrations were 165–793 μg/g for cyanidin, 196–880 μg/g for quercetin, and 19–152 μg/g for luteolin (García-Macías et al. 2007).

Bioavailability and Distribution of Phenolic Compounds

Vegetables, such as broccoli, cauliflower, white cabbage, lettuce, chinese cabbage, mug wort, carrot, chili pepper, onion, and tomato have been already analyzed for the presence of a large number of phenolic compounds (Bahorun et al. 2004; Nemeth and Piskula 2007; Young et al. 2005). Sources of dietary polyphenols are fruits, vegetables, beverages, and dietary supplements. In foods, flavonoids contribute to flavor and color characteristics. Pure procyanidins display both bitterness and astringency, and the balance between these sensations depends on the molecular weight. Tetrameric procyanidins have been shown to be the most bitter, while the more polymeric ones are more astringent on an equivalent weight basis. Bitterness is caused by an interaction between polar molecules and the lipid portion of the taste papillae membrane. Astringency results from nonspecific and, to some extent, irreversible hydrogen binding between *o*-diphenol and proline-rich proteins in the mouth (Spanos and Wrolstad 1992).

Polyphenol intake is different in particular countries and depends on preferences as to quality as well as quantity. Flavonoids including catechins, proanthocyanidins, and anthocyanins (and their oxidation products) account for approximately two-thirds of the total plant phenols, and phenolic acids for one-third (Scalbert and Williamson 2000). As a rough estimate, the total daily intake of polyphenols is between 150 and 1000 mg (Aherne and O'Brien 2002). However, the wide range of results is due to the diversity of dietary habits and the methodology of estimation applied. The mean flavonol intake by the German population was calculated to be 11.5 mg/day, mainly derived from fruits and vegetables, but also from black tea and red wine (Bohm et al. 1998). The average daily intake of flavonoids for the Dutch population, not representing total flavonoid intake, only that of three flavonol-type flavonoids (quercetin, myricetin, and kaempferol) and two flavone-type flavonoids (luteolin and apigenin), was estimated to be 23–25 mg per capita, with quercetin (16 mg/day) as the most consumed out of these five flavonoids (Hertog et al. 1993a,b, 1995). According to the U.S. Department of Health & Human Services, the average human daily intake of quercetin alone is 25 mg (Stavric 1994). Also in the "Seven Countries Study" (Hertog et al. 1995), quercetin was reported to account for a significant percentage of total daily flavonoid intakes.

The highest concentrations of quercetin expressed as aglycone were found in onions (284–486 mg/kg fresh edible part) and kale (110 mg/kg) (Hertog et al. 1992a,b, 1993), cherry tomatoes (17–203 mg/kg) (Crozier et al. 1993a), broccoli (30 mg/kg), green beans (39 mg/kg) (Hertog et al. 1992a,b), or in asparagus spears (142 mg/kg) (Makris and Rossiter 2001). In yellow onions, Makris and Rossiter (2001) found 300 mg/kg of quercetin, while Tsushida and Suzuki (1996) reported 227 mg/kg and as much as 793 mg/kg in red onion. Price and Rhodes (1997), however, found higher levels of quercetin in pink, yellow, and red onion varieties ranging 719–927 mg/kg. Red onions consumed by the Brazilian population have up to 1000 mg/kg quercetin (Arabbi et al. 2004). Lower quercetin levels (67.0–121.5 mg/kg) were found in edible parts of Hungarian onions (Lugasi and Hovari 2000) or white onions (Price and Rhodes 1997; Tsushida and Suzuki 1996). Crozier and others (1997b) found only 201 mg/kg of quercetin in edible parts of red onion but much greater quercetin amount in white onions (185–634 mg/kg). Total quercetin content in long-day cultivars was higher than in short-day cultivars, and this does not depend on the growing origin (Okamoto et al. 2006; Peffley et al. 2004).

Flavonols and their glycosides are present predominantly in the skin of the vegetables where they protect, among others, against UV radiations. However, the onion bulb grows under the soil at least partly; its skin, the nonedible dry peel, is also richer in total flavonoids compared to the edible flesh. Nine major compounds were found in dry onion skin with two dominating: quercetin aglycone and quercetin-4-glucoside (Ly et al. 2005; Suh et al. 1999). The flavonoids present in the peel are mainly aglycones due to flavonol glucoside hydrolysis during the peel formation (Price and Rhodes 1997; Takahma and Hirota 2000). Quercetin is concentrated in the dry skin of most onions where its oxidation products, 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxyphenyl glycosilic acid, impart the brown color and provide the onion bulbs protection from soil microbial infection (Takahma and Hirota 2000; Takahama et al. 2001). Several workers have described that the onion bulb contains a wide range of quercetin, isorhamnetin, and kaempferol derivatives in varying proportions with an increasing trend in the content of quercetin glucosides from the

inner to outer scales (Bilyk and Sapers 1985; Hirota et al. 1998; Patil and Pike 1995; Tsushida and Suzuki 1996; Wiczkowski et al. 2003).

The edible part of onion bulbs has quercetin-3-*O*-β-glucoside, quercetin-4-*O*-β-glucoside, quercetin-7-*O*-β-glucoside, quercetin-3,4-di*O*-β-glucoside, quercetin-3,7-di*O*-β-glucoside, and quercetin-7,4-di*O*-β-glucoside, as well as isorhamnetin (3-methoxy quercetin) derivatives -4-*O*-β-glucoside and -3,4-*O*-β-glucoside (Bonaccorsi et al. 2005; Park and Lee 1996; Price and Rhodes 1997). Kaempferol was found as -3-*O*-β-glucoside, -7-*O*-β-glucoside, as minor flavonoid kaempferol-3,4-di*O*-β-glucoside, and -4-*O*-β-glucoside (Tsushida and Suzuki 1996). Quercetin aglycone was detected in long-stored onions but only at levels less than 2% of the total quercetin (Price and Rhodes 1997). The red-purple color of red onions is given by the presence of anthocyanins in epidermal cells of the scale leaves in the form of four major anthocyanins: cyanidin-3-glucoside, cyanidin-3-laminaribioside, cyanidine-3-malonylglucoside, and cyanidin-3-malonyllaminaribioside (Donner et al. 1997; Wu and Prior 2005). Besides cyanidin derivatives constituting over 50% of the total anthocyanins, further delphinidin and petunidin derivatives were detected in the Tropea red onion (*Allium cepa* L.) (Gennaro et al. 2002). From the pigmented scales of red onion, quercetin-3,7,4-triglucoside was isolated and a dihydroflavonol, taxifolin-4-glucoside, was detected (Fossen et al. 1998).

Flavonoids are more abundant in the *Allium* genus (Fig. 32.8). The bulb onion, *A. cepa*, is characterized by higher concentrations of flavonoids compared

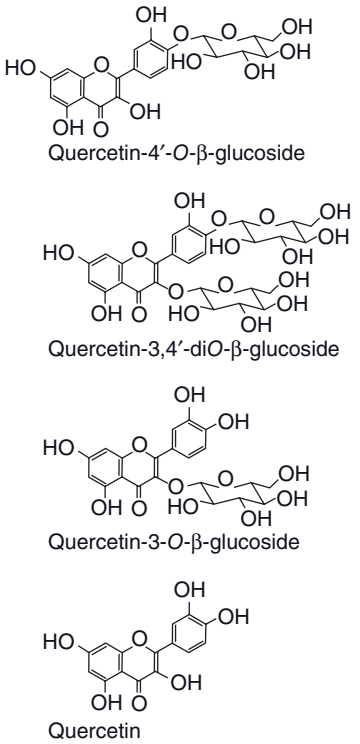

Figure 32.8. Structures of major quercetin glucosides and the aglycone found in onion (Nemeth and Piskula 2007).

with those of garlic (*Allium sativum*) and leek (*Allium porrum*) (Fattorusso et al. 2002). However, quercetin derivatives are the most abundant flavonols in vegetables; kaempferol glycosides can be prevalent or can even be the only flavonol type present, for example, in endive and in leek (Bohm et al. 1998). Leek has an exceptional position within the *Allium* genus as its flavonoid content is made up almost entirely from kaempferol derivatives. Fattorusso and others (2001) identified two kaempferol-related compounds in leek: kaempferol-3-*O*-[2-*O*-(*trans*-3-methoxy-4-hydroxycinnamoyl)-β-D-galactopyranosyl]-(1 → 4)-*O*-β-D-glucopyranoside and kaempferol-3-*O*-[2-*O*-(*trans*-3-methoxy-4-hydroxycinnamoyl)-β-D-glucopyranosyl]-(1 → 6)-*O*-β-D-glucopyranoside. Shallot, belonging to the *Aggregatum* group of the *A. cepa* L. (Le-Guen et al. 2002), also known as *Allium ascalonicum* Hort., grows in clusters of bulbs and was reported to have 940 mg/kg fresh weight of total flavonols (Fattorusso et al. 2002), the highest concentration among onion varieties.

Brassica species are reported to possess cancer-preventive properties that have been attributed to the flavonoids, and other phenolics also contribute to this capacity (Galati and O'Brien 2004; Le Marchand 2002). Several studies have reported the presence of polyphenolic compounds in different *B. oleracea* varieties (Llorach et al. 2003; Nielsen et al. 1993; Price et al. 1997, Vallejo et al. 2004). Those polyphenols consisted of complex flavonol glycosides, some of them being acylated derivatives, and hydrocycinnamic acid esters. Various glycosylated kaempferol derivatives from the external leaves of tronchuda cabbage (*B. oleracea* L. var. costata DC) were characterized by reversed-phase HPLC–DAD–MS/MS–ESI (electrospray ionization). These acylated derivatives are described for the first time in nature, with the exception of kaempferol 3-O-(sinapoyl) sophoroside.

Bahorun and others (2004) have analyzed 10 Mauritian vegetables, broccoli, cauliflower, white cabbage, lettuce, Chinese cabbage, mugwort, carrot, onion, tomato, and chili pepper, for their total phenol, flavonoid, proanthocyanidin, and vitamin C contents. Levels of total phenols in the vegetables varied between 132 and 1189 μg/g fresh weight and those of total flavonoids between 45 and 944 μg/g fresh weights, while proanthocyanidins were detected at very low levels in only a few vegetables. Quercetin was the dominant flavonoid aglycone in the hydrolyzed vegetable extracts, with values in the range of 15–390 μg/g fresh weight.

Consumption of plant foods, particularly fruits, vegetables, and cereal grains, is encouraged because they render beneficial health effects. Phenolics and polyphenolics are among the most desirable food bioactives because of their antioxidant activity, brought about by a number of pathways, or due to other mechanisms. The analysis of phenolics and polyphenolics makes their extraction possible purification and structure elucidation (Naczk and Shahidi 2007).

Phenolics and Health

Epidemiological and intervention studies have provided evidence of the beneficial health effects of dietary fruits and vegetables, and the beneficial effects have been attributed at least in part to secondary metabolites, including flavonoids and hydroxycinnamic acids (Nijveldt et al. 2001). The effects of selected flavonoids in reducing the risk of various diseases including cardiovascular disease, cancer,

atherosclerosis, and other age-related diseases have been demonstrated (Erlejman et al. 2006; Halliwell et al. 2005; Hodgson and Croft 2006), and it is thought that these phytochemicals may provide health benefits as antioxidants or by other mechanisms including effects on gene expression or cell signaling (Moon et al. 2006; Stangl et al. 2006). Therefore, increasing the levels of phenolic compounds in food plants has potential for improving the health of the population.

Vegetables contain several polyphenolic compounds that possess in general several biological properties. Flavonoids are mainly used as veinotonic. They have anti-inflammatory as well as antiallergic properties. Some of them have shown cytotoxic, antimitotic, antitumor, and potential cancer chemopreventive activities. Furthermore, some polyphenolic compounds have antioxidant activities, and they could possess a role in enhancing chemoprevention and in prolonging healthy life. Multiple mechanisms are undoubtedly involved in the protective effects of diets rich in vegetables (Young et al. 2005).

The results presented in this review are of potential importance in view of recent epidemiological studies indicating that polyphenol or flavanol intake is associated with a reduced risk of cancer and coronary heart disease and stroke. It has earleer been reported that at least 20% of coronary heart diseases are related to diet. Dietary factors are also responsible for 40–60% of cancer incidence and for 35% of cancer deaths (Johnson 2007; Singh et al. 2003). Vegetables and fruits have been found to be protective against coronary heart disease (Hertog et al. 1993a, 1995) and against a variety of cancers (Johnson 2007). The quantitative roles of antioxidants are not well known precisely in relation to their health benefits nor are the specific contributions of carotenoids, tocopherols, and ascorbic acid. However, evidence obtained with an *in vitro* oxidation model for heart disease has demonstrated that several plant flavonols, such as quercetin, myricetin, and rutin, are more powerful antioxidants than the traditional vitamins (Vinson et al. 1995). The health influences of flavonols have yet to be fully established. However, they have been shown to function in a way similar to antioxidant vitamins, to protect against lipoprotein oxidation *in vitro* (Negre-Salvayre and Salvayre 1992) and to have antiplatelet and antithrombotic actions (Cook and Samman 1996; Gryglewski et al. 1987). Therefore, there are grounds for encouraging people for the consumption of foods rich in polyphenols. It is evident with tomatoes, lettuce and onions, and in all probability with other produce, that there are very large varietal differences in phenolic compound concentration. Identification and incorporation of phenolic compound foods into the diet is clearly one means by which the intake of phytochemicals derived from fruits and vegetables could be increased markedly.

Recent years have seen an exponential increase in research on the absorption and metabolism of phenolic compounds, particularly flavonols. Evidence suggests that flavonol conjugates are absorbed to a greater extent than the parent aglycone. However, radio-labeled pure compounds are required before any firm conclusions can be drawn. It may be that the active antioxidant compounds are not the dietary flavonols per se, but their metabolites. If it is accepted that higher intakes of phenolic compounds from vegetables are associated with long-term health benefits, that opens new avenues for horticultural approaches toward health promotion by identifying and selecting varieties rich in phenolic compounds by optimizing growth and storage.

Summary

Phenolic compounds are widely distributed in the plant kingdom. Plant tissues may contain phenolic compounds up to several grams per kilogram. External stimuli such as microbial infections, UV radiation, and chemical stressors induce their synthesis. Polyphenolic compounds are secondary plant metabolites present in varying levels in commonly consumed vegetables. This section has highlighted work on chemistry, biosynthetic pathways, bioavailability, and health benefits of phenolic compounds. HPLC to identify and quantify common phenols and their sugar conjugates was described, and the results of a screening program into the phenol content of common products and vegetables was presented.

VEGETABLE AROMA

Introduction

The British Standards Institution defines flavor as the combination of taste and odor that may be influenced by painful heat–cold and tactile sensations. The flavor of vegetables is determined by taste and odor-active compounds. Taste is perceived on the tongue and odor in the olfactory system; this system is extremely sensitive and can detect odors in amounts of parts per trillion, whereas receptors on the tongue can detect flavor compounds in amounts of parts per hundred. Recent advances in olfactometric tools and volatile analysis have added to our understanding, but we still know little about the synergistic or antagonistic interactions between aroma compounds and non-volatile flavor compounds such as sugars, acids, and bitter compounds in fruits and vegetables. Many vegetables contain compounds adding bitterness, for example, isocoumarins and polyacetylenes in carrots and related vegetables (Czepa and Hofmann 2003, 2004) and sesquiterpene lactones in chicory and lettuce (Peters and Amerogen 1998; Sessa et al. 2000), and astringency, such as phenolic acids, flavonoids, alkaloids, and tannins (Mali and Borges 2003; Waterhouse 2002). Odor-active compounds have gained increasing attention because they contribute to the characteristic flavors of fresh and processed vegetables. This section contains information on the odor-active volatiles of vegetables.

The aroma and all the sensory characteristics of food represent only a fraction of the phenomena recognized by the individual when the food is consumed. The term aroma includes the global integral perception of all the senses that are involved in smell, taste, sight, and touch in consuming food. Aromatic substances are present in a wide range of chemical structures that stem from the main constituents of food. Aromas represent an extremely heterogenous group of molecules because of their botanical origin, their functional properties, and especially the chemical structure and the reactivity they exhibit. Several factors affect the volatile compound composition in vegetables, for example, genetics, maturity, growing conditions, and postharvest handling. Moreover, the preparation of the vegetables for consumption and the method for isolation of volatile compounds may change the volatile profile and key aroma compounds compared to nonprocessed vegetables. The most difficult problem in aroma research is to interpret the results of the volatile analysis, which gives information on the identity and the quantity of the volatile compounds collected from a given product. Several volatile compounds are not flavor active; that

is, they cannot be detected in the olfactory system, while others may have, even in trace amounts, significant effects on flavor owing to their low odor threshold values, which is defined as the minimum concentration needed to produce an olfactory response. A lot of effort has been made to detect the odor-active or character-impact compounds in vegetables by various techniques based on gas chromatography–olfactometry (GC-O) (Varming et al. 2004). The GC-O technique has been combined with techniques that measure the intensity of the odor-active compounds by dilution and determination of odor-detection threshold values (Acree 1993; Blank 2002; Mayer et al. 2003a,b; Varming et al. 2004) as in CHARM analysis and aroma extract dilution analysis (AEDA). Recently, the Osme method has been introduced to determine the quality, intensity, and duration of odor-active compounds. However, such techniques ignore synergism and antagonism between compounds; they seem to be the best methods to identify odor-active compounds in vegetables at present. Several vegetables contain a variety of aromatic compounds.

Aroma Compounds in Vegetables

Onion (*A. cepa*) The bulb of the onion contains more than 140 volatile compounds. The characteristic onion flavor develops when the cells are disrupted, allowing the enzyme alliinase to act upon the aroma precursors, (+)-*S*-alk(en)yl cysteine sulfoxides, producing many volatile sulfur compounds that contribute significantly to the aroma of raw onion (Granvogl et al. 2004; Maarse 1991). However, the chemistry of onion volatiles is quite complex because remarkable changes occur during storage and/or processing in the volatile spectrum owing to the disruption of the cell walls (Block et al. 1992). One of the most important flavor compounds in raw onions is thiopropanal-*S*-oxide, the lachrymatory factor (Ferary and Auger 1996). Some other important flavor compounds are 3,4-dimethyl-2,5-dioxo-2,5-dihydrothiophene and alkyl alkane thiosulfonates, such as propyl methanethiosulfonate and propyl propanethiosulfonate, with a distinct odor of freshly cut onions (Boelens et al. 1971). A number of thiosulfinates that have a sharp and pungent odor may also contribute to the flavor of onions. However, these compounds are rapidly decomposed to a mixture of alkyl and alkenyl monosulfides, disulfides, and trisulfides of which dipropyl disulfide, methyl (*E*)-propenyl disulfide, propyl (*E*)-propenyl disulfide, dipropyl trisulfide, and methyl propyl trisulfide are the most important contributors to the aroma of raw and cooked onions (Schulz et al. 1998; Tokitomo and Kobayashi 1992). Recently, 3-mercapto-2-methylpentan-1-ol was identified in raw and cooked onions, eliciting intense meat broth, sweaty, and onion- and leek-like odors (Granvogl et al. 2004; Widder et al. 2000).

Shallots (*A. ascalonicum*) The major aroma constituents in shallots (*A. ascalonicum*) are similar to those found in *A. cepa*. The most important aroma compounds present in shallots are dipropyl disulfide, propyl (*E*)-propenyl disulfide, methyl propyl trisulfide, dimethyl trisulfide, and dipropyl trisulfide (D'Antuono et al. 2002; Maarse 1991).

Garlic (*A. sativum*) In garlic, more than 30 volatiles compounds have been identified (Maarse 1991; Mondy et al. 2002). The characteristic flavor of crushed raw garlic is due to the formation of dialkyl thiosulfinates by the action of alliinase upon *S*-

alk(en)yl cysteine sulfoxides. Allicin, which is formed from alliin (*S*-allyl cysteine sulfoxides), is the most abundant and important dialkyl thiosulfinate formed in garlic (Maarse 1991). However, allicin is very unstable and will undergo nonenzymatic disproportionation and form symmetrical and mixed monosulfides, disulfides, and trisulfides, many of which contribute to the garlic flavor (Maarse 1991). Volatile sulfur *Allium* flavor compounds found in garlic include allicin, di-2-propenyl disulfide, methyl 2-propenyl disulfide, dimethyl trisulfide, methyl 2-propenyl trisulfide, and di-2-propenyl trisulfide (Edris and Fadel 2002; Lee et al. 2003; Mondy et al. 2002).

Leek (*Allium ampeloprasum *var. porrum) The edible portion of leek has more than 90 volatile compounds, which include numerous sulfur-containing volatile compounds. *S*-alk(en)yl cysteine sulfoxides are converted to their respective thiosulfinates or propanethial-*S*-oxide by the action of the enzyme alliinase (Krest et al. 2000; Lancaster et al. 2000). These are responsible for the odor of freshly cut leeks (Block et al. 1992; Ferary and Auger 1996; Nielsen and Poll 2004). Thiosulfinates simply transform into thiosulfonates, which then convert in various monosulfides, disulfides, and trisulfides. 1-Propanethiol, dipropyl disulfide, dipropyl trisulfide, methyl (*E*)-propenyl disulfide, and propyl (*E*)-propenyl disulfide are the most important sulfur-containing aroma compounds having leek aroma notes in fresh and blanched leek (Boelens et al. 1971; Jacobsson et al. 2004; Maarse 1991, Ulrich et al. 1998, Widder et al. 2000).

Products of the lipoxygenase (LOX) pathway or compounds formed by autoxidation of fatty acids are also important for leek aroma (Boelens et al. 1971; Jacobsson et al. 2004). Volatile compounds of the LOX pathway are not pronounced in the aroma profile of freshly cut leeks containing a high content of thiosulfinates and thiopropanal-*S*-oxide (Nielsen et al. 2003). The most important volatile products obtained from fatty acids and perceived by GC-O of raw or cooked leeks are pentanal, hexanal, decanal, and 1-octen-3-ol (Jacobsson et al. 2004; Maarse 1991; Nielsen et al. 2003; Ulrich et al. 1998; Widder et al. 2000).

Broccoli (*B. oleracea *var. italica) There are more than 40 volatile compounds in raw and cooked broccoli (*B. oleracea* var. italica). The most influential aroma compounds found in broccoli are sulfides, isothiocyanates, aliphatic aldehydes, alcohols, and aromatic compounds (Jacobsson et al. 2004; Maarse 1991; Ulrich et al. 1998). Broccoli has a significant amount of sulfurous aroma compounds, which are formed from glucosinolates and amino acid precursors (Chin and Lindsay 1994; Dan et al. 1997a,b; Kubec et al. 1998). The strong off-odors of broccoli are associated with volatile sulfur compounds, such as methanethiol, hydrogen sulfide, dimethyl disulfide, and trimethyl disulfide (Dan et al. 1997b; Derbali et al. 1998; Forney and Jordan 1999). Some more volatile compounds that have also been reported as important to broccoli aroma and odor are dimethyl sulfide, hexanal, (*Z*)-3-hexen-1-ol, nonanal, ethanol, methyl thiocyanate, butyl iso-thiocyanate, 2-methylbutyl isothiocyanate, and 3-isopropyl-2-methoxypyrazine (Derbali et al. 1998; Forney and Jordan 1999; Jacobsson et al. 2004).

Brussels Sprout (*B. oleracea *var. gemmifera) The breakdown products of glucosinolates are predominant in brussels sprouts and represent about 80–90% of the volatiles in headspace samples (Van Langenhove et al. 1991). The residual

volatiles are mostly sulfur compounds (Van Langenhove et al. 1991). The compounds likely to be associated with the aroma of brussels sprouts are 2-propenyl isothiocyanate, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Maarse 1991; Van Langenhove et al. 1991).

Cabbage (*B. oleracea *var. capitata) Cabbage contains nearly 160 volatile compounds in the raw, cooked, and dehydrated material and includes aliphatic alcohols, aldehydes, and esters as well as isothiocyanates and other sulfur-containing compounds (Chin and Lindsay 1993; Kushad et al. 1999; Maarse 1991). 2-Propenyl isothiocyanate is generally considered one of the desirable flavor compounds in cabbage where it provides characteristic fresh cabbage notes and hotness. However, the component appears to be important in very fresh cabbage since it is found to be the major flavor-bearing sulfur compound detected soon after blending (Chin and Lindsay 1993). Few more important compounds present in raw cabbage are methanethiol, dimethyl trisulfide, ethanol, methyl acetate, ethyl acetate, hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol (Chin and Lindsay 1993; Maarse 1991).

Cauliflower (*B. oleracea *var. botrytis) Nearly 80 volatile compounds have been reported from raw and cooked cauliflower. Among the compounds potentially active in cooked cauliflower, certain sulfides such as methanethiol, dimethyl sulfide, and dimethyl trisulfide have often been incriminated in objectionable sulfurous aromas and overcooked off-flavors (Chin and Lindsay 1993; Forney et al. 1991; Maruyama 1970). Additional aldehydes have been found to be the most abundant cauliflower volatiles, with nonanal as a major component (Chin and Lindsay 1993; Derbali et al. 1998). It has earlier been reported that volatile compounds such as 2-propenyl isothiocyanate, dimethyl trisulfide, dimethyl sulfide, and methanethiol are the key odorants of cooked cauliflower "sulfur" odors, whereas different glucosinolates are correlated with bitterness intensity (Engel et al. 2002).

Cucumber (*Cucumis sativus*) The fruit of the cucumber plant contains approximately 30 volatile compounds, with aliphatic alcohols and carbonyl compounds being most abundant (Maarse 1991). Fresh cucumber flavor is produced as a result of the enzymatic degradation of linoleic and linolenic acid after the tissue is disrupted, by which (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal mainly are produced (Grosch and Schwarz 1971). (*E*,*Z*)-2,6-Nonadienal is the main flavor volatile of cucumber fruit, with (*E*)-2-nonenal as the second most important compound (Buescher and Buescher 2001; Schieberle et al. 1990).

Pumpkin (*Cucurbita pepo*) The fruit of pumpkin (*C. pepo*) has about 30 compounds in the volatile extracts of raw pumpkin, with the major classes of compounds being aliphatic alcohols and carbonyl compounds, furan derivatives, and sulfur-containing compounds. Hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, and 2,3-butanedione have been reported as important for the flavor of freshly cooked pumpkins (Maarse 1991).

Potato (*S. tuberosum*) Approximately 50 compounds have been reported to contribute to raw potato aroma. Raw potatoes have a high content of LOX, which catalyzes the oxidation of unsaturated fatty acids into volatile degradation products

(Galliard and Phillips 1971). These reactions occur as the cells are disrupted, for example, during peeling or cutting. It has been demonstrated that the freshly cut raw potatoes contain (*E*,*Z*)-2,4-decadienal, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-octenal, and hexanal, which are all products of LOX-initiated reactions of unsaturated fatty acids (Josephson and Lindsay 1987; Petersen et al. 1998a). Futher, it was investigated that two compounds represent typical potato aroma in raw potato: methional and (*E*,*Z*)-2,6-nonadienal (Petersen et al. 1998a). Some more important volatiles in raw potatoes are also produced via the LOX pathway. These compounds are are 1-penten-3-one, heptanal, 2-pentyl furan, 1-pentanol, and (*E*,*E*)-2,4-heptadienal (Petersen et al. 1998b). Pyrazines such as 3-iso-propyl-2-methoxypyrazine could be responsible for the earthy aroma of potato (Maarse 1991).

Tomato (*L. esculentum*) The fruit of the tomato plant has more than 400 volatile compounds (Hayata et al. 2002; Maneerat et al. 2002). No character-impact compound has been identified in tomatoes, although 2-isobutylthiazole is unique to tomato flavor. The most important compounds in tomatoes are 3-methylbutanal, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 3-methyl-1-butanol, 1-hexanol, (*Z*)-3-hexen-1-ol, 1-penten-3-one, 6-methyl-5-hepten-2-one, β-ion-one, β-damascenone, 2-phenylethanol, methyl salicylate, furaneol, and 2-isobutyl-thiazole, and of these, (*Z*)-3-hexenal and β-ionone have the highest odor units (Bezmann et al. 2003; Buttery and Takeoka 2004; Hayata et al. 2002; Maneerat et al. 2002; Maul et al. 2000; Mayer et al. 2003a,b).

Pea (*Pisum sativum*) The seed and immature pod of the pea plant contains about 120 volatile compounds. These compounds, 1-hexanol, 1-propanol, 2-methylpropanol, 1-pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and (*Z*)-3-hexen-1-ol, are present in the highest concentrations (Jakobsen et al. 1998; Maarse 1991). Compounds contributing to the aroma profile of peas seem to be grouped in two main categories: (1) the fatty acid breakdown products, which contribute to the pea aroma with "strong, green," "perfume, sweet," "orange, sweet," and "mushroom" odors, and (2) the methoxypyrazines, responsible for the characteristic pea aroma also associated with bell pepper (Jakobsen et al. 1998). The most important volatile compounds of the first category include hexanal, (*E*)-2-heptenal, (*E*)-2-octenal, 1-hexanol, and (*Z*)-3-hexen-1-ol, whereas the compounds of the second category are 3-alkyl-2-methoxypyrazines, such as 3-isopropyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 5-methyl-3-isopropyl-2-methoxypyrazine, and 6-methyl-3-isopropyl-2-methoxypyrazine (Jakobsen et al. 1998; Maarse 1991).

Carrots (*D. carota*) The characteristic aroma and flavor of carrots are mainly due to volatile compounds, although nonvolatile polyacetylenes and isocoumarins contribute significantly to the bitterness of carrots (Czepa and Hofmann 2003, 2004). More than 90 volatile compounds have been repoted from carrots (Alasalvar et al. 2001; Kjeldsen et al. 2001, 2003; Schnitzler et al. 2003; Yoo et al. 1997). The carrot volatiles consist mainly of terpenoids in terms of numbers and amounts and include monoterpenes, sesquiterpenes, and irregular terpenes. Monoterpenes and

sesquiterpenes account for about 98% of the total volatile mass in carrots (Kjeldsen et al. 2001). The composition of different volatiles such as α-pinene, sabinene, myrcene, limonene, β-ocimene, γ-terpinene, *p*-cymene, terpinolene, β-caryophyllene, α-humulene, (*E*)-γ-bisabolene, and β-ionone is responsible for the flavor properties. These compounds are considered to be the main flavor compounds of raw carrots (Alasalvar et al. 2001; Howard et al. 1995; Kjeldsen et al. 2001, 2003; Schnitzler et al. 2003; Tóth Markus and Takács-Hájos 2001). Few of the odor sensations characteristic of the volatiles are "carrot top," "terpene like," "green," "earthy," "fruity," "citrus like," "spicy," "woody," and "sweet." Monoterpenes like sabinene, myrcene, and *p*-cymene seem to be important contributors to "green," "earthy," or "carrot top" flavors with relatively high odor activity values. Sesquiterpenes like β-caryophyllene and α-humulene contribute to "spicy" and "woody" notes, whereas a "sweet" note is mainly due to β-ionone (Kjeldsen et al. 2003).

Celery (*Apium graveolens *var. dulce) and Celeriac (*A. graveolens *var. rapaceu) Cultivated celery and celeriac are closely related members of the Apiaceae family. These vegetables are eaten raw in salads or are cooked. Terpenes and phthalides are the volatile compounds responsible for the aroma of celery and celeriac. Phthalides are present in lower quantity as compared with terpenes, but their contribution to celery aroma is dominant. Nearly 165 volatile compounds have been described in celery and celeriac (Deng et al. 2003; Habegger and Schnitzler 2000; Rao et al. 2000; Thappa et al. 2003; Tirillini et al. 2004). The major aroma components of celery are 3-butylphthalide and 3-butyltetrahydrophthalide (sedanolide) with strong characteristic celery aromas (Macleod et al. 1988; Thappa et al. 2003). Some more important volatile compounds found in celery and celeriac include (*Z*)-3-hexen-1-ol, myrcene, limonene, α-pinene, γ-terpinene, 1,4-cyclohexadiene,1,5,5-trimethyl-6-methylene-cyclohexene,3,7,11,15-tetramethyl-2-hexadecen-1-ol, and α-humulene (Deng et al. 2003; Rao et al. 2000; Tirillini et al. 2004).

Parsley (*Petroselinum crispum*) More than 80 compounds have been reported in the volatile fraction and in the aromatic volatiles of parsley. These are mainly monoterpenes and the aromatics myristicin and apiole. It is suggested that the characteristic odor of parsley is due to the presence of *p*-mentha-1,3,8-triene, myrcene, 3-*sec*-butyl-2-methoxypyrazine, myristicin, linalool, (*Z*)-6-decenal, and (*Z*)-3-hexenal (Masanetz and Grosch 1998b). Moreover, β-phellandrene, 4-isopropenyl-1-methylbenzene, and terpinolene contribute significantly to the parsley flavor (Macleod et al. 1985). Studies have shown that a decrease in the intensities of parsley-like and green notes in the odor profile during storage is particularly due to losses of *p*-mentha-1,3,8-triene, myrcene, and (*Z*)-6-decenal (Masanetz and Grosch 1998a).

Parsnip (*Pastinaca sativa*) The major classes of compounds identified in raw and cooked parsnip are monoterpenoids, aliphatic sulfur compounds, and 3-alkyl-2-methoxypyrazines (Maarse 1991). It has been reported that volatile compounds such as terpinolene, myristicin, and 3-*sec*-butyl-2-methoxypyrazine are important contributors to the flavor of parsnip (Maarse 1991).

Horseradish (*Armoracia rusticana*) Horseradish extract comprises 14 different components including isothiocyanates, thiocyanates, and cyanides (Jirovetz et al. 2002; Tokarska and Karwowska 1983).

Rocket Salad (*Eruca sativa*) The aroma compounds of rocket salad headspace samples of fresh leaves were analyzed using gas chromatography (GC), GC-MS, and olfactometry. Over 50 constituents of the *Eruca* headspace could be identified to be essential volatiles responsible for the characteristic intense green, herbal, nutty, and almond-like, Brassicaceae-like (direction of cabbage, broccoli, and mustard), and horseradish-like aroma of these salad leaves. These aroma compounds are especially isothiocyanates, and derivatives of butane, hexane, octane, and nonane have been identified as 4-methylthiobutyl isothiocyanate, *cis*-3-hexen-1-ol, *cis*-3-hexenyl butanoate, 5-methylthiopentyl isothiocyanate, *cis*-3-hexenyl 2-methylbutanoate, and 5-methylthiopentanenitrile (Jirovetz et al. 2002).

Biosynthesis of Some Volatile Compounds in Vegetables

It is clear from the previous section that several volatile compounds are produced in vegetables during maturation and preparation such as cutting, chewing, and mild heat treatment. Due to cutting, chewing, and mild heat treatment, the release of various volatile compounds is taking place and it is an uncontrolled process. Here, enzymes are mixed with primary and secondary metabolites that are separated in the intact tissue. Volatile compounds formed by anabolic or catabolic pathways include fatty acid derivatives, terpenes, and phenolics. However, volatile compounds formed during tissue damage are typically produced through enzymatic degradation and/or autoxidation reactions of primary and/or secondary metabolites and include amino acids, lipids, glucosinolates, and terpenoids.

Compounds from Amino Acids Some of the volatile compounds are produced by the action of enzymes on the amino acids when the tissue of the vegetable is damaged. This seems to be true particularly for sulfur-containing amino acids in vegetables of the Alliaceae and Brassicaceae families. Various aromas of freshly cut *Allium* species (Alliaceae) are dominated by different sulfur-containing volatile compounds originating from the decomposition of the odorless nonvolatile precursors, (+)-*S*-alk(en)yl cysteine sulfoxides, by the action of alliinase (EC 4.4.1.4) as shown in Figure 32.9 (Krest et al. 2000; Lancaster et al. 2000; Nielsen et al. 2003). Because of the compartmentation of allinase in the vacuole and the cysteine sulfoxides in the cytoplasm, volatiles are not produced until cell rupture, for example, by cutting into slices. The products of the enzyme action are pyruvate, ammonia, and various sulfenic acids depending on the (+)-*S*-alk(en)yl cysteine sulfoxides present in the tissue. At least five different cysteine sulfoxides are commonly present in *Allium* species (Fig. 32.9). The sulfenic acids are highly reactive and will quickly combine to form thiosulfinates, which are responsible for the odor of freshly cut *Allium* species. These thiosulfinates are also unstable molecules and will rearrange into disulfides and thiosulfonates. The thiosulfonates also release sulfur dioxide to give the corresponding monosulfides and disulfides. These compounds can be rearranged into monosulfides and trisulfides to form the final products of the reaction as a combination of monosulfides and polysulfides. Further, the amino acid

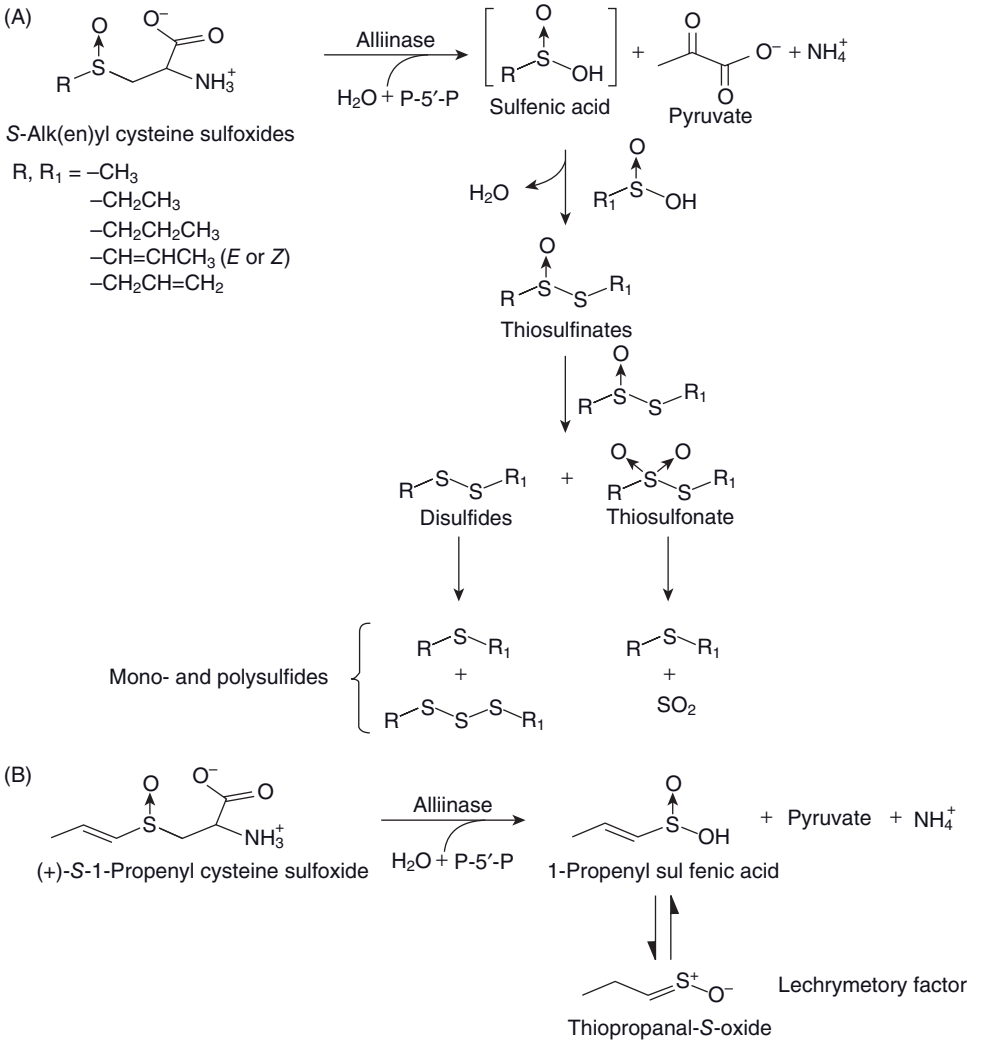

Figure 32.9. Enzymatic production of sulfur-containing flavor compounds in *Allium* species from amino acid flavor precursors. (A) *S*-Alk(en)yl cysteine sulfoxides and (B) (+)-*S*-1-propenyl cysteine sulfoxide (isoalliin) *P*-5′-*P* pyridoxal-5′-phosphate (Krest et al. 2000; Lancaster et al. 2000; Nielsen et al. 2003).

(*E*)-*S*-1-propenyl cysteine sulfoxide (isoalliin) can, apart from taking part in the formation of polysulfides as described earlier, result in the formation of thiopropanal-*S*-oxide, the lachrymatory factor. Thiopropanal-*S*-oxide is also unstable and rearranges spontaneously to produce propanal and sulfur. Propanal may undergo an aldol condensation with a further propanal molecule and may result in the formation of 2-methyl-2-pentenal and other volatile aldehydes (Nielsen et al. 2003).

These heat-treated amino acids may also couple to some other food components, particularly with sugars. The major types of volatile compounds formed from amino–sugar interactions include Strecker degradation aldehydes, alkyl pyrazines, alkyl

thiazolines and thiazoles, and other heterocycles (Maarse 1991). These amino acids are also precursors for some branched chain aliphatic compounds such as 2-methyl-1-butanol and 3-methyl-1-butanol; those are formed during amino acid catabolism (Graham and Eastmond 2002).

Compounds Formed from Glucosinolates Gucosinolates are present in various vegetables, particularly from the cabbage family (Brassicaceae). Glucosinolates are thioglucosides that consist of a common basic skeleton containing a β-thioglucose group, a side chain, and a sulfonated oxime moiety (Fig. 32.10). If the plant tissue is damaged, for example, by cutting or chewing, glucosinolates are hydrolyzed enzymatically by the action of myrosinase (EC 3.2.3.1), which is physically separated from the glucosinolates in intact plant tissue. The products of this reaction are initially isothiocyanates, nitriles, glucose, and a sulfate (Fig. 32.10). Some glucosinolates also give rise to the formation of thiocyanates. The nature of the hydrolysis products depends primarily on the side chain of the glucosinolate, the conditions of the hydrolysis, such as pH, and the presence of cofactors (Bones and Rossiter 1996; Verkerk et al. 2001).

In the cabbage family, the major breakdown products of the glucosinolates are 2-propenyl isothiocyanate, 3-butenyl isothiocyanate, and the corresponding nitriles. The shredding of cabbage tissue in the preparation of coleslaw is particularly effective in bringing about the enzymatic conversion of the glucosinolates. The nitriles can also be generated by the thermal decomposition of the glucosinolates. Volatile isothiocyanates and their corresponding nitriles are important flavor compounds, in particular in vegetables of the cabbage family. At low pH, the formation of nitrile is favored, whereas neutral or high pH favors the formation of the isothiocyanate.

Compounds Obtained by Degradation of Fatty Acids Fatty acids are important parts of the cell membranes. These are precursors for a large number of volatile compounds of which many are important character-impact aroma compounds

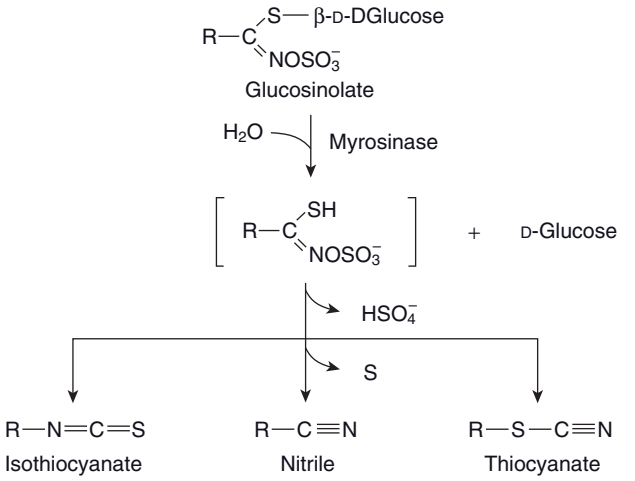

Figure 32.10. Products of thioglucosidase (myrosinase) hydrolysis of glucosinolates (Bones and Rossiter 1996; Verkerk et al. 2001).

responsible for the fresh, green, and fruity notes of vegetables. Degradation of fatty acids occurs mainly by three different oxidative routes: (1) β-oxidation, (2) oxidation by the LOX pathway, and (3) autoxidation. However, fatty acids do not accumulate in healthy plant tissue and therefore, the initial phase in the oxidative degradation process of fatty acids is their liberation by acyl hydrolases before an oxidative degradation (Tomás-Barberán and Robins 1997). β-Oxidation is a classical biochemical pathway, involved in fatty acid degradation (Aguedo et al. 2004; Graham and Eastmond 2002), which typically occurs in intact tissue during ripening of vegetables. β-Oxidation acts on acylcoenzyme A (acetyl-CoA) and consists of a four-step reaction sequence, yielding an acyl-CoA, which has two carbons less and an acetyl-CoA. This sequence is repeated several times until the complete breakdown of the compound occurs (Fig. 32.11). Depending on many factors, the breakdown can be stopped, resulting in the liberation of medium- or short-chain-length volatile compounds. These metabolites can exit between the pathway β-oxidation cycles or inside the sequence. This can produce a variety of volatile saturated and unsaturated compounds such as lactones, esters, alcohols, ketones, and acids (Fig. 32.11). The volatiles produced by the LOX pathway and autoxidation are typically volatile aldehydes and alcohols that are responsible for the fresh and green sensorial flavor. The LOX pathway is responsible for the synthesis of such volatile compounds in response to stress, during ripening, or after damage of the plant tissue. Precursors for the LOX (EC 1.13.11.12) catalyzed reactions are C18-polyunsaturated fatty acids with a (*Z*,*Z*)-1,4-pentadiene moiety such as linoleic and α-linolenic acids that are typically oxidized into 9-, 10-, or 13-hydroperoxides depending on the specificity of the LOX catalyst. Hydroperoxide lyase (HPL) can catalyze the cleavage of such compounds into mainly C6, C9, and C10 aldehydes. These reduced compounds can be further

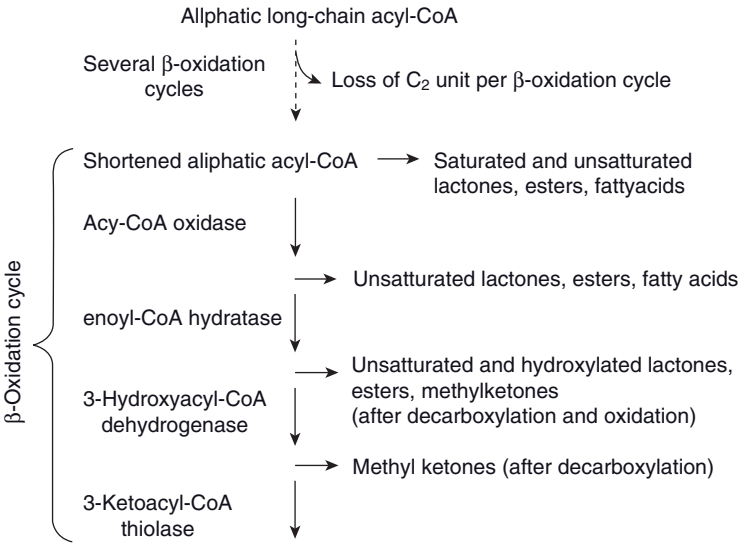

Figure 32.11. Enzymatic degradation of fatty acids by the β-oxidation cycle and formation of various types of aroma compounds in vegetables (Aguedo et al. 2004; Graham and Eastmond 2002).

converted into the corresponding alcohols by alcohol dehydrogenase (EC 1.1.1.1) (Haslbech and Grosch 1985; Yilmaz 2001). However, the production of volatile compounds by the LOX pathway depends on the plants as they have different sets of enzymes, pH in the cells, fatty acid composition of cell walls, and so on (Fig. 32.12).

Several compounds derived from the enzyme-catalyzed oxidative breakdown of unsaturated fatty acids may also be produced by autoxidation (Chan 1987). While the enzymatically produced hydroperoxides in most cases yield one hydroperoxide as the dominant product, nonenzymatic oxidation of unsaturated fatty acids yields a mixture of hydroperoxides that differ in the position of the peroxide group and in the geometrical isomerism of the double bonds. If the number of double bonds increases, the number of oxidation and oxygen-addition sites increases proportionally, and thus the number of possible volatile degradation products increases (German et al. 1992). Autoxidation of linoleic acid produces the 9- and 13-hydroperoxides, while the linolenic acid in addition also produces 12 and 16 hydroperoxides (Ho and Chen 1994). Hexanal and 2,4-decadienal are the primary oxidation products of linoleic acid, whereas autoxidation of linolenic acid produces 2,4-heptadienal as the important product. However, the autoxidation of such aldehydes leads to the formation of other volatile compounds (Chan 1987).

It has earlier been reported that unsaturated fatty acids undergo oxidative breakdown during cooking and that their volatile compounds present in cooked products

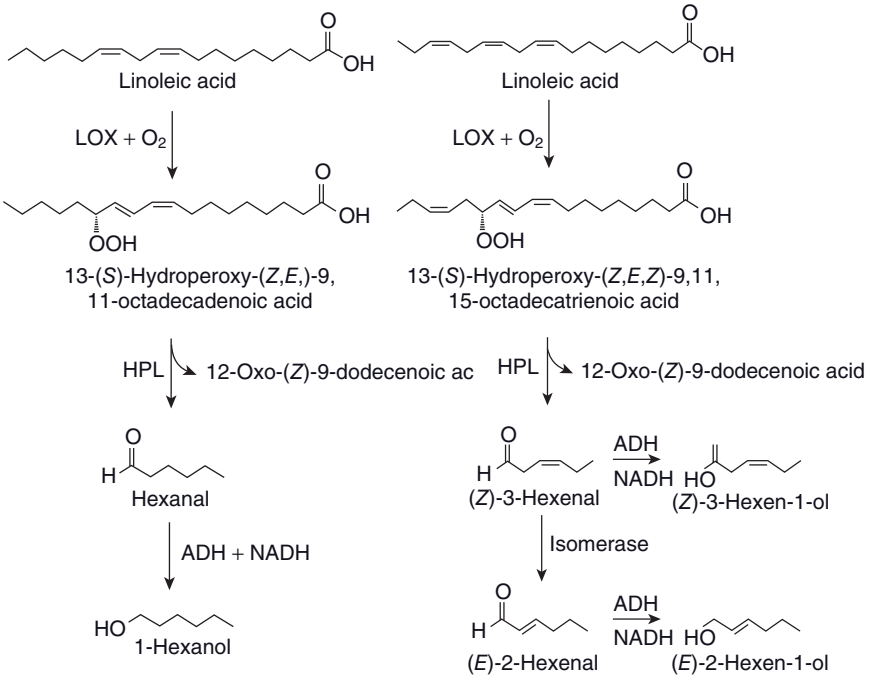

Figure 32.12. Pathway for the enzymatic degradation of linoleic acid and linolenic acid via the lipoxygenase (LOX) pathway to C6 key aroma compounds in fruits and vegetables responsible for green notes. HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase (Haslbeck and Grosch 1985; Yilmaz 2001).

are generally the same as in the raw product (Picardi and Issenberg 1973). However, not much is known about the thermal fatty acid degradation, but possibly, it involves degradation of preformed hydroperoxides in the raw product and/or oxidation of such volatile compounds, that is, 1-octen-3-ol occurs in raw cut mushroom, while 1-octen-3-one cannot be detected. Moreover, 1-octen-3-one is found relatively in large amounts in cooked mushroom (Picardi and Issenberg 1973).

Compounds of Terpenoid Origin Terpenoids are widely distributed among vegetables and fruits and in some vegetables, for example, carrots. These compounds are mainly responsible for the flavor. There are two main types of terpenoids that may contribute significantly to the flavor of vegetables and these are (1) monoterpenes and sesquiterpenes and (2) irregular terpenes produced by catabolistic pathways and/or autoxidation. The monoterpenes and sesquiterpenes are formed by anabolic processes and are therefore present in intact plant tissue (Verkerk et al. 2001). Tissue disruption therefore does not normally alter the profile of monoterpenes and sesquiterpenes significantly in the raw product, although changes in the concentration of some monoterpenes and sesquiterpenes may occur owing to oxidation and release of glycoside-bound oxygenated terpenoids.

α-Terpineol and terpinen-4-ol might result from oxidation of terpinolene, and further, it cannot be ruled out that some monoterpenes and sesquiterpenes, such as geraniol and geranial, may arise from oxidative cleavage of carotenoids. Finally, glycoside-bound oxygenated terpenoids that are released enzymatically may be a source of volatile oxygenated terpenoids, especially in vegetables such as carrot and tomato during ripening or cell disruption (Stahl-Biskup et al. 1993). The formation of some irregular terpenes cannot be explained by anabolic pathways in plants. These terpenoids are primarily oxidative degradation products of the carotenoids. The oxidative breakdown of carotenoids seems somewhat related to the oxidative breakdown of unsaturated fatty acids as has been discussed in an earlier section. As with fatty acids, carotenoid oxidation occurs whenever the plant tissues are damaged and/or during senescence (ripening or bleaching). The volatile degradation products generated obviously depend on the carotenoids present in the different vegetables (Aguedo et al. 2004; Bonnie and Choo 1999; Winerhalter and Rouseff 2001). For example, the tomato volatiles 6-methyl-5-hepten-2-one, geranyl acetone, and farnesyl acetone may result from the oxidative cleavage of acyclic carotenoids (Fig. 32.13A). Similarly, α-ionone, β-ionone, and β-damascenone probably result from the oxidative breakdown of cyclic carotenoids (Fig. 32.13B), and as for other terpenoids, they may exist in intact plant tissue bound as glycosides (Stahl-Biskup et al. 1993). Heating (cooking) seems to produce certain terpenoids. In some vegetables, such as tomatoes and potatoes, there is a considerable increase in the formation of some terpene alcohols, including linalool, α-terpineol and terpinen-4-ol during heat treatments.

Phenols and Related Compounds Several volatile phenols and other related compounds are found in vegetables and some of them are potent aroma compounds. The majority of volatile phenols and related compounds in plants are formed mainly through the shikimic acid pathway. Some aroma compounds are formed after oxidative cleavage of acyclic carotenoids (e.g., lycopene, phytofluene, and phytoene) and cyclic carotenoids (e.g., α-carotene and β-carotene) as aglycones or are bound as

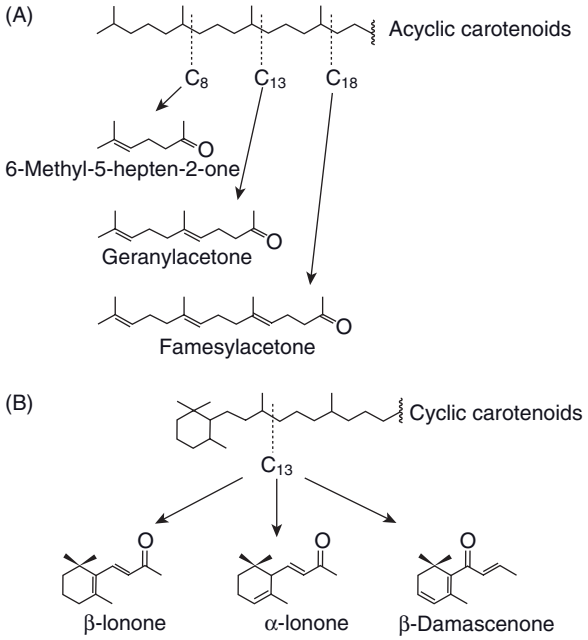

Figure 32.13. Formation of some aroma compounds after oxidative cleavage of (A) acyclic carotenoids (e.g., lycopene, phytofluene, and phytoene) and (B) cyclic carotenoids (e.g., α-carotene and β-carotene) (Aguedo et al. 2004; Bonnie and Choo 1999; Stahl-Biskup et al. 1993; Winerhalter and Rouseff 2001).

glycosides that can be liberated by enzymatic hydrolysis (Stahl-Biskup et al. 1993). However, many of the phenols and related compounds, in particular the phenylpropanoids, originate from some of the "building blocks" of lignin such as ferulic acid and *p*-coumaric acid. These compounds are not breakdown products of lignin. Generally, the volatile phenols and related compounds are substituted benzene derivatives with methoxy and phenolic groups often with an allyl, a vinyl, or an aldehyde group. Common flavor compounds of this group are eugenol, vanillin, myristicin, apiole, elemicin, and benzaldehyde.

Summary

A large number of volatile compounds are responsible for the aroma of vegetables because they have strong penetration odors with low threshold values. Recent advances in methods for measuring flavor release in complex matrices and sensory techniques combined with advanced chemometric methods may give some answers in the future to this central aspect of flavor science. A complete understanding of the aroma chemistry and biochemistry of volatile components of vegetables is important in order to improve the flavor quality of fresh and processed produce that complies with the consumer needs for better quality vegetable products. A large number of factors affect the volatile compound composition in vegetables, for example, genetics, maturity, growing conditions, and postharvest handling. It has been

demonstrated that various vegetables contain a variety of volatile aroma compounds. Some of the vegetables rich in aroma compounds are onion, shallots, garlic, leek, broccoli, brussels sprout, cabbage, cauliflower, cucumber, pumpkin, potato, tomato, pea, carrots, celery, celeriac, parsley, parsnip, horseradish, and rocket salad. These vegetables contain different types of aroma compounds derived from sulfur-containing amino acids, fatty acids, glucosinolates, terpenoid, and phenolic compounds. The biosynthesis of such aroma compounds requires different pathways.

REFERENCES

Acree TE. 1993. Handbook of meat, poultry and seafood quality. In Acree TE, Teranishi R (eds.), *Flavor Science. Sensible Principles and Techniques*, Vol. 23. Washington, DC: American Chemical Society, pp. 120–354.

Aguedo M, Ly MH, Belo I, Teixeira JA, Belin J-M, Waché Y. 2004. The use of enzymes and microorganisms for the production of aroma compounds from lipids. *Food Technol Biotechnol* 42(4):327–336.

Aherne SA, O'Brien NM. 2002. Dietary flavonols: Chemistry, food content, and metabolism. *Nutrition* 18:75–81.

Alasalvar C, Grigor JM, Zhang DL, Quantick PC, Shahidi F. 2001. Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol-fed rat. *J Agric Food Chem* 49:1410.

Al Babili S, Hartung W, Kleinig H, Beyer P. 1999. CPTA modulates levels of carotenogenic proteins and their mRNAs and affects carotenoid and ABA content as well as chromoplast structure in *Narcissus pseudonarcissus* flowers. *Plant Biol* 1:607–12.

Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A* 90:7915–22.

Antuono D, Moretti LF. 2002. Influence of assessors' qualities and analytical conditions on gas chromatography- olfactometry analysis. *Genet Res Crop Evol* 49:175–83.

Arabbi PR, Genovese I, Lajolo FM. 2004. Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. *J Agric Food Chem* 52:1124–31.

Arai Y. 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 130:2243–50.

Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N. 2000. Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 130:2243–50.

Bahorun T, Luximon RA, Crozier A, Aruoma OI. 2004. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. *J Sci Food Agric* 84:1553–61.

Bartley GE, Scolnik PA. 1995. Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *Plant Cell* 7:1027–38.

Bendich A. 1993. Biological functions of carotenoids. In Canfield LM, Krinsky NI, Olson JA (eds.), *Carotenoids in Human Health*. New York: NewYork Academy of Sciences, pp. 61–7.

Bezman Y, Mayer F, Takeoka GR, Buttery RG, Ben-Oliel G, Rabinowitch HD, Naim M. 2003. Differential effects of tomato (*Lycopersicon esculentum* mill) matrix on the volatility of important aroma compounds. *J Agric Food Chem* 51:722–6.

Bilyk A, Sapers GM. 1985. Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues. *J Agric Food Chem* 33(2):226–28.

Bitsch R. 1996. Pflanzenphenole and their gesundheitliche Wirkung. *Natwiss Rundesch* 2:47–51.

Blank I. 2002. Sensory-directed flavor analysis. In Marsili R (ed.), *Flavor, Fragrance, and Odor Analysis*, Vol. 25. New York: Marcel Dekker, pp. 297–378.

Block E, Naganathan S, Putman D, Zhao S. 1992. HPLC analysis of thiosulfinates from onion, garlic, wild garlic (Ramsons), leek, scallion, shallot, elephant (great-headed) garlic, chive, and chinese chive. *J Agric Food Chem* 40:2418–30.

Boelens M, de Valois PJ, Wobben HJ, van der Gen A. 1971. Volatile flavor compounds from shallots. *J Agric Food Chem* 19:984–98.

Bohm H, Boeing H, Hempel J, Raab B, Kroke A. 1998. Flavonole, flavone und anthocyane als natürliche antioxidantien der nahrung und ihre mögliche rolle bei der prävention chronischer erkrankungen. *Z Ernahrungswiss* 37:147–63.

Boileau TWM. 2002. Bioavailability of all-trans and cis-isomers of lycopene. *Exp Biol Med* 227:914–9.

Boileau W-M, Boileau AC, Erdman JW Jr. 2002. Bioavailability of all-trans and cis-isomers of lycopene. *Exp Biol Med* 227:914–19.

Bonaccorsi P. 2005. Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *J Agric Food Chem* 53:2733–40.

Bonaccorsi P, Caristi C, Gargiulli C, Leuzzi U. 2005. Flavonol glucoside profile of southern Italian red onion (*Allium cepa* L.). *J Agric Food Chem* 53(7):2733–40.

Bones AM, Rossiter JT. 1996. The myrosinase-glucosinolate system: An innate defense system in plants. *Physiol Plant* 97(1):194–208.

Bonnie TYP, Choo YM. 1999. The impact of ozone, isoprene and propene on antioxidant levels in two leaf classes of velvet bean (*Mucuna pruriens* L.). *J Oil Palm Res* 2:62–71.

Botella-Pavia P, Rodriguez-Concepcion M. 2006. Carotenoid biotechnology in plants for nutritionally improved foods. *Physiol Plant* 126:369–81.

Bramley PM. 2002. Regulation of carotenoid formation during tomato fruit ripening and development. *J Exp Bot* 53:2107–13.

Brown CR, Culley D, Yang CP, Durst R, Wrolstad R. 2005. Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. *J Am Soc Hortic Sci* 130:174–80.

Brown MJ. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am J Clin Nutr* 80:396–403.

Brown MJ, Ferruzzi MG, Nguyen ML, Cooper DA, Eldridge AL, Schwartz SJ, White WS. 2004. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *J Nutr* 80:396–403.

Buescher RH, Buescher RW. 2001. Effect of oxygen and free fatty acid on cucumber flavour generation. *J Food Sci* 66:357–62.

Buijsse B, Feskens EJ, Schlettwein-Gsell D, Ferry M, Kok FJ, Kromhout D, de Groot LC. 2005. Plasma carotene and α-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: The survey in Europe on nutrition and the elderly, a concerted action (SENECA). *Am J Clin Nutr* 82:879–86.

Burkhardt PK, Beyer P, Wünn J, Klöti A, Gregory AA, Schledz M, von Lintig J, Potrykus I. 1997. Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonar-*

cissus) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. *Plant J* 11(5):1071–8.

Buttery RG, Takeoka GR. 2004. Broccoli storage under low-oxygen atmosphere: Identification of higher boiling volatiles. *J Agric Food Chem* 52:6264–71.

Camara B, Hugueney P, Bouvier F, Kuntz M, Moneger R. 1995. Biochemistry and molecular biology of chromo-plast development. *Int Rev Cytol* 163:175–247.

Castenmiller JJM. 1999. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. *J Nutr* 129:349–355.

Castenmiller JJM, West CE, Linssen JPH, van het Hof KH, Voragen AGJ. 1999. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. *J Nutr* 129:349–355.

Chan HWS. 1987. Detection of short-chain hydroxyaldehydic compounds as pentafluorbenzyloxime derivatives in bovine liver. *Chem Phys Lipid* 77:113–19.

Chenard CH, Kopsell DA, Kopsell DE. 2005. Nitrogen concentration affects nutrient and carotenoid accumulation in parsley. *J Plant Nutr* 28:285–97.

Chin HW, Lindsay RC. 1993. Mechanisms of formation of volatile sulfur compounds following the action of cysteine sulfoxide lyases. *J Food Sci* 58:835–9.

Chin HW, Lindsay RC. 1994. Physiological and quality response of brussels sprouts to storage in controlled atmospheres. *Sulfur Comp Food* 564:90–7.

Chitchumroonchokchai C, Schwartz SJ, Failla ML. 2004. Assessment of lutein bioavailability from meals as a supplement using simulated digestion and Caco-2 human in testinal cells. *J Nutr* 134:2280–6.

Clinton SK et al. 1996. Cis–trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol Biomarkers Prev* 5:823–33.

Combs GF. 1998. Vitamin A. In *The Vitamins: Fundamental Aspects in Nutrition and Health*, 2nd Ed. San Diego, CA: Academic Press, pp. 107–53.

Cook NC, Samman S. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *J Nutr Biochem* 7:66–76.

Cookson PJ, Kiano JW, Shipton CA, Fraser PD, Romer S, Schuch W, Bramley P, Pyke KA. 2003. Increases in cell elongation, plastid compartment size and phytoene syn-thase activity underlie the phenotype of the high pigment1 mutant of tomato. *Planta* 217:896–903.

Crozier A, Jensen E, Lean MEJ, McDonald MS. 1997a. Quantitative analysis of flavonoids by reversed-phase high performance liquid chromatography. *J Chromatogr A* 761: 315–21.

Crozier A, Lean MEJ, McDonald MS, Black C. 1997b. Quantitative analysis of the flavonol content of commercial tomatoes, onions, lettuce and celery. *J Agric Food Chem* 43: 590–5.

Cunningham FX. 2002. Regulation of carotenoid synthesis and accumulation in plants. *Pure Appl Chem* 74:1409–17.

Cunningham FX, Gantt E. 1998. Genes and enzymes of carotenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol* 49:577–83.

Czepa A, Hofmann T. 2003. Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree. *J Agric Food Chem* 51:3865–73.

Czepa A, Hofmann T. 2004. Quantitative studies and sensory analyses on the influence of cultivar, spatial tissue distribution, and industrial processing on the bitter off-taste of carrots (*Daucus carota* L.) and carrot products. *J Agric Food Chem* 52:4508–14.

Dan K, Nagata M, Yamashita I. 1997a. Effects of cooling method, prepacking and top-icing on the quality of brussels sprouts. *J Jpn Soc Hortic Sci* 66:621–35.

Dan K, Todoriki S, Nagata M, Yamashita I. 1997b. A color atlas of post-harvest diseases and disorders of fruits and vegetables. *J Jpn Soc Hortic Sci* 65:867–71.

Daniel O, Meier MS, Schlatter J, Frischknecht P. 1999. Selected phenolic compounds in cultivated plants: Ecologic functions, health implications, and modulation by pesticides. *Environ Health Perspect* 107(Suppl. 1):109–14.

D'Antuono LF, Moretti A, Neri R. 2002. Evaluation of "di Romagna" and "French grey" shallots for quality characters connected to bulb colour and volatile oil content and composition. *Genet Res Crop Evol* 49:175–82.

de Azevedo CH, Rodriguez-Amaya DB. 2005a. Carotenoid composition of kale as influenced by maturity, season and minimal processing. *J Sci Food Agric* 85:591–7.

de Azevedo CH, Rodriguez-Amaya DB. 2005b. Carotenoids of endive and New Zealand spinach as affected by maturity by, season and minimal processing. *J Food Comp Anal* 18:845–55.

DellaPenna D. 1999. Carotenoid synthesis and function in plants: Insights from mutant studies in *Arabidopsis thaliana*. *Pure Appl Chem*. 71:2205–12.

Deng CH, Song GX, Zheng XH, Hu YM, Zhang XM. 2003. Solid-phase micro-extraction followed by gas chromatography-mass spectrometry analysis of the volatile components of *Flos chrysanthemi indici* in different growing areas. *Chromatographia* 57:805–10.

Derbali E, Makhlouf J, Vezina LP. 1998. Biosynthesis of sulfur volatile compounds in broccoli seedlings stored under anaerobic conditions. *Postharvest Biol Technol* 13:191–204.

Deruere J, Romer S, d'Harlingue A, Backhaus RA, Kuntz M, Camara B. 1994. Fibril assembly and carotenoid over-accumulation in chromoplasts: A model for supramolecu-lar lipoprotein structures. *Plant Cell* 6:119–33.

DiMascio P. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 274:532–8.

DiMascio P, Kaiser S, Sies H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 274:532–8.

Diretto G, Al-Babili S, Tavazza R, Papacchioli V, Beyer P, Giuliano G. 2007. Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. *PLoS One* 2:e350.

Diretto G, Tavazza R, Welsch R, Pizzichini D, Mourgues F, Papacchioli V, Beyer P, Giuliano G. 2006. Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. *BMC Plant Biol* 6:13.

Donner H. 1997. Separation and characterization of simple and malony-lated anthocyanins in red onions, *Allium cepa* L. *Food Res Int* 30:637–43.

Donner H, Gao L, Mazza G. 1997. Separation and characterization of simple and malonylated anthocyanins in red onions, *Allium cepa* L. *Food Res Int* 30:637–43.

Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA. 2005. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of beta-carotene and lutein. *J Exp Bot* 56:81–9.

Edris AE, Fadel HM. 2002. Investigation of the volatile aroma components of garlic leaves essential oil. Possibility of utilization to enrich garlic bulb oil. *Eur Food Res Technol* 214:105–7.

Emenhiser C, Beecher GR, Whittaker NF. 1996. Separation of geometrical carotenoid isomers in biological extracts using a polymeric C30 column in reverse-phase liquid chromatography. *J Agric Food Chem* 44:3887–93.

Engel E, Baty C, Corre D, Souchon I, Martin N. 2002. Flavor-active compounds potentially implicated in cooked cauliflower acceptance. *J Agric Food Chem* 50:6459–467.

Erlejman AG, Fraga CG, Oteiza PI. 2006. Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. *Free Radic Biol Med* 41:1247–56.

Fattorusso E. 2001. The flavonoids of leek, *Allium porrum*. *Phytochemistry* 57:565–9.

Fattorusso E. 2002. Chemical composition of shallot (*Allium ascalonicum* Hort.). *J Agric Food Chem* 50:5686–90.

Fattorusso E, Lanzotti V, Taglialatela-Scafati O, Cicala C. 2001. The flavonoids of leek, *Allium porrum*. *Phytochemistry* 57:565–9.

Fattorusso E, Iorizzi M, Lanzotti V, Taglialatela-Scafati O. 2002. Chemical composition of shallot (*Allium ascalonicum* Hort.). *J Agric Food Chem* 50:5686–90.

Faulks RM, Southon S. 2005. Challenges to understanding and measuring carotenoid bioavailability. *Biochim Biophys Acta* 1740:95–100.

Ferary S, Auger J. 1996. What is the true odor of cut *Allium*? Complementary of various hyphenated methods: Gas chromatography-mass spectrometry and high performance liquid chromatographymass spectrometry with particle beam and atmospheric pressure ionization interfaces in sulphenic acids rearrangement components discrimination. *J Chromatogr A* 750:63–74.

Ferrer JL, Austin MB, Stewart C Jr, Neol JP. 2008. Structure and function of enzyme involve in the biosynthesis of phenylpropanoids. *Plant Physiol Biochem* 46:356–70.

Ferreres F, Llorach R, Gil-Izquierdo A. 2004. Characterization of the interglycosidic linkage in di-, tri-, tetra-and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J Mass Spectrom* 39:312–21.

Ferreres F, Valenta OP, Llorach R, Pinheiro C, Cardoso L, Pereira J, Sousa C, Seabra RM, Andrade PB. 2005. Phenolic compounds in external leaves of tronchuda cabbage (*Brassica oleracea* L. var. costata DC). *J Agric Food Chem* 53:2901–7.

Finley JW. 2005. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols and seleno-compounds. *Ann Bot (Lond)* 95:1075–96.

Formica JV, Regelson W. 1995. Review of the biology of quercetin and related bioflavonoids. *Food Chem Toxicol* 33:1061–80.

Forney CF, Jordan MA. 1999. Anaerobic production of methanethiol and other compounds by brassica vegetables. *HortScience* 34:696–9.

Forney CF, Mattheis JP, Austin RK. 1991. Volatile compounds produced by broccoli under anaerobic conditions. *J Agric Food Chem* 39:2257–9.

Fossen T. 1998. Flavonoids from red onion (*Alliumcepa*). *Phytochemistry* 47:281–5.

Fossen T, Pedersen AT, Andersen OM. 1998. Flavonoids from red onion (*Alliumcepa*). *Phytochemistry* 47:281–5.

Frank HA, Cogdell RJ. 1996. Carotenoids in photosynthesis. *Photochem Photobiol* 63:257–64.

Fraser PD, Bramley PM. 2004. The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 43:228–265.

Fraser PD, Romer S, Shipton CA, Mills PB, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM. 2002. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proc Natl Acad Sci U S A* 99:1092–7.

Fray RG, Khachik F. 1995. Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by restricting metabolites from the gibberellin pathway. *Plant J* 8:693–701.

Fray RG, Wallace A, Fraser PD, Valero D, Hedden P, Bramley PM, Grierson D. 1995. Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. *Plant J* 8:693–701.

Galati G, O'Brien PJ. 2004. Potential toxicity of flavonoids and other dietary phenolics: Significance for their chemopreventive and anticancer properties. *Free Radic Biol Med* 37:287–303.

Galliard T, Phillips DR. 1971. Identification of a specific isoform of tomato lipoxygenase (tomloxc) involved in the generation of fatty acid-derived flavor compounds. *Biochem J* 24:431–6.

Garcia AL, Bialek-Bylka GE, Hiyama T, Yumoto K, Koyama Y. 2003. Retinoid-and carotenoid-enriched diets influence the ontogenesis of the immune system in mice. *Immunology* 110:180–7.

García-Macías P, Ordidge M, Vysini E, Waroonphan S, Battey NH, Gordon MH, Hadley P, John P, Julie A, Lovegrove JA, Wagstaf A. 2007. Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (*Lollo rosso*) due to cultivation under plastic films varying in ultraviolet transparenc. *J Agric Food Chem* 55:10168–72.

Gartner C, Stahl W, Sies H. 1997. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am J Clin Nutr* 66:116–22.

Gennaro L. 2002. Flavonoid and carbohydrate contents in Tropea red onions: Effects of home like peeling and storage. *J Agric Food Chem* 50:1904–10.

Gennaro L, Leonardi C, Esposito F, Salucci M, Maiani G, Quaglia G, Fogliano V. Flavonoid and carbohydrate contents in tropea red onions: Effects of home like peeling and storage. *J Agric Food Chem* 50:1904–10.

Gerjets T, Sandmann G. 2006. Ketocarotenoid formation in transgenic potato. *J Exp Bot* 57:3639–45.

Goldman IL, Kader AA, Heintz C. 1999. Influence of production, handling, and storage on phytonutrient content of foods. *Nutr Rev* 57:46–52.

Graham IA, Eastmond PJ. 2002. Pathways of straight and branched chain fatty acid catabolism in higher plants. *Prog Lipid Res* 41:156–81.

Granado F, Olmedilla B, Blanco I. 2003. Nutritional and clinical relevance of lutein in human health. *Br J Nutr* 90:487–502.

Granvogl M, Christlbauer M, Schieberle P. 2004. Quantitation of the intense aroma compound 3-mercapto-2-methylpentan-1-ol in raw and processed onions (*Allium cepa*) of different origins and in other *allium* varieties using a stable isotope dilution assay. *J Agric Food Chem* 52:27–97.

Grosch W, Schwarz JM. 1971. Linoleic and linolenic acid as precursors of the cucumber flavor. *Lipids* 6:351–2.

Gross J. 1991. *Pigments in Vegetables: Chlorophylls and Carotenoids*. New York: AVI/Van Nostrand Reinhold.

Grusak MA, DellaPenna D. 1999. Improving the nutrient composition of plants to enhance human nutrition and health. *Annu Rev Plant Physiol Plant Mol Biol* 50:133–61.

Gruszecki WI. 1999. Carotenoids in membranes. In Frank HA, Young AJ, Britton G, Cogdell RJ (eds.), *The Photochemistry of Carotenoids*. Dordrecht: Kluwer, pp. 363–79.

Gryglewski RJ, Korbut R, Robak J, Swies J. 1987. On the mechanism of anti-thrombotic action of flavonoids. *Biochem Pharm* 36:317–22.

Habegger R, Schnitzler WH. 2000. Relationship between differential adsorption of odorants by the olfactory mucus and perception of odour mixtures. *Obst Gemüse Kartoffelverarbeitung* 85:162–74.

Halliwell B, Rafter J, Jenner A. 2005. Health promation by flavonoids, tocophenols, tocotrienols and other phenol: Direct or indirect effect? Antioxidant or not? *Am J Clin Nutr* 81:268S–276S.

Hanum T, Sinha NK, Guyer DE, Cash JN. 1995. Onion flavor recovery in the process of adsorption and supercritical carbon dioxide desorption. *Food Chem* 54:205–18.

Harborne JB, Williams CA. 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55:481–504.

Haslbeck F, Grosch W. 1985. HPLC analysis of all positional isomers of the monohydroperoxides formed by soybean lipoxygenases during oxidation of linoleic acid. *J Food Biochem* 9:1–14.

Hayata Y, Maneerat C, Kozuka H, Sakamoto K, Ozajima Y. 2002. Flavor volatile analysis of "House Momotaro" tomato fruit extracts at different ripening stages by Porapak Q column. *J Jpn Soc Hortic Sci* 71:473–79.

Heldt HW. 1997. *Pflanzenbiochemie*. Heidelberg: Spektrum Akademischer Verlag.

Herrmann K. 1988. On the occurrence of flavonols and flavone glycosides in vegetables. *Z Lebensm Unters Forsch* 186:1–5.

Hertog MGL, Hollman PCH, Katan MB. 1992a. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 41:2379–83.

Hertog MGL, Hollman PCH, Venema DP. 1992b. Optimization of quantitative HPLC determination of potentially anticarcinogenic flavonoids in fruits and vegetables. *J Agric Food Chem* 40:1591–8.

Hertog MGL, Fesens EJM, Hollman PCH, Katan MB, Kromhout D. 1993a. Dietary antioxidant flavonoids and the risk of coronary heart disease: The Zutphen elderly study. *Lancet* 342:1007–11.

Hertog MGL, Hollman PCH, van de Putte B. 1993b. Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *J Agric Food Chem* 41:1242–6.

Hertog MGL, Kromhout D, Aravansis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen, M, Simic BS, Toshima H, Feskens EJM, Hollman PCH, Katan MB. 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the "Seven Country Study". *Arch Intern Med* 155:381–6.

Hirota S, Shimosa T, Takahama U. 1998. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *J Agric Food Chem* 46:3497–502.

Ho CT, Chen Q. 1994. Lipids in food flavors. In Ho CT, Hartman TG (eds.), *Lipids in Food Flavors*. Newark, NJ: American Chemical Society and Rutgers University, pp. 2–24.

Hochmuth GJ, Brecht JK, Bassett MJ. 1999. Nitrogen fertilization to maximize carrot yield and quality on a sandy soil. *HortScience* 34:641–5.

Hodgson JM, Croft KD. 2006. Dietary flavonoids: Effects on endothelial function and blood pressure. *J Sci Food Agric* 86:2492–8.

Holick CN, De Vivo I, Feskanich D, Giovannucci E, Stampfer M, Michaud DS. 2005. Intake of fruits and vegetables, carotenoids, folate, and vitamins A, C, E and risk of bladder cancer among women (United States). *Cancer Causes Control* 16:1135–45.

Horner JD, Gosz JR, Cates RG. 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *Am Nat* 132:869–83.

Howard LR, Michal DA. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J Agric Food Chem* 48:1713–20.

Howard LR, Talcott ST, Brenes CH, Villalon B. 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (Capsicum species) as influenced by maturity. *J Agric Food Chem* 48:1713–20.

Howitt CA, Pogson BJ. 2006. Carotenoid accumulation and function in seeds and non-green tissues. *Plant Cell Environ* 29:435–45.

Hughes DA. 1999. Effects of carotenoids on human immune function. *Proc Nutr Soc* 58:713–18.

Humphries JM, Khachik F. 2003. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruits, vegetables, wheat, and pasta products. *J Agric Food Chem* 51:1322–7.

Ishida BK, Roberts JS, Chapman MH, Burri BJ. 2007. Processing tangerine tomatoes: Effects on lycopene-isomer concentrations and profile. *J Food Sci* 72:C307–12.

Ito Y, Khachik F. 2006. Cardiovascular disease mortality and serum carotenoid levels: A Japanese population-based follow-up study. *J Epidemiol* 16:154–60.

Ito Y, Kurata M, Suzuki K, Hamajima N, Hishida H, Aoki K. 2002. Carotenoid bioavailability and bioconversion. *Annu Rev Nutr* 22:483–504.

Ito Y, Kurata M, Suzuki K, Hamajima N, Hishida K, Aoki K. 2006. Cardiovascular disease mortality and serum carotenoid levels: A Japanese population-based follow-up study. *J Epidemiol* 16:154–60.

Ito Y, Wakai K, Suzuki K, Ozasa K, Watanabe Y, Seki N, Ando M, Nishino Y, Kondo T, Ohno Y, Tamakoshi A. 2005. Lung cancer mortality and serum levels of carotenoids, retinol, tocopherols, and folic acid in men and women: A case-control study nested in the JACC study. *J Epidemiol* 15:S140–S149.

Jacobsson A, Nielsen T, Sjöholm I. 2004. Influence of temperature, modified atmosphere packaging, and heat treatment on aroma compounds in broccoli. *J Agri Food Chem* 52:1607–14.

Jakobsen HB, Hansen M, Christensen MR, Brockhoff PB, Olsen CE. 1998. Isolation of a carotenoid-containing sub-membrane particle from the chloroplastic envelope outer membrane of pea (*Pisum sativum*). *J Agric Food Chem* 46:3727–32.

Jirovetz L, Smith D, Buchbauer G. 2002. Aroma compound analysis of *Eruca sativa* (Brassicaceae) SPME headspace leaf samples using GC, GC-MS, and olfactometry. *J Agric Food Chem* 50:4643–6.

Johnson B, Hammond R, Yeum K-J. 2001. Expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate. *Food Chem* 14:36–8.

Johnson EJ, Hammond BR, Yeum KJ, Qin J, Wang XD, Castaneda C, Snodderly DM, Russell RM. 2000. Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr* 71(6):1555–62.

Johnson IT. 2007. Phytochemicals and cancer. *Proc Nutr Soc* 66:207–15.

Josephson DB, Lindsay RC. 1987. Retro-aldol related degradations of 2,4-decadienal in the development of staling flavors in fried foods. *J Food Sci* 52:328–35.

Kabagambe EK, Niyogi KK, Björkman O, Grossman AR. 2005. Some dietary and adipose tissue carotenoids are associated with the risk onnon fatal acute myocardial infarction in Costa Rica. *J Nutr* 135:1763–9.

Kanner J, Frankel EN, Grant R, German JB, Kinsella JE. 1994. Natural antioxidants in grapes and wines. *J Agric Food Chem* 42:64–9.

Kelemen LE, Cerhan JR, Lim U, Davis S, Cozen W, Schenk M, Colt J, Hartge P, Ward MH. 2006. Vegetables, fruit, and antioxidant-related nutrients and risk of non-Hodgkin lymphoma: A national cancer institute-surveillance, epidemiology, and end results population-based case-control study. *Am J Clin Nutr* 83:1401–10.

Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY, Katz NB. 2002. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Exp Biol Med* 227:845–51.

Khachik F, Beecher GR, Whittaker NF. 1986. Separation, identification, and quantification of the major carotenoids and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J Agric Food Chem* 34:603–16.

Kimura M, Rodriguez-Amaya DB. 2003. Carotenoid composition of hydroponic leafy vegetables. *J Agric Food Chem* 51:2603–7.

Kjeldsen F, Christensen LP, Edelenbos M. 2001. The chlorophyll a/b-binding protein inserts into the thylakoids independent of its cognate peptide. *J Agric Food Chem* 49:4342–52.

Kjeldsen F, Christensen LP, Edelenbos M. 2003. Changes in volatile compounds of carrots (*Daucus carota* L.) during refrigerated and frozen storage. *J Agric Food Chem* 51:5400–7.

Klöti A, Rabbani S, Beyer P, Lintig J, Hugueney P, Kleinig H. 1998. Induced beta-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. *Plant Physiol* 116:1239–48.

Kopas-Lane LM, Warthesen JJ. 1995. Carotenoid photostability in raw spinach and carrots during cold storage. *J Food Sci* 60:773–6.

Kopsell DA, Kopsell DE. 2006. Accumulation and bioavailability of dietary carotenoids in vegetable crops. *Trends Plant Sci* 11:499–507.

Kopsell DA, Schweigert FJ, Worm M. 2004. Variation in lutein, β-carotene, and chlorophyll concentrations among *Brassica oleracea* cultigens and seasons. *HortScience* 39:361–4.

Kopsell DE, Kopsell DA, Randle WM, Coolong TW, Sams CE, Curran-Celentano J. 2003. Kale carotenoids remainst able while flavor compounds respond to changes in sulfur fertility. *J Agric Food Chem* 51:5319–25.

Koyama Y, Fujii R. 1999. Cis–trans carotenoids in photosynthesis: Configurations, excited-state properties and physiological functions. In Frank HA, Young AJ, Britton G, Cogdell RJ (eds.), *The Photochemistry of Carotenoids*. Dordrecht: Kluwer, pp. 161–88.

Krest I, Glodek J, Keusgen M. 2000a. Effects of cysteine-containing compounds on biosynthesis of triacylglycerol and cholesterol and anti-oxidative protection in liver from mice consuming a high-fat diet. *J Agric Food Chem* 48:3753–67.

Krest I, Glodek J, Keusgen M. 2000b. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J Agric Food Chem* 48:3753–60.

Krinsky NI, Johnson EJ. 2005. Carotenoid actions and their relation to health and disease. *Mol Aspects Med* 26(6):459–516.

Kubec R, Drhova V, Velisek J. 1998. Thermal degradation of *S*-methylcysteine and its sulfoxides: Important flavor precursors of *Brassica* and *Allium* vegetables. *J Agric Food Chem* 46:4334–40.

Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, Jeffery EH, Kushad M, Wallig MA, Juvik JA. 1999. Carotene, tocopherol, and ascorbate in sub species of *Brassica oleracea*. *J Agric Food Chem* 47:1576–81.

Kurlich AC, Juvik JA. 1999. Quantification of carotenoid and tocopherol antioxidants in *Zea mays*. *J Agric Food Chem* 47:1948–55.

Kushad MK, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, Jeffery EH. 1999. A comparison of the aromas of six coffees. *J Agric Food Chem* 47:1541–8.

Lancaster JE, Shaw ML, Joyce MDP, McCallum JA, McManus MT. 2000. A novel alliinase from onion roots. Biochemical characterization and cDNA cloning. *Plant Physiol* 122:269–79.

Landrum JT, Bone RA. 2001. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 385:28–40.

Lanzotti V. 2006. Review: The analysis of onion and garlic. *J Chromatogr A* 1132:3–22.

Lee SN, Kim NS, Lee DS. 2003. Comparative study of extraction techniques for determination of garlic flavor components by gas chromatography-mass spectrometry. *Anal Bioanal Chem* 377:749–56.

Lefsrud MG, Kopsell DA, Augé R, Both AJ. 2005. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. *HortScience* 40:2026–30.

Lefsrud MG, Kopsell DA, Kopsell DE, Curran-Celentano J. 2006a. Biomass production and pigment accumulation in kale grown under increasing photo-periods. *HortScience* 41:603–6.

Lefsrud MG, Kopsell DA, Kopsell DE, Curran-Celentano J. 2006b. Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. *Physiol Plant* 127:624–31.

Le-Guen L, Saos F. 2002. In vitro development in shallot (*Allium cepa* L. group Aggregatum): Effects of anti-gibberellins, sucrose and light. *Ann Bot* 89:419–25.

Le Guen-Le Saos F, Hourmant A, Esnault F, Chauvin JE. 2002. In vitro development in shallot (*Allium cepa* L. group Aggregatum): Effects of anti-gibberellins, sucrose and light. *Ann Bot* 89:419–25.

Le Marchand L. 2002. Cancer preventive effects of flavonoidssa review. *Biomed Pharm* 56:296–301.

Li L, Paolillo DJ, Parthasarathy MV, Dimuzio EM, Garvin DF. 2001. A novel gene mutation that confers abnormal patterns of beta-carotene accumulation in cauliflower (*Brassica oleracea* var. botrytis). *Plant J* 26:59–67.

Li L, Van Eck J. 2007. Metabolic engineering of carotenoid accumulation by creating a metabolic sink. *Transgenic Res* 16:581–5.

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J. 2004. Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proc Natl Acad Sci U S A* 101:9897–902.

Livny O, Reifen R, Levy I, Madar Z, Faulks R, Southon S, Schwartz B. 2003. β-Carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. *Eur J Nutr* 42:338–45.

Llorach R, Gil-Izquierdo A, Ferreres F, Tomás-Barberán Llorach R, Gil-Izquierdo A, Ferreres F, Tomás-Barberán FA. 2003. HPLC-DAD-MS/MS ESI characterization of unusual highly glycosylated acylated flavonoids from cauliflower (*Brassica oleracea* L. var. botrytis) agro-industrial byproducts. *J Agric Food Chem* 51:3895–9.

Lu S, Eck Van J, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J, Kochian LV, Kupper H, Earle ED, Cao J, Li L. 2006. The cauliflower Or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of beta-carotene accumulation. *Plant Cell* 18:3594–605.

Lugasi A, Hovari J. 2000. Flavonoid aglycones in foods of plant origin I: Vegetables. *Acta Aliment* 29:345–52.

Ly TN et al. 2005. Antioxidative compounds from the outer scales of onion. *J Agric Food Chem* 53:8183–9.

Maarse H. 1991. *Volatile Compounds in Foods and Beverages*, Vol. 21. New York, Basel, Hong Kong: Marcel Dekker, pp. 5678–91.

Macleod AJ, Macleod G, Subramanian G. 1988. Biotransformation of the sesquiterpenoid β-selinene using the plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. *Phytochemistry* 27:373–82.

Macleod AJ, Snyder CH, Subramanian G. 1985. Apparatus for quantitative headspace analysis of characteristic odorants of baguettes. *Phytochemistry* 24:2623–8.

Makris DP, Rossiter JT. 2001. Domestic processing of onion bulbs (*Alliumcepa*) and asparagus spears (*Asparagus officinalis*). Effect on flavonol content and antioxidant status. *J Agric Food Chem* 49:3216–22.

Mali S, Borges RM. 2003. Phenolics, fibre, alkaloids, saponins, and cyanogenic glycosides in a seasonal cloud forest in India. *Biochem Syst Ecol* 31:1221–46.

Maneerat C, Hayata Y, Kozuka H, Sakamoto K, Osajima Y. 2002. Application of the porapak q column extraction method for tomato flavor volatile analysis. *J Agric Food Chem* 50:3401–4.

Mares-Perlman JA, Klein R. 1999. Diet and age-related macular degeneration. In Taylor A (ed.), *Nutritional and Environmental Influences on the Eye*. Boca Raton, FL: CRC Press, pp. 181–214.

Martínez-Sánchez A, Gil-Izquierdo A, Gil MI, Ferreres F. 2008. A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species. *J Agric Food Chem* 56:2330–40.

Maruyama FT. 1970. Volatile compounds characterizing the aroma of fresh Atlantic and Pacific oysters. *J Food Sci* 35:540–52.

Masanetz C, Grosch W. 1998a. Crust aroma of baguettes. I. Key odorants of baguettes prepared in two different ways. *Z Lebensm-Unters Forsch A* 206:114–53.

Masanetz C, Grosch, W. 1998b. Key odorants of parsley leaves (*Petroselinum crispum* [Mill.] Nym. ssp. crispum) by odour activity values. *Flav Frag J* 13:115–24.

Maul F, Sargent SA, Sims CA, Baldwin EA, Balaban MO, Huber DJ. 2000. Chilling injury adversely affects aroma volatile production in mango during fruit ripening. *J Food Sci* 65:1228–36.

Mayer F, Takeoka G, Buttery R, Nam Y, Naim M, Bezman Y, Rabinowitch H. 2003a. Fruits and vegetables of moderate climate. *ACS Symp Ser Am Chem Soc* 1:136–44.

Mayer F, Takeoka G, Buttery R, Nam Y, Naim M, Bezman Y, Rabinowitch H. 2003b. Effects of low-oxygen atmosphere on ethanolic fermentation in fresh-cut carrots. *Freshness Shelf Life Foods* 836:144–54.

Mondy N, Duplat D, Christides JP, Arnault I, Auger J. 2002. Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction-liquid extraction and gas chromatography-mass spectrometry. *J Chromatogr A* 963:89–93.

Moon YJ, Wang XD, Morris ME. 2006. Dietary flavonoids: Effects on xenobiotic and carcinogen metabolism. *Toxicol In Vitro* 20:187–210.

Mortensen A, Skibsted LH, Truscott TG. 2001. The interaction of dietary carotenoids with radical species. *Arch Biochem Biophys* 385:13–19.

Mou B. 2005. Genetic variation of beta-carotene and lutein contents in lettuce. *J Am Soc Hortic Sci* 130:870–6.

Naczk M, Shahidi F. 2007. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J Pharm Biomed Anal* 43:798–803.

Nail PS, Niyogi KK, Björkman O, Grossman AR. 2003. Genetic manipulation of carotenoid pathway in higher plants. *Curr Sci* 85:1423–30.

Negre-Salvayre A, Salvayre R. 1992. Quercetin prevents the cytotoxicity of oxidised LDL on lymphoid cell lines. *Free Radic Biol Med* 12:101–6.

Nemeth K, Piskula MK. 2007. Food content, processing, absorption and metabolism of onion flavonoids. *Crit Rev Food Sci Nutr* 47:397–409.

Nesterenko S, Sink KC. 2003. Carotenoid profiles of potato breeding lines and selected cultivars. *HortScience* 38:1173–7.

Nicolle C, Simon G, Rock E, Amouroux P, Remesy C. 2004. Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. *J Amer Soc Hort Sci* 129:523–9.

Nielsen GS, Larsen LM, Poll L. 2003. Chemical and functional properties of food. *J Agric Food Chem* 51:1970–2012.

Nielsen GS, Poll L. 2004. Determination of odor active aroma compounds in freshly cut leek (*Allium ampeloprasum* var. bulga) and in long-term stored frozen unblanched and blanched leek slices by gas chromatography olfactometry analysis. *J Agric Food Chem* 52:1642–54.

Nielsen JK, Olsen CE, Petersen MK. 1993. Acylated flavonol glycosides from cabbage leaves. *Phytochemistry* 34:539–44.

Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen AM. 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *Am J Clin Nutr* 74:418–26.

Niyogi KK, Björkman O, Grossman AR. 1997. Chlamydomonas xanthophyll cycle mutants identified by video imaging of chlorophyll fluorescence quenching. *Plant Cell* 9:1369–80.

Noteborn HP, Jansen Benito S, Mengelers MJB. 1997. Oral absorption and metabolism of quercetin and sugar-conjugated derivatives in specific transport systems. *Cancer Lett* 114:175–7.

Okamoto D, Noguchi Y, Muro T, Morishita M. 2006. Genetic variation of quercetin glucoside content in onion (*Allium cepa* L.). *J Jpn Soc Hortic Sci* 75:100–8.

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silver-stone AL, Drake R. 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat Biotechnol* 23:482–7.

Park YK, Lee CY. 1996. Identification of isorhamnetin 4-glucoside in onions. *J Agric Food Chem* 44:34–6.

Patil BS, Pike LM. 1995. Distribution of quercetin content in different rings of various coloured onion (*Allium cepa* L.) cultivars. *J Hortic Sci* 70:643–50.

Peffley EB, Lombard KA, Geoffriau E. 2004. Total quercetin content in onion: survey of cultivars grown at various locations. *Horttechnology* 14:628–30.

Peters AM, Amerongen AV. 1998. Relationship between levels of sesquiterpene lactones in chicory and sensory evaluation. *J Amer Soc Hort Sci* 123(2):326–9.

Petersen MA, Poll L, Larsen LM. 1998a. Comparison of volatiles in raw and boiled potatoes using a mild extraction technique combined with GC odour profiling and GC-MS. *Food Chem* 61:461–6.

Petersen MA, Poll L, Larsen LM. 1998b. Effects of high CO_2 level on the titres of aminobutyric acid, total polyamines and some pathogenesis-related proteins in cherimoya fruit stored at low temperature. *Food Chem* 61:461–5.

Picardi SM, Issenberg P. 1973. Volatile compounds from *Pencillium* sp. contributing musty-earthy notes to brie and camembert cheese flavors. *J Agric Food Chem* 21:959–89.

Price KR, Casuscelli F, Colquhoun IJ, Rhodes MJC. 1997. Hydroxycinnamic acid esters from broccoli florets. *Phytochemistry* 45:1683–7.

Price KR, Rhodes MJC. 1997. Analysis of the major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. *J Sci Food Agric* 74:331–9.

Rao AV, Ali A. 2007. Biologically active phytochemicals in human health: *Lycopene. Inter J Food Prop* 10:279–88.

Rao AV, Shen H. 2002. Effect of low dose lycopene intake only copene bioavailability and oxidative stress. *Nutr Res* 22:1125–31.

Rao LJM, Nagalakshmi S, Naik JP, Shankaracharya NB. 2000. Studies on character impact odorants of coffee brews. *J Food Sci Technol-Mysore* 37:631–5.

Reboul E, Borel P, Mikail C, Abou C, Charbonnier M, Caris-Veyrat C, Goupy P, Portugal D, Lairon MJ. 2005. Enrichment of tomato paste with 6% tomato peel increases lycopene and β-carotene bioavailability in men. *J Nutr* 135:790–4.

Rodriguez-Amaya DB. 1999. Changes in carotenoids during processing and storage of foods. *Arch Latinoam Nutr* 49:38–47.

Romer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM. 2000. Elevation of the provitamin A content of transgenic tomato plants. *Nat Biotechnol* 18:666–9.

Römer S, Lübeck J, Kauder F, Steiger S, Adomat C, Sandman G. 2002. Genetic engineering of azeaxanthin-rich potato by antisense inactivation and co-suppression of a carotenoid epoxidation. *Metab Eng* 4:263–72.

Ross JA, Kasum CM. 2002. Dietary flavonoids: Bioavailability, metabolic effects, and safety. *Ann Rev Nutr* 22:19–34.

Russo VM, Howard LR. 2002. Carotenoids in pungent and non-pungent peppers at various developmental stages grown in the field and glass house. *J Sci Food Agric* 82:615–24.

Rusznyák S, Szent-Gryörgi A. 1936. Vitamin nature of flavones. *Nature* 4(138):798.

Sandmann G. 2001. Carotenoid biosynthesis and biotechnological applications. *Arch Biochem Biophys* 385:4–12.

Sandmann G, Romer S, Fraser PD. 2006. Understanding carotenoid metabolism as a necessity for genetic engineering of crop plants. *Metab Eng* 8:291–302.

Scalbert A, Williamson G. 2000. Dietary intake and bioavailability of polyphenols. *J Nutr* 130:2073S–85S.

Schaub P, Al-Babili S, Drake R, Beyer P. 2005. Why is golden rice golden (yellow) instead of red? *Plant Physiol* 138:441–50.

Schieberle P, Ofner S, Grosch W. 1990. A comparison of the aromas of six coffees characterized by conventional profiling, free-choice profiling and similarity scaling methods. *J Food Sci* 55:193–209.

Schlatter J, Luethy J. 1986. Vorkomen and Toxizitate Von Phytoalexinen in Pflanzlichen Lebensmitteln. *Mitt Geb Lebensmitteleunters Hyg* 77:404–19.

Schnitzler WH, Broda S, Schaller RG. 2003. Descriptive profiling versus direct similarity assessments of soft drinks. *J Appl Bot* 77:53–8.

Schulz H, Kruger H, Liebmann J, Peterka H. 1998. Distribution of volatile sulfur compounds in an interspecific hybrid between onion (*Allium cepa* L.) and leek (*Allium porrum* L.). *J Agric Food Chem* 46:5220–43.

Seifried HE, McDonald SS, Anderson DE, Greenwald P, John A, Milner JA. 2003. The antioxidant conundrum in cancer. *Cancer Res* 63:4295–8.

Sessa RA, Bennett MH, Lewis MJ, Mansfield JW, Beale MH. 2000. Metabolite profiling of sesquiterpene lactones from Lactura species. Major latex components are novel oxalate and sulfate conjugates of lactucin and its derivatives. *J Biol Chem* 275:26877–84.

Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY. 1999. Seed-specific overexpression of phytoene syn-thase: Increase in carotenoids and other metabolic effects. *Plant J* 20:401–12X.

Shi J, Le Maguer M. 2000. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Crit Rev Biotechnol* 204:293–334.

Simonne AH, Simonne EH, Eitenmiller RR, Mills HA, Green NR. 2000. Retention and changes of soy isoflavones and carotenoids in immature soybean seeds (Edamame) during processing. *J Agric Food Chem* 48:6061–9.

Simonne AH, Smith M, Weaver DB, Vail T, Barnes S, Wei CI. 1997. Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). *J Food Comp Anal* 10:299–311.

Singh B, Bhat TK, Singh B. 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. Review. *J Agric Food Chem* 51:5579–97.

Slimestad R, Fossen T, Vagen IM. 2007. Onions: A sourse of uniques diatry flavanoids. *J Agric Food Chem* 55:1067–80.

Sommerburg O, Keunen JEE, Bird AC, van Kuijka FJGM. 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: The macular pigment in human eyes. *Br J Ophthalmol* 82:907–10.

Spanos GA, Wrolstad RE. 1992. Phenolics in apple, pear, and white grape juices and their changes with processing and storage-A review. *J Agric Food Chem* 40:1478–87.

Stahl-Biskup E, Intert F, Holthuijzen J, Stengele M, Schulz G. 1993. Glycosidically bound volatiles—A review 1986–1991. *Flav Frag* 8:61–75.

Stangl V, Lorenz M, Stangl K. 2006. The role of tea and tea flavonoids in cardiovascular health. *Mol Nutr Food Res* 50:218–28.

Stavric B. 1994. Quercetin in our diet: From potent mutagen to probable anti-carcinogen. *Clin Biochem* 27:245–8.

Suh HJ, Lee JM, Cho JS, Kim YS, Chung SH. 1999. Radical scavenging compounds in onion skin. *Food Res Int* 32:659–64.

Takahma U, Hirota S. 2000. Deglucosidation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales. *Plant Cell Physiol* 41:1021–9.

Takahama U, Oniki T, Hirota S. 2001. Phenolic components of brown scales of onion bulbs produce hydrogen peroxide by autooxidation. *J Plant Res* 114: 395–402.

Tang L, Jin T, Zeng X, Wang J-S. 2005. Lycopene inhibits the growth of human and rogen-independent prostate cancer cells in vitro and in BALB/cnudemice. *J Nutr* 135:287–90.

Tavani A, Gallus S, Negri E, Parpinel M, La Vecchia C. 2006. Dietary intake of carotenoids and retinol and the risk of myocardial infarction in Italy. *Free Radic Res* 40:659–64.

Taylor M, Ramsay G. 2005. Carotenoid biosynthesis in plant storage organs: Recent advances and prospects for improving plant food quality. *Physiol Plant* 124:143–51.

Thappa RK, Dhar AK, Balyan SS, Khan S, Raina P, Dhar PL, Choudhary DK. 2003. Characterization of volatiles in rambutan fruit (*Nephelium lappaceum* L.). *J Food Sci Technol-Mysore* 40:426–31.

Tirillini B, Pellegrino R, Pagiotti R, Pocceschi N, Menghini L. 2004. Volatile compounds in different cultivars of *Apium graveolens* L. *Ital J Food Sci* 16:477–82.

Tokarska B, Karwowska K. 1983. The role of sulphur compounds in evaluation of flavouring value of some plant raw materials. *Nahrung* 27:443–7.

Tokitomo Y, Kobayashi A. 1992. Volatile flavor compounds formed in an interspecific hybrid between onion and garlic. *Biosci Biotechnol Biochem* 56:1865–71.

Tomás-Barberán FA, Robins RJ. 1997. *Evolution of Juice Anthocyanins during Ripening of New Selected Pomegranate (Punica granatum) Clones*. *Phytochemistry of Fruits and Vegetables*, Vol. 21. Oxford: Clarendon, pp. 1438–2385.

Tóth Markus M, Takács Hájos M. 2001. Headspace-solid-phase microextraction in the analysis of the volatile fraction of aromatic and medicinal plants. *Acta Aliment* 30:219–21.

Touvier M, Kesse E, Clavel-Chapelon F, Boutron-Ruault MC. 2005. Dual association of beta-carotene with the risk of tobacco-related cancers in a cohort of French women. *J Natl Cancer Inst* 97:1338–44.

Tsushida T, Suzuki M. 1996. Content of flavonol glucosides and some properties of enzymes metabolizing the glucosides in onion. Flavonoids in fruits and vegetables. Part II. *Nippon Shokuhin Kagaku Kogaku Kaishi* 43:642–9.

Ulrich D, Krumbein A, Schonhof I, Hoberg E. 1998. Comparison of two sample preparation techniques for sniffing experiments with broccoli (*Brassica oleracea* var. *italica* Plenck). *Nahrung Food* 42:392–4.

Unlu NZ. 2005. Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocadooravocado oil. *J Nutr* 135:431–6.

Unlu NZ, Bohn T, Clinton ST, Schwartz SJ. 2005. Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocado or avocado oil. *J Nutr* 135:431–6.

Updike AA, Schwartz SJ. 2003. Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. *J Agric Food Chem* 51:6184–90.

Vallejo F, Tomás-Barberán FA, Ferreres F. 2004. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. italica) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. *J Chromatogr A* 1054:181–93.

Van Langenhove HJ, Cornelis CP, Schamp NM. 1991. Effects of O_2 and CO_2 partial pressure on senescence of oat leaves and broccoli miniflorets. New Zealand. *J Sci Food Agric* 55:483–92.

Varming C, Petersen, MA, Poll L. 2004. Fruits and vegetables of moderate climate. *J Agric Food Chem* 52:1647–867.

Verkerk R, Dekker M, Jongen WMF. 2001. Post-harvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. *J Sci Food Agric* 81:953–8.

Vinson JA, Jang J, Dabbagh YA, Serry MM, Cai S. 1995. Plant polyphenols exhibit lipoprotein-bond antioxidant activity using an in vitro oxidation model for heart disease. *J Agric Food Chem* 43:2798–9.

Vishnevetsky M, Ovadis M, Itzhaki H, Levy M, Libal WY, Adam Z, Vainstein A. 1996. Molecular cloning of a carotenoid-associated protein from *Cucumis sativus* corollas: Homologous genes involved in carotenoid sequestration in chromoplasts. *Plant J* 10:1111–18.

Vishnevetsky M, Ovadis M, Vainstein A. 1999. Carotenoid sequestration in plants: The role of carotenoid-associated proteins. *Trends Plant Sci* 4:232–5.

Walle T. 2004. Absorption and metabolism of flavonoids. *Free Radic Biol Med* 36:829–37.

Waterhouse AL. 2002. Wine phenolics. *Ann N Y Acad Sci* 957:21–36.

Wiczkowski W, Nemeth K, Bucinski A, Piskula MJ. 2003. Bioavailability of quercetin from flesh scales and dry onion skin of onion in rats. *Pol J Food Nutr Sci* 12:95–9.

Widder S, Lüntzel CS, Dittner T, Pickenhagen W. 2000. 3-Mercapto-2 methylpentan-1-ol, a new powerful aroma compound. *J Agric Food Chem* 48:418–23.

Wintherhalter P, Rouseff R. 2001. Carotenoid-derived aroma compounds: An introduction. In Wintherhalter P, Rouseff R (eds.), *Carotenoid-Derived Aroma Compounds*, Vol. 12. Washington, DC: American Chemical Society, pp. 1–42.

Wu K, Schwartz SJ, Platz EA, Clinton SK, Erdman JW, Ferruzzi JMG, Willett WC, Giovann EL. 2003. Variations in plasma lycopene and specific isomers over time in a cohort of U.S. men. *J Nutr* 133:1930–6.

Wu XL, Prior RL. 2005. Identification and characterization of antho-cyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United Sates: Vegetables, nuts and grains. *J Agric Food Chem* 53:3101–13.

Yeum KJ, Russell RM. 2002. Carotenoid bioavailability and bioconversion. *Annu Rev Nutr* 22:483–504.

Yilmaz E. 2001. Oxylipin pathway in the biosynthesis of fresh tomato volatiles. *Turk J Biol* 25:351–60.

Yoo KS, Pike LM, Hamilton BK. 1997. Sensory characterization of ewe's milk cheeses using direct and indirect similarity measures: A comparison. *HortScience* 32:714–21.

Young AJ, Lowe GM. 2001. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* 385:20–7.

Young JE, Zhao X, Carey EE, Welli R, Yang SS, Wang W. 2005. Phytochemical phenolics in organically grown vegetables. *Mol Nutr Food Res* 49:1136–42.

Zaripheh S, Erdman JW. 2002. Factors that influence the bioavailability of xanthophylls. *J Nutr* 132:531–4.

Zeb A, Mehmood S. 2004. Carotenoid content from various sources and their potential health applications. *Pak J Nutr* 3:199–204.

CHAPTER 33

Traditional and New Analytical Methodology

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INTRODUCTION

The sensory properties of food are important determinants in the choice of foodstuffs by the consumer, and flavor plays a prominent role, with clear links to consumer preferences. Flavor may be defined as the combination of taste and odor, sensations of pain, heat, and cold (chemesthesis or trigeminal sensitivity), and tactile sensation. Sensory analysis is clearly the most valid means of measuring flavor characteristics. Applied to vegetable flavor, sensory evaluation is a prominent descriptive tool that is used widely in academia and industry. However, measuring flavor also means primarily analyzing volatile compounds that are sensed in the nose at the olfactory receptors either via the orthonasal (odor) or retronasal (aroma) routes when foods are eaten. The main reason for this is the major importance of aroma in the overall flavor of a food, as is easily demonstrated by the difficulties encountered by subjects attempting to identify a particular flavor if the airflow through their nostrils is prevented, and the fact that volatile components are more amenable to conventional instrumental analysis than nonvolatile compounds. The analysis of food flavors has dramatically improved over the past 50 years, mainly through the invention of new work-up procedures, modern separation techniques (high-resolution gas chromatography [GC] on fused silica capillaries, high-performance liquid chromatography), and more sensitive spectroscopic methods (mass spectrometry, infrared spectroscopy, nuclear magnetic resonance).

Instrumental analysis of aroma volatiles has been the subject of important specialized treatises (for the most relevant literature on the subject, see Ho and Manley 1993; Mussinan and Morello 1998; Marsili 1997; Stephan et al. 2000; van Ruth 2001b; Reineccius 2002, 2006; Deibler and Delwiche 2004).

Aroma compounds are mainly hydrophobic, and instrumental analysis of volatiles must consider, as a first step, an extraction method suitable for separating these

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hydrophobic volatiles from the food matrix in order to obtain information that is representative of the studied material. However, as no single method yields a "true" picture of a food aroma (Reineccius 2002, 2006), complete identification of flavor volatiles is rarely attained with a single sample preparation. Generally, a combination of two or more separation and identification techniques is needed, especially for analysis of complex matrixes such as fruits and vegetables, but isolation and analysis of aroma remain challenging (Teranishi 1998). Moreover, not only may the extraction step lead to artifacts, but the total volatile content in most cases is very difficult to relate to the flavor profile determined by a panel or experienced by a consumer. Therefore, it appears much more efficient to concentrate efforts on the identification of those compounds that are really relevant to the perceived flavor. As no universal extraction method exists, some authors claim that it is essential to choose a method that yields an extract as representative as possible of the sensory properties of the food (Abbott et al. 1993a,b; Etiévant and Langlois 1998; Etiévant et al. 1994; Mehinagic et al. 2003). Once this extraction method has been chosen, the next steps involve various hyphenated techniques coupled to GC among which include gas chromatography–olfactometry (GC-O), which plays a prominent role in determining the key volatile compounds that contribute significantly to the flavor of the food (Lee 2003; Leland et al. 2001), and gas chromatography–mass spectrometry (GC-MS), which is essential for the identification of those key odorants.

As it is still not known how the various volatiles combine to produce an overall sensory impression, it is particularly difficult to predict an aroma perception on the basis of GC-O data only. Demanding recombination experiments have to be undertaken (Buettner and Schieberle 2001a,b; Grosch 2001), even though significant advances have been made recently in the comprehension of odorant mixtures (Atanasova et al. 2004, 2005). Moreover, interactions between taste and aroma (Noble 1996) and interactions of trigeminal sensations with taste and aroma (Green 1996) occur and play an important role in global flavor perception. However, methods that allow direct analysis of flavor molecules released in the mouth during consumption have been developed in recent years (Roberts and Taylor 2000; Taylor and Linforth 1996). Development of instrumental techniques and data obtained recently for volatile flavor compounds will be presented, with a particular attention to vegetable flavors, with the aim to explain more closely the relationship between aroma perception and food volatile composition.

Finally, specific instrumental techniques have been developed for the global analysis of food flavor. The methods currently used in the quality control of food flavor are still usually based on sensory evaluation by a panel of experts. These panels are able to monitor the quality of a particular food, to detect defects, and to compare samples for classification purposes. Nevertheless, obtaining results rapidly at low cost using instruments could be desirable. The so-called electronic noses based on gas sensor technology, despite some important drawbacks for some of them (Schaller et al. 2000), are theoretically able to perform some classification tasks (Schaller et al. 1998), and some applications for the analysis of fruits and vegetables have been developed (see, for instance, Gomez et al. 2008 for tomatoes). However, two other global analysis methods based on mass spectrometry seem more powerful and reliable for classification purposes. The first one analyzes total headspace (HS) using a mass spectrometer, without any prior GC separation (Vernat and Berdagué 1995). This method is often referred to as a mass-based electronic nose. Alternatively,

HS sampling may be replaced by solid phase microextraction (SPME) of food volatiles (Marsili 1999). Both sampling methods, followed directly by mass spectrometry (Pérès et al. 2003), have found applications for the rapid characterization of food flavor (for fruit and vegetable flavor, see, e.g., Berna et al. 2004, 2005 for tomato). The second method is pyrolysis mass spectrometry (Aries and Gutteridge 1987), where a small food sample is pyrolyzed at up to 500°C. The resulting volatile fraction, characteristic of the flavor but also of the matrix composition, is analyzed by a mass spectrometer. For all the rapid instrumental methods used for classification, a pattern or fingerprint is obtained for each sample, and extensive data treatment, either by conventional multivariate statistics or by artificial neural networks, is necessary for classification and quality control purposes (Aries and Gutteridge 1987; Pérès et al. 2003).

CHARACTERIZATION OF AROMA (VOLATILES)

Sample Treatment

Volatile aroma compounds in foodstuffs are typically hydrophobic, are generally distributed in a heterogeneous manner throughout a food matrix, and are present at low or even traces (<10 μg/kg) concentrations. Their analysis requires homogenization of the sample prior to extraction, where isolation procedures adapted to hydrophobic material dispersed in trace amounts in the food are required. These compounds can be isolated from liquid or solid material by an extraction or distillation procedure. Next, they can be separated by liquid chromatography or by GC. Finally, these components are identified by their chromatographic retention behavior and by different spectroscopic methods.

Isolation Techniques

A number of different techniques, ranging from conventional solvent extraction and distillation to the newly developed direct thermal desorption (DTD) and SPME (or stir bar methodology) are available for isolating flavors from diverse food systems. Each of these techniques has its own applications with advantages and limitations.

Direct Extraction Procedures In direct extraction procedures, the components are extracted due to their distribution between two different phases. The techniques most commonly employed are liquid–liquid extraction (LLE), solid phase extraction (SPE), and HS extraction.

LLE This is a process for separating components in solution by their distribution between two immiscible liquid phases (Robbins and Cusack 1997). This extraction method, frequently followed by concentration under nitrogen or in a rotary evaporator, was one of the earliest methods used to recover flavor compounds from foods.

The basis for separation by this two-phase system is the selective distribution of compounds between two liquid phases. Distribution is characterized by the partition coefficient, K, defined as the concentration in the low-density phase, C_L, divided by the concentration in the high-density phase, C_H:

$$K = C_L / C_H$$

The selection of the solvent for extraction is one of the most important criteria in LLE. Solvents differ in their extraction capabilities depending on their own and the chemical structure of the solutes. The desired properties of solvents are a high distribution coefficient, good selectivity toward the solutes, and little or no miscibility with the feed solution. Other factors affecting solvent selection are boiling point, density, interfacial tension, viscosity, corrosiveness, flammability, toxicity, stability, compatibility with product, availability, and cost. Even though LLE is frequently replaced by other solvent-free techniques, it is still used in flavor and aroma analysis, especially for the collection of preliminary data.

Lopez and Gomez (2000) addressed some operational parameters in the application of LLE to extract aroma from wines. They compared several solvents (diethyl ether, n-pentane, freon-11, 1:1 ether/pentane, 1:1 ether/hexane, and dichloromethane) for the extraction of odorant terpenic components from "artificial wine" (12% v/v ethanol in water). It was concluded that dichloromethane and 1:1 ether/pentane are the best solvents for the extraction of these analytes from wine. This study also showed that the use of diethyl ether, which is employed to extract aroma from wine, does not result in a very high recovery of the following compounds: ethyl butanoate, 2-methyl propanol, 3-methyl butanol, hexanol, and so on, in comparison with freon-11, and dichloromethane. Today, the reference technique for the extraction of volatile components from wine is continuous LLE (Villen et al. 1995; Zhou et al. 1996). In this method, all volatile compounds (low, medium, and high volatility) have a high partition coefficient to the organic phase. However, it requires solvent evaporation, which in some cases involves loss or degradation of some of the components and formation of others that were not originally in wine (Ortega-Heras et al. 2002). Petersen and others (1998) compared the aromas of raw and boiled potatoes using the LLE on a mixture of peeled shredded potatoes with diethyl ether/pentane as solvent. GC-MS and GC–sniffing analysis of the extracts showed that the change in aroma during the boiling of potatoes depends on compounds both from lipid oxidation and from other types of reactions, for instance, the Strecker degradation.

Such extractions, using organic solvents, are simple and direct, but there are several valid objections to the method:

- The high-purity solvents are expensive; the organic solvent must be scrupulously purified (Ferreira et al. 1993) in order to avoid artifacts.
- The "perfect" solvent does not exist as no solvent can extract all the compounds.
- In general, the extracts are analyzed by GC, by injection in split mode, getting rid of the excess solvent. This means that to reach the sensitivity required, the substances that are being quantified often have to be concentrated. During this concentration step, discrimination processes can take place. Not only can the impurities from the organic solvent be concentrated, but also only some of the compounds will be concentrated and some of the highly volatile substances will be lost. Thus, not all of the compounds claimed to be present will still be there (Grob and Müller 1987).
- Moreover, many organic solvents are toxic, flammable, and undesirable pollutants.

All these objections can be avoided if another type of solvent is used: a supercritical fluid.

Supercritical Fluid Extraction (SFE) This has been regarded as a promising alternative technique to other more conventional extraction procedures, chiefly because the dissolving power of supercritical fluids can be adjusted by regulating the pressure and temperature conditions employed. Carbon dioxide (CO_2) is the most commonly used supercritical fluid in the food industry because of its low critical point ($T_c = 31.1°C$ and $P_c = 7.38$ MPa). Moreover, this fluid is nontoxic (except at high concentrations in air), nonflammable, chemically stable, and available in high purity at low cost. It is an inert gas that does not react with food constituents and is easily removable from the extract. This explains why CO_2 has been widely used for the aroma analysis of different spices, fruits, or vegetables (Blanch et al. 1994; Diaz-Maroto et al. 2002; Mau et al. 2003; Polesello et al. 1993; Saito et al. 1991). In fact, one of the main advantages afforded by SFE is the retention of volatile substances at temperatures below 0°C using a CO_2-based cryogenic system and the transfer of analytes from the extraction solvent (supercritical CO_2) to the reconstitution solvent without the need for aggressive solvents and treatments.

Sonsuzer and others (2004) optimized the isolation of aroma compounds from *Thymbra spicata*, a thyme-like plant, using SFE extraction. The parameters to optimize were temperature (40, 50, and 60°C), pressure (8, 10, and 12 MPa), and time (30, 60, and 90 min). Dependent variables were yield and monoterpene, sesquiterpene, and oxygenated monoterpene contents. The most significant parameter was pressure. An increase in pressure increased the extraction yield. An increase in temperature produced a decrease in the solubility of oil components. The extraction of sesquiterpenes and oxygenated compounds was more difficult due to their higher molecular weight and polarity, respectively, as compared with monoterpenes. Supercritical CO_2 is a poor extractor for polar substances. Therefore, for such analytes, the addition of modifiers or the use of other fluids, such as supercritical water, is advisable. Kubatova and others (2001) compared water–SFE and CO_2–SFE to hydrodistillation for the separation of aromatic essential oils from savory and peppermint. They showed that water–SFE was highly selective for polar oxygenated flavor compounds compared to CO_2–SFE and hydrodistillation.

SPE This is defined as a process for extraction of the analyte from a liquid matrix to a solid support (adsorbent) by adsorption. The adsorbents used in SPE are bonded silica and various polymeric resins of varying hydrophobicity and with different selectivities. This technique can be directly applied to isolate odorants from liquid or liquefiable samples, such as beverages, fruit pulp, and vegetable tissues. The advantage of this technique in comparison to LLE is that the recovery of analytes is done with a small quantity of solvent. Thus, the obtained extract needs almost no concentration, which is necessary with LLE. The sensitivity of SPE is very high.

A typical contemporary application of SPE to aroma analysis was presented by Lopez and others (2002), who studied the extraction of minor and trace volatile compounds in wine. Wine samples were passed through an SPE resin and the elution carried out with dichloromethane. The extracts were directly analyzed by GC–ion trap MS without further concentration. The recovery in the SPE isolation was higher than 90% for many extracted volatiles, except for guaiacol, vanillin,

2,6-dimethoxyphenol, and 4-vinylphenol. For samples such as fruits or other solids, SPE can be combined with isolation techniques such as distillation. Boulanger and Crouzet (2000) used the XAD-2 resin to recover the volatiles extracted previously by distillation from cupuaçu pulp. Mehinagic and others (2003) combined SPE with vacuum hydrodistillation in order to concentrate the aqueous extract obtained from this isolation technique on fresh apple fruit. The aqueous extract was passed through styrene divinylbenzene-based SPE columns. The adsorbed volatiles were then recovered by elution with pentane/diethyl ether and were analyzed by GC–flame ionization detector (FID) and GC-MS. In this study, SPE was used as a concentration technique and was compared to another concentration technique, LLE. The study showed that both techniques gave very similar results.

HS Extraction This can be defined as a method used for obtaining information about the composition, the nature, or state of liquid and solid bodies by the analysis of the gas phase with which they come into contact (Ioffe 1984). This technique has been applied widely in food science (Macku et al. 1988; Wang et al. 1996; Young and Hovis 1990). The technique of HS extraction of volatiles can be divided into three groups (Fig. 33.1):

1. *Static HS (Fig. 33.1A).* This procedure involves the equilibration of the volatile analytes within the sample with the vapor phase at a defined temperature. The method requires rigid control of the sample temperature, sample withdrawal, and other parameters like time and pressure. The concentration of the analytes in the phases does not change once the state of equilibrium is attained. However, the state of equilibrium is disturbed temporarily upon sampling. Therefore, the volume of the sample as well as the method of withdrawal must be chosen with precaution. The samples analyzed by this procedure can be liquid and solid solutions.

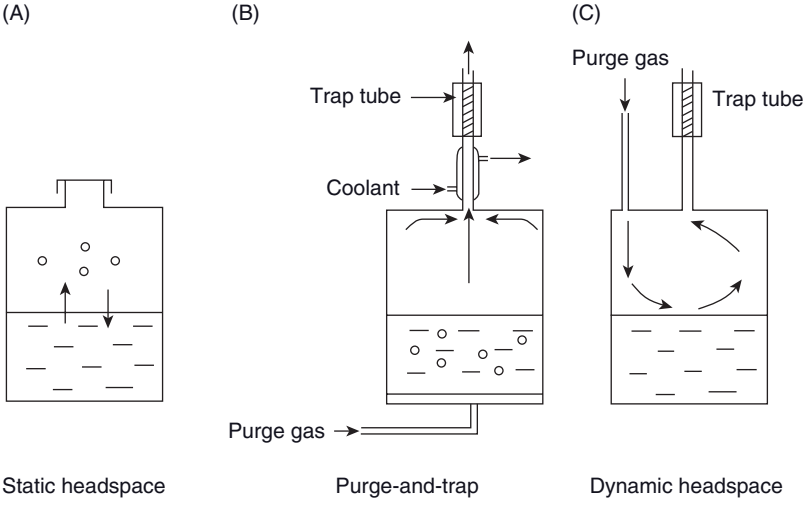

Figure 33.1. Diagram of three different methods of headspace extraction.

2. *Purge-and-Trap HS (Fig. 33.1B).* This procedure involves passing a carrier gas through the liquid material for a selected period of time. Generally, this gaseous effluent is passed through a suitable trapping medium, inert to the stripping gas, where the volatiles are trapped and are subsequently eluted by a solvent or thermally desorbed into the gas chromatograph . This procedure is suitable for liquid products and solutions.
3. *Dynamic HS (Fig. 33.1C).* This procedure is close to the purge-and-trap procedure except that the gaseous effluent is passed over and not through the material. The surface contact between gas and material is less and it may take longer for the analysis. However, this technique is suitable for extractions on solid matrices that cannot be solubilized.

The static HS technique fails when trace components or components with very low vapor pressure are analyzed. The application of this technique is limited by low sensitivity (Wampler 1997). In this case, the concentration of the analytes in the gas phase can be improved by increasing the temperature (Kieckbusch and King 1979; Przyjazny et al. 1983) and by adding salts (Seto 1994). Other factors can improve the extraction of volatiles: increasing the volume of the sample, increasing the equilibration time, or stirring.

The dynamic HS methods resolve this problem of sensitivity as the equilibrium between the food and the HS is constantly renewed (Buttery and Ling 1996). Variations of the dynamic HS method include

- sample purging, where the volatiles are bubbled or swept with a carrier gas;
- sample trapping, where the purged volatiles are trapped by physical (cold) or chemical adsorption (using various adsorbents);
- the relative temperature and duration of purging and trapping; and
- the desorption method, either choice of solvent or thermal desorption.

Desorption of trapped analytes for subsequent analysis can be performed either by elution, with a small quantity of an appropriate solvent, or by using online automated desorption devices, which are more suitable for routine analysis. The choice of desorption method is dependent on the volatile compounds to be extracted. Buttery (1993) studied the liberation of Z-3-hexenal from fresh tomatoes using dynamic HS extraction. To elute trapped compounds, ether was used rather than the more common thermal desorption method, which could cause molecular rearrangement of Z-3-hexenal. However, thermal desorption has several advantages compared to solvent desorption: improved detection limits, no interference of the solvent peak on chromatograms, and a simpler procedure. Moreover, desorption can be automated, which allows the analysis of the evolution of different products over time. Dirinck and others (1989) used this technique, coupled with GS/MS, to study the aroma development in apples during the complete ripening process. They showed that the dynamic HS sampling of the volatiles from intact fruits is a convenient procedure for the study of the influence of different external and internal factors on aroma development in ripening fruits.

Agelopoulos and others (1999) studied the temporal emission pattern of the volatile compounds released by the leaves of potatoes and broad beans by using

dynamic HS coupled to GS/MS. They showed that thermal desorption provided better detectability of volatile compounds than solvent desorption and therefore required reduced sampling times. The introduction of artifacts produced by degradation of the sorbent can be a major difficulty in dynamic HS extraction coupled to thermal desorption (Canac-Arteaga et al. 2000).

Distillation Methods Steam distillation and hydrodistillation are traditional distillation procedures for the isolation of volatile aromatic compounds from food and detached parts of plants. Being simple and straightforward procedures, they are still extensively applied for flavor characterization either alone or combined with other procedures. Distillation is usually carried out in two slightly different ways:

- In the first, the matrix to be extracted is mixed or suspended with water in a suitable vessel fitted with a condenser and, while the mixture is boiled, a condensate phase is collected. This procedure is called hydrodistillation.
- In the second procedure, steam is passed through a vessel containing a mixture of the matrix in water to yield a similar condensate. This procedure is called steam distillation.

Simultaneous Distillation Extraction (SDE) SDE, also known as the Likens–Nickerson (Likens and Nickerson 1964) method (Fig. 33.2), is a widespread distillation-based sample preparation method for the chemical analysis of fragrance and aroma.

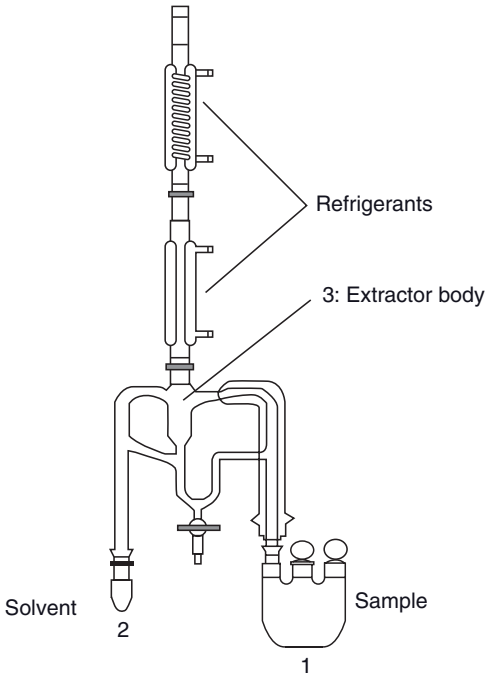

Figure 33.2. Likens–Nickerson apparatus.

The Likens–Nickerson apparatus has been used on different products and has been modified by a number of laboratories, but the principle stays the same (Blanch et al. 1993; Godefroot et al. 1981; Pollien and Chaintreau 1997; Pollien et al. 1998; Schultz et al. 1977). A sample, along with distilled water, is contained in flask 1, and flask 2 receives a suitable volume of an extracting solvent (dichloromethane, chloroform, etc.). Flasks 1 and 2 are heated; the solvent and water vapors are conducted to the extractor body (3), where they are condensed thanks to the refrigerants. In this operation, aroma compounds are removed from the matrix by water vapor directly and are transferred to the organic phase when the liquids condense together. Both water and solvent are collected in the extractor body after condensation and then return to their flasks, allowing continuous reflux.

Factors affecting the extraction of volatiles by SDE are the choice of solvent (density and selectivity), the temperature of heating, and the length of extraction.

Andrade and others (2000) used SDE in order to analyze the aromatic composition of 15 varieties of cultivated mango, originating from different regions. They showed that mango cultivars could be classified into three aroma groups: the first group is rich in α-terpinolene (eight varieties, originating from Sri Lanka, Australia, and Florida); the second is rich in Δ3-carene (three varieties, originating from Venezuela); and the third group is rich in myrcene and (Z)-β-ocimene (four varieties, originating from India and Sri Lanka, respectively). Similar studies were carried out on the jackfruit (*Artocarpus heterophyllus* Lam.) grown in the Amazon. Maia and others (2004) studied the difference between the aroma composition of "hard" and "soft" jackfruits and showed that the aroma concentrate of hard fruits was dominated by isopentyl valerate (28.4%) and butyl isovalerate (25.6%), while that of soft fruits was dominated by isopentyl isovalerate (18.3%), butyl acetate (16.5%), and ethyl isovalerate (14.4%). Another interesting study was described by Blanch and others (1996). They investigated the potential of the SDE technique for the rapid enrichment of wine aroma compounds. Several aspects concerning the extraction and concentration of volatiles from aqueous-alcoholic samples were studied, and three different operating modes were explored: SDE at normal pressure, SDE at reduced pressure, and SDE involving the concentration of dynamic HS due to purging the sample with an inert gas. They concluded that the three investigated operation modes were suitable for the aroma analysis of wine. SDE at normal pressure provided recoveries ranging from 79% to 100% for some compounds previously reported as wine aroma constituents (e.g., isoamyl acetate, ethyl hexanoate, terpinolene, 1-hexanol, and benzaldehyde). This operation method, however, demands a higher sample temperature than the other two operating modes, which is an important aspect to consider if thermolabile solutes are analyzed or if thermally generated artifacts form from the matrix during heating.

Steam Distillation This can be carried out under atmospheric or reduced pressure in order to avoid the thermal degradation of products. The principle is the same: volatile compounds as well as water vapor are liberated from the product and are led into one or more cooled traps. The extraction under atmospheric pressure is attractive because the rate of extraction of volatiles is higher and the compounds are directly condensed in water. However, the products must be heated so that the water can be evaporated, and this produces many artifacts (degradation of some labile compounds, such as esters and lactones, and formation of new compounds).

This technique is therefore reserved for the extraction of essential oils from spices and from aromatic or medicinal plants. Saritas and others (2001) isolated the essential oils from aromatic lichens by hydrodistillation of fresh and dried plant parts. Combining GS/MS and ^{13}C-NMR techniques, they identified several terpenoid and aliphatic compounds in their extracts, including two unreported sesquiterpenes.

For other products, this technique is not recommended. It is preferable to use vacuum hydrodistillation. Figure 33.3 shows one of the pieces of apparatus proposed by Forss and Holloway (1967) for vacuum hydrodistillation. This apparatus was used for the extraction of volatiles from fresh products such as apples (Mehinagic et al. 2003). A quantity of the fragrance-generating sample, along with distilled water, is contained in flask 1, while flask 2 receives most of the condensed water cooled by the system of refrigeration. Some very polar compounds are condensed in flask 2 with water vapor, and others are captured in traps plunged into liquid nitrogen.

The application of this technique to model solutions showed that it can extract more than 80% of substances with boiling points lower than 150°C at concentrations of less than 1 ppm (Forss and Holloway 1967). Vacuum hydrodistillation is very useful for studying fresh products as the sample does not need to be heated. In fact, in order to avoid cooked notes and artifacts, flavor analysis of fresh products, which are eaten in the raw state, needs to be carried out in a very gentle way. Güntert and others (1998) compared three extraction methods: vacuum hydrodistillation, dynamic HS, and the SDE method on different fruits: yellow passion fruit, strawberry, raspberry, pear, cherry, and so on. They showed that dynamic HS extracts are more dominated by the lower boiling point components, while the vacuum hydrodistilled extracts are dominated by the higher boiling point components. They found that vacuum hydrodistillation clearly offers superior performance on fruits with respect to the sensory impression of the resulting extracts. Sensory evaluation of the vacuum HS extracts of various fruits led to very fruit-typical descriptions and, consequently, the qualitative and quantitative flavor patterns of the analyzed fruits represented the genuine fruit flavors with no artifacts.

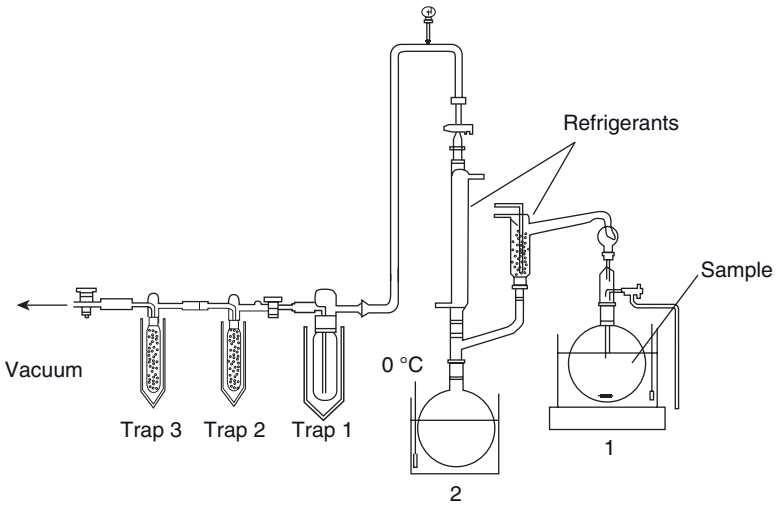

Figure 33.3. Apparatus for vacuum distillation proposed by Forss and Holloway (1967).

Sorptive Extraction Methods All the extraction procedures used to isolate the volatile fraction from the food matrix should be adapted to the analysis of trace levels of hydrophobic molecules generally present in a polyphasic medium while minimizing losses of highly volatile molecules and preventing modification of compounds or the formation of artifacts.

SPME First developed for the extraction of volatile organic compounds in water (see Lord and Pawliszyn 2000 for an authoritative review on the technology), this method has been applied in the last 10 years to the isolation of aroma compounds from food (Harmon 1997; Kataoka et al. 2000; Pillonel et al. 2002; Reineccius 2002). Numerous applications deal with fruit flavors, for instance, strawberry (Ibanez et al. 1998; Song et al. 1998; Reid et al. 2004; Ulrich et al. 1997a,b), apple (Matich et al. 1996; Schulz et al. 2003), orange juice (Da Porto et al. 2003; Jia et al. 1998; Rega et al. 2003; Steffen and Pawliszyn 1996), kiwifruits (Wan et al. 1999), Brazilian fruits (Augusto et al. 2000), cantaloupe (Beaulieu and Grimm 2001), black currant (Ruiz del Castillo and Dobson 2002), pear, peach, and apricot (Riu-Aumatell et al. 2004). Some applications relate to vegetables, for instance, onions and leeks (Mondy et al. 2002), tomato (Song et al. 1998), and others to vegetable oils (Cavalli et al. 2004; Jelen et al. 2000; Keszler et al. 1998; Mildner-Szkudlarz et al. 2003). SPME partitions analytes between a liquid or a vapor phase and a thin solid phase adsorbent, of which there are several choices in terms of polarity and film thickness (Kataoka et al. 2000). Adsorbents are coated on inert fibers, generally associated with a syringe that serves as a direct injection device (Harmon 1997; Kataoka et al. 2000). The method, which is an equilibrium one, can be performed either in the direct extraction mode (immersion of the fiber in sample matrix, generally in an aqueous solution or suspension) or more conventionally in an HS configuration. It can be automated very easily, but the extraction of the solutes depends on polarity, volatility, partition coefficients, sample volume, temperature, and the nature of the adsorbent coating material. The mechanisms affecting the analysis of flavor volatiles by SPME have been reviewed (Holt 2001). Therefore, the technique exhibits a certain degree of selectivity but with the advantages of sensitivity, ease of use, no solvent, and small sample volume (Harmon 1997; Kataoka et al. 2000; Pillonel et al. 2002; Reineccius 2002). Nevertheless, each extraction step, that is, extraction mode (direct or HS), selection of fiber coatings, extraction setup (concentration, time, agitation, temperature), and desorption, gains through a careful optimization for each application (Bicchi et al. 2000; Doleschall et al. 2003; Ferreira and de Pinho 2003; Kataoka et al. 2000; Liu and Yang 2002). SPME, used for the first time for the analyses of food volatile compounds in the mid-1990s (Chin et al. 1996; Pelusio et al. 1995; Yang and Peppard 1994), has since been used in significant applications on food aroma (Bredie and Petersen 2006; Kataoka et al. 2000; Le Quéré and Etiévant 2003; Pillonel et al. 2002; and references cited therein). SPME has demonstrated several advantages in terms of rapidity and simplicity over conventional extraction methods in some comparative studies carried out in the analysis of strawberry (Ulrich et al. 1995), fruit juices (Miller and Stuart 1999), or olive oils (Cavalli et al. 2003; Kanavouras et al. 2005). If HS SPME shows always better retention capacities than static HS (Miller and Stuart 1999), hence a better sensitivity, LLE (Ulrich et al. 1995), and dynamic HS with thermal desorption of Tenax® traps (Kanavouras et al. 2005) provide higher efficiency. Analyzing volatiles directly by immersion of the fiber in

highly complex matrices could damage the fiber, and SPME is therefore used almost always in the HS mode. Comparison of direct SPME and HS SPME of Camembert cheese volatiles obtained after cryotrapping of the aqueous phase under vacuum showed only a slight reduction in sensitivity using HS SPME compared to direct SPME (Jaillais et al. 1999). Compared to other HS extraction procedures, it is very often concluded that SPME is more appropriate for routine quality control due to its simplicity, repeatability, and low cost (Cavalli et al. 2003). That is probably why the method has been widely used in recent works on food aroma (Bredie and Petersen 2006; Le Quéré and Etiévant 2003; and references cited therein).

The main limitation of SPME is the relatively low extraction yield due to the relatively small amount of sorbent available on the syringe needle (typically ca. 0.5 μL). Some artifact formation has also been noticed. Artifacts due to the formation of Maillard products during the desorption step were noticed in the flavor analysis of strawberry and apple fruits (Verhoeven et al. 1997). A significant reduction in artifact formation was obtained by rinsing the fiber with water prior to thermal desorption. However, the formation of artifacts is often unavoidable, and intrinsic artifact formation during the analysis of volatile amines (Lestremau et al. 2001) and volatile sulfur compounds (Lestremau et al. 2004) in air has been reported. Recently, new internally cooled fibers have been described for the analysis of five tropical fruits (Carasek and Pawliszyn 2006). By reducing the operating adsorption and desorption temperatures, it is claimed that artifact formation is significantly reduced, and it was found that the cold fiber was the most appropriate one for the purpose of extracting volatile compounds from the five fruit pulps studied.

Stir Bar Sorptive Extraction (SBSE) A novel extraction technique that uses up to 200 μL of the sorbent polydimethylsiloxane (PDMS), SBSE was developed to reduce the disadvantage of a small absorption phase volume (Baltussen et al. 1999). This new technique consists of a glass-coated magnetic stir bar (typically 5-mm film thickness, 10-mm length) with a coating of PDMS that is spun in an aqueous medium for a predefined time (Baltussen et al. 1999). After completion of the extraction step, the stir bar is placed in a thermodesorption system and is desorbed at the head of a GC column after cryo-refocusing of the extracted material (Baltussen et al. 1999). A complete set of coated stir bars (called Twister™) and thermodesorption system is commercially available from Gerstel GmbH (Gerstel, Müllheim a/d Ruhr, Germany). As expected, the recoveries were higher for SBSE than for SPME (Baltussen et al. 1999), and the detection limits were found in the low nanogram per liter range for a wide selection of volatile and semivolatile analytes (Baltussen et al. 1999). SBSE has been used for the measurement of volatiles from a wide variety of liquid foods (Pillonel et al. 2002 and references cited therein) including, recently, coffee brew (Bicchi et al. 2002), wine (Kittel et al. 2004), and lemon beverages (Tredoux et al. 2000). Despite careful optimization of the method, some precision problems have been observed with very volatile and/or soluble compounds (Ibañez and Sola 2006). Artifacts due to the thermal desorption step have also been described in the flavor analysis of onions (Granvogl and Schieberle 2006).

Headspace Sorptive Extraction (HSSE) As an extension to SBSE, HSSE has been developed (Tienpont et al. 2000) to overcome the limitation of HS-SPME in terms of extraction capacity. Limits of detection in the nanogram per liter range have been

obtained for the analyses of volatiles of some food samples (Tienpont et al. 2000). HSSE bars coated with ca. 55-µL PDMS (commercially available from Gerstel GmbH) are suspended in the HS of the sample (Bicchi et al. 2002), and after sampling completion, the bars are thermally desorbed in a thermal desorption unit connected to a gas chromatograph (Bicchi et al. 2002). As expected, when comparing HS extractions of coffee (Bicchi et al. 2002) and olive oil (Cavalli et al. 2003) volatiles, HSSE bars showed a higher concentration capacity than SPME fibers due to the higher amount of polymeric coating. However, like SBSE, HSSE needs a thermal desorption unit to be handled and therefore requires a significant investment, compared to SPME if used in manual mode (automation is possible at a cost), without avoiding possible artifact formation. Nevertheless, as SBSE and HSSE coated bars are less subject to deterioration than SPME fibers, they can be applied easily to the analyses of both HS and liquid (Bicchi et al. 2002).

GC

As stated earlier, the choice of volatile isolation technique depends on the physical and biochemical properties of the product, as well as on the molecular characteristics of the volatiles to be isolated. However, it must not be forgotten that the obtained extracts will be analyzed by different techniques that necessitate different conditions too. In this chapter, we will focus on the standard approach to the analysis of volatiles by GC.

GC is suited to this role due to its excellent separating powers and extreme sensitivity (Eiceman et al. 1992; Stevenson et al. 1996).

GC is basically a separation technique and the separation of compounds occurs when the extract is injected into a mobile phase (inert gas). The mobile phase carries the injected extract through a stationary phase, which is composed of chemicals that can selectively attract compounds. Every gas chromatograph includes a source of gas as the mobile phase, an inlet to deliver the sample to a column, the column where separation occurs, an oven as a thermostat for the column, a detector to register the presence of a chemical in the column effluent, and a data system to record and display the chromatogram (Eiceman 2000). The concept of GC as well as its instrumentation is discussed in detail in the literature (Eiceman 2000; Jennings 1987). Each of these components contributes to the overall efficiency of a GC separation. The carrier gas must be chemically inert and purified from moisture and oxygen because most columns do not tolerate these impurities when operated over 100°C. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of the detector that is used. The suite of gas chromatographic detectors includes the following: FID, thermal conductivity detector (TCD), electron capture detector (ECD), thermoionic detector, photoionization detector (PID), flame photometric detector, chemiluminescence detector, and some more unusual and expensive detectors like atomic emission detector (AED). The most popular detector used in flavor and fragrance analysis is the FID because of its high sensitivity and universal applicability. Alternatively, selective detectors such as the nitrogen-phosphorus and the flame photometric detector (or chemiluminescence detector) can be used to detect nitrogen and sulfur compounds, respectively, in complex mixtures. These detectors can be used separately or in conjunction with the normal detector. High-resolution

columns (typically 25 m or longer) are mandatory in most applications using relatively nonpolar phases, although more polar phases may assist with difficult separations (Sides et al. 2000).

The principle of GC is that volatile compounds, passed over a stationary phase, to which they have some tendency to bind, will be "slowed" compared to a gas that passes over the same surface, but has no tendency to bind. The time that it takes for a compound to pass through the column is called its retention time. Under constant GC conditions, the retention time of a compound remains constant. This retention time is characteristic of the component and therefore it could be used to identify the component, but, most frequently, absolute retention time is unreliable, and so relative retention times are often calculated. Relative retention time is obtained by relating the retention time of an unknown compound to that of a standard compound or a series of standard compounds. The most commonly used system is that developed by Kovats (1958), who used n-paraffins as standards. These compounds have, by definition, an index equal to the number of carbon atoms multiplied by 100. The original Kovats indices were developed by using isothermal and isobaric conditions. Van den Dool and Kratz (1963) extended retention indices to programmed temperature analyses. Jennings and Shibamoto (1980) presented a list of programmed temperature retention indices for a group of common flavor and fragrance compounds. Another important set of retention indices has also been published by Kondjoyan and Berdagué (1996).

The gas chromatographic step provides considerable information about the identification of a compound. Tabulated lists of retention times for thousands of compounds are available both in book form and in computer libraries. However, GC cannot completely characterize a compound since more than one substance may have the same retention time.

A more definitive answer is obtained by coupling the gas chromatographic technique with mass spectrometry, which is capable of providing a great deal of additional information about the eluted substances. In practice, while there are many pairs of compounds with similar retention times and many pairs of compounds with similar mass spectra, the combination of retention time and mass spectra usually provides a definitive identification of a compound.

The principle of mass spectrometry is that a gaseous compound can be ionized and partially fragmented by an electron beam, and the mass-to-charge ratios (m/z) of the resulting charged fragments can be measured. Many excellent books giving more details about this technique are available (McFadden 1973; Silverstein et al. 1974; Watson 1985). Because compounds fragment in a set pattern according to their chemical structure, the mass spectrum of an unknown component can be identified by comparing it to a spectral library of known molecules. Mass spectral libraries are available commercially, for example, Wiley and National Institute of Standards and Technology (NIST) libraries.

Many studies have used GS/MS to analyze the complex aroma of wine (Villen et al. 1995), fruits (Andrade et al. 2000; Elmaci and Altug 2002; Rocha et al. 2000), and vegetables (Petersen et al. 1998). However, it must be remembered that many, if not most, of the volatile compounds in a typical chromatogram are not aroma active at the concentrations found in flavor extracts. Volatile compounds that contribute to aroma can be localized in the gas chromatogram of the flavor extract and determined on the basis of their odor activities by GC-O (see, for instance, Schieberle

1991). This method, which aims at identifying key aroma compounds and which involves simultaneous "sniffing" of the effluent from the GC column and identifying of the eluting compounds by odor nature, is detailed hereafter.

Identifying Key Aroma Compounds

Representativeness As already outlined, because there is no universally applicable aroma isolation method, none of the extraction techniques described above yields an aroma isolate that truly represents either qualitatively or quantitatively the aroma profile of a food (Reineccius 2002). It is therefore necessary to choose the isolation procedure best suited to address the problem faced: determination of the complete aroma profile, identification of key odorants or off-flavors, monitoring aroma changes with time in foods, or prediction of sensory properties (Reineccius 2002). When the ultimate aim of a particular study was the identification of the compounds that are important for flavor (the key odorants), it was claimed that the most reliable results would be obtained if the odor of the extract resembles closely that of the food itself (Etiévant and Langlois 1998; Etiévant et al. 1994). It is, for instance, advisable to prevent oxidation during the extraction step by the addition of a suitable antioxidant, especially when oxidation of sensitive compounds may alter the odor of the extracts (Escudero and Etiévant 1999). Different sensory methods, which necessitate a trained sensory panel, can be used to check the sensory representativeness of the food extract odors (Etiévant et al. 1994). When an estimation of the relative importance of key constituents in a single sample is required, a similarity test is preferred. The panelists are asked to score the similarity of the odor of the extracts obtained by different methods to the odor of the food itself used as reference on an unstructured scale. This approach was applied to various foodstuffs including, for instance, wine (Abbott et al. 1993a; Bernet et al. 1999; Etiévant et al. 1994), cheese (Etiévant et al. 1994; Le Quéré et al. 1996), tomato (Etiévant and Langlois 1998), champagne (Escudero and Etiévant 1999), vinegar (Charles et al. 2000), edible algae (Le Pape et al. 2004), black currant (Boccorh et al. 2002), and apple (Mehinagic et al. 2003). The similarity test can be completed by a descriptive analysis of the extracts (Le Quéré et al. 1996; Moio et al. 1995) or even by a quantitative descriptive analysis (QDA) of the extracts compared to a QDA of the food samples (Abbott et al. 1993b; Le Guen et al. 2000; Le Pape et al. 2004; Mehinagic et al. 2003).

When different food samples have to be compared, triangle tests and overall matching tests are preferred. The different samples are presented as control samples, and extracts from the samples, presented in random order, have to be matched with controls. This approach was initially done on beer extracts (Abbott et al. 1993b). A key point in these evaluations of representativeness is the choice of a suitable matrix for testing the olfactory character of the extracts. For fat-containing food like cheese or butter, the best results have been obtained when the extracts are added to an emulsion, that is, a matrix similar to food in terms of fat composition (Etiévant et al. 1994). Since, generally, a combination of techniques should be used to obtain a reasonably complete view of an aroma profile (Reineccius 2002), it is noteworthy that substantial efforts have been made recently toward sensory evaluation of HS or SPME extracts. Thus, edible red algae volatiles have been desorbed from the dynamic HS Tenax trap and collected in evacuated brown flasks (Le Pape et al.

2004), and solvent-free extracts from apples have been collected by preparative GC in Teflon bags half filled with nitrogen (Mehinagic et al. 2003). The easiest and most promising technique in this field is probably "direct GC-O" (i.e., without a chromatographic column), where a complete HS or SPME extract is directly evaluated at the sniffing port of a gas chromatograph (Lecanu et al. 2002). This has been recently applied for orange juice (Rega et al. 2003) and apricot extracts (Guillot et al. 2003).

GC-O The analytical technique that uses a human nose as a detector and is known as GC-O (sometimes referred to as "GC–sniffing") has received considerable attention during the past 25 years in aroma research (see, e.g., Blank 1997; Lee 2003; Leland et al. 2001). The selectivity of this specific detector is based only on the odorous properties of the individual compounds separated by high-resolution GC. As the most abundant volatiles may have little, if any, odor of significance in a food (Mistry et al. 1997), GC–sniffing has been an invaluable tool for identifying potentially key compounds in aroma extracts.

The first aim of the technique is to discriminate the odorous compounds from the many background volatile components. The so-called aromagram constructed from the chromatogram obtained by simply smelling a GC effluent (Blank 1997; Reineccius 2002) is a potential interface with sensory analysis, as odor descriptors detected at the GC–sniffing port can be compared to the descriptors generated by sensory panelists from the original food. This method is particularly efficient for identifying off-flavors. Selection of key odorants or character-impact compounds in a food is another objective of GC-O. Quantitative approaches (the true GC-O) based on odor detection thresholds or on odor intensity have been developed and are the subject of specialized treatises (Lee 2003; Leland et al. 2001; Mistry et al. 1997; van Ruth 2001a).

Three different methods have been developed for GC-O: dilution analyses based on determination of detection thresholds, detection frequency methods, and intensity measurement methods. Original dilution methods, combined hedonic aroma measurement (CHARM) analysis, developed by Acree and coworkers (1984), and aroma extract dilution analysis (AEDA), developed by Grosch and coworkers (Ullrich and Grosch 1987), are essentially screening methodologies since the methods, based only on detection thresholds determination, violate certain sensory rules and psychophysical laws (Grosch 2001; Reineccius 2002; and references cited therein). They can be used to identify those single odorous compounds that are most likely to contribute to the complex odor of a food. Originally developed by McDaniel and coworkers (1990), the OSME method is basically a cross-modal technique aimed at measuring the perceived odor intensity of eluting volatiles. In OSME and in other cross-modality matching methods (Etiévant et al. 1999; Guichard et al. 1995), results are not based on odor detection thresholds, and only one concentration of the extract is evaluated by a panel, contrarily to dilution methods where several dilutions of the extract are evaluated. Results can be subjected to statistical analysis, and more consistent results are obtained when panelists are trained (Callement et al. 2001). The detection frequency methods, originally developed by Roozen and coworkers (Linssen et al. 1993), and now referred to as nasal impact frequency (NIF) or surface of nasal impact frequency (SNIF) since the work of Chaintreau and coworkers (Pollien et al. 1997), also use a group of assessors who

simply have to note when they detect an odor in a single GC run (i.e., also at only one concentration). The GC peaks being detected as odorous by the greatest number of assessors are considered to be the most important. Not being based on real odor intensities, the method has important drawbacks, especially when all the odorous compounds are present above their sensory threshold for all the assessors (Reineccius 2002). However, a study on Gewürztraminer wines from Alsace showed that the odor intensities measured by OSME using the finger-span cross-modality matching method were well correlated to the detection frequencies (Bernet 2000; Etiévant and Chaintreau 2001). According to these authors, the theoretical saturation limitation when using detection frequencies is practically reachable for only 10% of the detected odors (Etiévant and Chaintreau 2001).

Nevertheless, each of the methods described above has its advantages and weaknesses. Only three studies have compared all the methods for their performance (Le Guen et al. 2000; Serot et al. 2001; van Ruth and O'Connor 2001). In all cases, the results obtained with the different techniques were found to be very similar and well correlated. Finally, the choice of a GC-O method depends on the objective of the study, on the quality of the panel, and on the time scheduled for the analyses (Le Guen et al. 2000). Dilution techniques are clearly time-consuming; intensity methods give better results with a trained panel (Callement et al. 2001; Le Guen et al. 2000), while detection frequency methods are the least demanding but also the least precise (Le Guen et al. 2000). A comparative critical review may be found in Etiévant and Chaintreau (2001).

The aim of any GC-O experiment is to determine the relative odor potency of volatiles present in an aroma extract or fraction and to prioritize compounds for identification then usually performed using GC coupled to mass spectrometry (GC-MS). Mass spectrometry is also used for quantification purposes through the use of a stable isotope dilution assay (Blank et al. 1999; Milo and Blank 1998; and references cited therein). Such a precise quantification is required for the determination of odor activity values (OAVs) generally calculated when using AEDA (Grosch 1994, 2001). OAVs, calculated as the ratio of concentrations to odor thresholds, despite their limitations in terms of psychophysical validity (Mistry et al. 1997), give a good indication of the respective contributions of key odorants to the aroma of foods. They are the basis of the first attempts of using recombination studies to validate impact odorants sensorially in model foods (Grosch 1994). Aroma recombination studies are the important last step in sensorially validating the analytical data obtained by GC-O and for the quantification of key odorants of foods (Mistry et al. 1997). Many examples of models used in recombination experiments may be found in an authoritative review (Grosch 2001). Preparation of aroma models was found simpler for liquid foods than for solid foods because in that case, it is not easy to reproduce the composition and distribution of the nonvolatile fraction of the food matrix (Grosch 2001). However, for cheese models, for instance, either bland unripened cheese (Grosch 1994; Kubickova and Grosch 1998; Preininger et al. 1996) or especially designed odorless model cheeses (Salles et al. 1995) have been successfully used to incorporate potential key odorants. Thus, the branched-chain volatile fatty acids, 4-methyloctanoic and 4-ethyloctanoic acids, were confirmed to be essential to the typical goaty note of goat cheese (Le Quéré et al. 1996), and their retronasal aroma thresholds were determined using a cheese model (Le Quéré and Salles 2001; Salles and Le Quéré 1998; Salles et al. 2002). GC-O allows odor

evaluation of individual compounds, but a large loss of sensory properties is encountered when odorants are mixed (Grosch 2001). To understand the perceptual interactions of odorants, recombination studies in model foods or psychophysical experiments are necessary. Masking or enhancing properties of important odorants may be evaluated during GC-O with a new method called original aroma simultaneously input to the sniffing port (OASIS) (Hattori et al. 2003). It consists of evaluating complex odors at the sniffing port by delivering an original aroma directly at the sniffing port while a GC-O experiment is running. It is then possible to know how the individual components separated in the GC and sensed at the sniffing port influence the original odor notes. The authors have demonstrated that even high odor threshold compounds may affect an original Japanese green tea aroma (Hattori et al. 2003).

The GC-O methods that have been developed during the past 25 years, combined with either aroma extracts, HS or even SPME (Dufour et al. 2001), have facilitated the identification of potent odorants in numerous foodstuffs, for example, olive oil (Guth and Grosch 1993) or French fries (Wagner and Grosch 1997). Hundreds of scientific papers have been published on the subject, and it is out of the scope of this chapter to review all of them (for the most recent review, see d'Acampora Zellner et al. 2008). The most recent development combines comprehensive two-dimensional GC (Tranchida et al. 2004) to olfactometry (GC-GC-O). With the benefit of the high resolution gained through the use of two-dimensional GC (Chaintreau et al. 2006) that reveals numerous coelutions, some interesting results have been obtained, for example, with perfumes (d'Acampora Zellner et al. 2007) or essential oils (Eyres et al. 2007).

DYNAMIC METHODS IN FLAVOR ANALYSIS

Supposing that the "best" extraction and identification methods are used, trying to correlate the quantified flavor components in a food to the sensory perception experienced when eating this food is very often unsuccessful. In other words, it is not enough to know the exact composition of food in terms of flavor compounds to understand perfectly the perception of its flavor. Perception of flavor is a dynamic process (Piggott 2000). During food consumption, the concentration of aroma compounds at the olfactory epithelium varies with time as they are released progressively from the food matrix during chewing. Release kinetics depends on the composition of the food matrix and on individual mastication behavior. Sensory methods, such as time–intensity, have been used to study the time-related aspects of flavor perception (Piggott 2000).

Release of Volatiles *In Vivo*

Methods that measure volatiles directly in the mouth or in the nose have been developed to obtain data that could reflect the pattern of aroma molecules released from food and effectively present at the olfactory epithelium during consumption. These methods have been reviewed in an authoritative edited book (Roberts and Taylor 2000). Among the various approaches aimed at sampling aroma from the nose (nosespace), the collection of expired air samples on Tenax traps provided the

first robust results (Linforth and Taylor 1993; Taylor and Linforth 1994). Applied to tomatoes (Linforth et al. 1994) and to strawberry (Ingham et al. 1995), the method showed significant differences compared to HS sampling, underlining the importance of maceration in the mouth.

By overlapping the sampling time periods, release curves can be constructed and temporal changes reflecting relative concentrations of volatiles at a particular moment during consumption can be determined (Linforth et al. 1996). Correlation of accumulated data with sensory time–intensity data has been demonstrated (see for instance Delahunty et al. 1996).

Real-time *in vivo* flavor release was demonstrated some time ago using mass spectrometric breath-by-breath analysis with an optimized membrane separator interfaced to a mass spectrometer operated in electron impact mode (Overbosch 1987). Sensory time–intensity data measured in parallel for the perception of 2-pentanone in vegetable oil showed a clear adaptation effect, the stimulus being present in exhaled air long after the perception ended (Overbosch 1987). The method was also used by Soeting and Heridema (1988) and was extensively reviewed by Overbosch and others (1991). However, membrane separator techniques have important drawbacks in terms of selectivity and sensitivity (Taylor et al. 2000).

More recently, atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been developed to monitor aroma release during chewing (reviewed by Taylor et al. 2000). Air from the nose (nosespace) is sampled directly into the APCI-MS source through an interface making real-time breath-by-breath analysis routinely possible (Roberts and Taylor 2000; Taylor and Linforth 1996; and references cited therein). Therefore, by combining time–intensity sensory studies with nosespace analysis, it is now possible to relate temporal parameters of aroma release to perception (de Kok and Smorenburg 1999; Linforth and Taylor 2006; Linforth et al. 2000; Salles et al. 2003; Taylor and Hort 2004). Perceptual interactions of aroma with sapid compounds may also be studied by this method through controlled delivery of both aroma and sapid molecules to panelists (Cook et al. 2004; Taylor and Hort 2004). The APCI-MS method has been extensively reviewed in detail in specialized treatises (Roberts and Taylor 2000; Taylor 2002; Taylor and Linforth 2003). The method has been applied to tomato flavor (Brauss et al. 1998) and more recently to French fries (van Loon et al. 2005) or to compare the aroma volatiles emitted *in vivo* of the yellow-fleshed kiwifruit at two different stages of eating ripeness (Friel et al. 2007). Atmospheric pressure chemical ionization (APCI) sources may also be connected to a tandem mass spectrometer (MS/MS) such as an ion trap with the selectivity and structural capability benefits of MS/MS (Haahr et al. 2003; Le Quere et al. 2006; Sémon et al. 2003).

Another powerful chemical ionization method is proton transfer reaction mass spectrometry (PTR-MS). Originally developed by Lindinger's group (Lindinger et al. 1993) for online trace gas analysis, it consists of a three-chamber system. In the first chamber, nearly pure H_3O^+ ion is generated by electrical discharges in water vapor. A small electric field drives H_3O^+ ions through an orifice into a drift tube, where chemical ionization takes place, while neutral volatiles are introduced into the drift tube. Volatile compounds with proton affinities exceeding that of water (166.5 kcal/mol) ionize by proton transfer from H_3O^+ and are accelerated into the third chamber, the mass spectrometer. The specificity of PTR-MS compared to other chemical ionization approaches is that the generation of the reactant ion and the

chemical ionization process are spatially and temporally separated. Individual optimization is therefore possible and quantification is made easier (Yeretzian et al. 2000b). Since the early works on HS flavor volatiles that used PTR-MS (Yeretzian et al. 2000a,b), applications developed rapidly and a fourth specific international conference was organized in 2009 in Obergurgl, Austria (Hansel and Dunkl 2009). Results may be found in the current literature on HS (Blank et al. 2003; Mayr et al. 2003b), in nosespace studies on banana (Mayr et al. 2003c), strawberry (van Ruth et al. 2005), and other foodstuffs (Buettner et al. 2008; Roberts et al. 2003, 2004), and in model mouth (van Ruth et al. 2003, 2004) applications.

In vitro Measurements: Model Mouth Systems

Numerous mechanical devices that aim to mimic the processes that occur in the mouth during eating have been developed (Piggott 2000 and references cited therein). These "model mouths" are often variants of dynamic HS analysis, but their aim is to obtain time-resolved data similar to those obtained during *in vivo* studies. The various parameters like temperature, airflow, mastication rate, and addition of artificial saliva can be varied to study their effects on volatile flavor release (Rabe et al. 2002; van Ruth and Roozen 2000; van Ruth et al. 1995). The main advantages of model mouths are the large quantities of food samples that can be handled overcoming some sensitivity problems encountered when monitoring volatiles at low concentrations (Taylor 2002) and the suppression of interindividual variations, always encountered *in vivo*, which can be detrimental to a robust interpretation of the data. The release of volatile flavor compounds from the retronasal aroma simulator (RAS), originally developed by Roberts and Acree (1995), has been compared with flavor release *in vivo* using APCI-MS detection in both cases (Deibler et al. 2001). While delivering higher concentrations of volatiles than from human breath, the RAS gave a good approximation of time-averaged flavor release in the mouth, with volatile compounds present at similar ratios (Deibler et al. 2001). The model mouth device, originally developed by Roozen and coworkers to study the rehydration of bell peppers (van Ruth et al. 1994), French beans, and leeks (van Ruth et al. 1995), has been used to investigate changes in flavor composition in real time during mastication of ripe and unripe bananas (Mayr et al. 2003a) or strawberry (van Ruth et al. 2005). In a recent study, this model mouth system has been compared to the RAS in terms of the effects of oral physiological characteristics on the release of aromas as a function of the physicochemical properties of model emulsions (Geary et al. 2004). Both have been found suitable for the study of oral parameters on aroma release (Geary et al. 2004) with confirmed limits for the temporal dimension of the release (Deibler et al. 2001; Geary et al. 2004). Real-time data, comparable to those obtained *in vivo*, have also been obtained with a computerized apparatus that follows the temporal dimension of flavor release from liquid food (Rabe et al. 2002). More recently, the release of apple volatile compounds has been related to mastication using a specially designed model mouth system (Arvisenet et al. 2006). A new chewing simulator with improved performance to mimic a human mouth has also been recently described (Salles et al. 2007).

Flavor release and flavor perception are dynamic processes and must be studied using dynamic methods (Piggott 2000). Dynamic techniques have been developed to study the parameters of flavor release from foods. Parallel increased applications

of dynamic sensory methods provide a better understanding of food flavor. However, further work is needed to improve our knowledge of various interactions arising at different levels in the process of food consumption, for example, interactions between food ingredients (Taylor 2002) and interactions at the perceptual levels such as taste–aroma interactions (Given and Paredes 2002; Noble 1996; Taylor 2002), or trigeminal interferences (Green 1996; Given and Paredes 2002), as these play a fundamental role in overall flavor perception.

GLOBAL AND FAST ASSESSMENT OF FLAVOR

The methods currently used to evaluate and control the quality of food flavor are still essentially based on sensory evaluation by panels of experts. These trained panels are able to handle such difficult tasks like quality monitoring through descriptive analysis, off-flavor detection, and comparison of samples for classification purposes. It could be interesting to substitute instruments for humans, which could give quicker answers at reduced costs.

"Electronic Nose"

Evaluation of aroma release from food using gas sensors, the so-called electronic noses or e-noses, is theoretically feasible (Hodgins 1997; Mielle 1996; Schaller et al. 1998). Electronic noses are generally composed of arrays of nonspecific gas sensors that are based on different physical principles (Hodgins 1997; Mielle 1996; Schaller et al. 1998). The most common sensors are semiconducting metal oxides and conducting organic polymers, and they all give rise to nonspecific responses with particular patterns. Therefore, pattern recognition software, using either standard multivariate data analyses or artificial neural network technology, must be used for data treatment and for final presentation of the results (Hodgins 1997; Schaller et al. 1998). The e-nose is particularly attractive for quality control applications where conformity/nonconformity answers are expected (Mielle 1996). Discriminative studies have been conducted on all types of food with some success (Schaller et al. 1998) and some results obtained for fruit juices (Bleibaum et al. 2002; Shaw et al. 2000). However, some problems occurred with the repeatability of the system that could be possibly related to the product itself, the sampling technique, or the moisture content of the air used for sampling, precluding its use in routine tests (Schaller et al. 1998). Metal oxide semiconductor technology, despite some poisoning problems affecting the sensors, seems more reliable than conducting organic polymer sensors that show poor sensitivity to volatile components, the main problem of these sensors lying, however, with their instability (Schaller et al. 2000). The dangers of creating false classifications due to noise in the e-nose have also been emphasized (Goodner et al. 2001). Nevertheless, despite some success in some classification tasks conducted to monitor fruit maturity (Benedetti et al. 2008; Gomez et al. 2006; Pathange et al. 2006) and quality during shelf life (Berna et al. 2004; Benedetti et al. 2008; Saevels et al. 2004), electronic noses hardly meet the requirements of the food industry in terms of precision, reproducibility, sensitivity, and stability (Mielle et al. 2000). Moreover, the sensors are known to deteriorate or can be poisoned, therefore changing their response. Even with frequent calibration, the inherent

weaknesses of the technique make the perennial properties of the built databases problematic. Giving a global response, these instruments cannot be used to identify single odorants or to differentiate samples with subtle differences in distinctive sensory attributes. For instance, a recent study showed that e-nose based on metal oxide sensors revealed poor prediction performance in monitoring the storage shelf life of tomato (Gomez et al. 2008). Therefore, in off-flavor studies where identification of the off-flavor compound is a prerequisite and in quality control assessment, they may be used successfully only after recognizing their inherent weaknesses (Mielle et al. 2000; Reineccius 2002).

Mass Spectrometry-Based Systems

For classification purposes, two other global and fast analytical methods, based on mass spectrometry, have been used for food products and seem more powerful and reliable than electronic noses. The first consists of a global analysis of an HS sample by a mass spectrometer operated in electron ionization mode, without any GC separation. The feasibility of the method was originally demonstrated for rapid classification of four rather different French cheeses (Vernat and Berdagué 1995). This method is often referred to as an "MS-based electronic nose" or an "MS e-nose." The mass patterns obtained, considered as fingerprints of the food products analyzed, also need data treatment, either by conventional multivariate analyses or by artificial neural networks. SPME may be used as a preconcentration technique instead of HS sampling (Marsili 1999). A review on the subject appeared in 2003 (Pérès et al. 2003). Applied to rapid assessment of apple quality during shelf life, MS e-nose data clearly indicated the presence of both shelf life and storage history trend; trend e-nose measurements did not show. Therefore, the e-nose data had poorer prediction performance than those based on the MS e-nose data (Saevels et al. 2004). During the study of shelf life and cultivar effect on tomato aroma profile, MS e-nose data indicated a clear change in aroma profile with shelf life: change that could not be demonstrated by a quartz microbalance-based e-nose (Berna et al. 2004). A clear distinction between cultivars based on MS e-nose was obtained; however, based on e-nose-only measurements, it was difficult to discriminate between tomato varieties (Berna et al. 2004). However, some results demonstrated the potential of the e-nose and the HS fingerprint MS to complement routine sensory analysis of tomatoes (Berna et al. 2005). Recently, HS has been directly connected to a PTR-MS with some success to study the influence of dehydration on the subsequent reconstitution of mandarin juices (van Ruth et al. 2008). HS MS was also used for the classification of red wines with the aim to overcome traditional time-consuming methods (Dirinck et al. 2006).

Developed in the 1980s for food applications, direct pyrolysis MS is another method that delivers "fingerprints," which can be used for classification/authentication purposes (Aries and Gutteridge 1987). With this method, a tiny food sample is pyrolyzed rapidly at temperatures up to 530°C and the resulting volatile fraction, characteristic of the flavor but also of the matrix breakdown, is analyzed immediately by a mass spectrometer operated in low-energy electron ionization mode. Here again, a complex mass pattern is obtained for each sample and several data preprocessing steps are often necessary to select a reduced number of mass fragments that allow satisfactory classification. Curie point pyrolysis mass spectrometry

with associated multivariate data analysis techniques is considered as a powerful classification tool in microbiology for the recognition of microorganisms (Talon et al. 2002 and references cited therein) and in food science (Aries and Gutteridge 1987; Pérès et al. 2002; and references cited therein). A clear advantage of the method is that it provides a specific fingerprint of the food matrix, which can be potentially related to textural parameters (Pérès et al. 2002).

REFERENCES

Abbott N, Bayonove C, Etiévant P, Issanchou S. 1993a. Production of wine extracts for chemical and sensory analysis with an odour representative of that of the original wine. In Bayonove C, Flanzy C, Sapis JC (eds.), *Connaissance Aromatique des Cépages et Qualité des Vins*. Montpellier: Revue Française d'Oenologie, pp. 419–26.

Abbott N, Etiévant P, Langlois D, Lesschaeve I, Issanchou S. 1993b. Evaluation of the representativeness of the odor of beer extracts prior to analysis by GC eluate sniffing. *J Agric Food Chem* 41:777–80.

Acree TE, Barnard J, Cunningham DG. 1984. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem* 14:273–86.

Agelopoulos N, Hooper A, Maniar S, Pickett J, Wadhams L. 1999. A novel approach for the isolation of volatile chemicals released by individual leaves of a plant in situ. *J Chem Ecol* 25:1411–25.

Andrade EHA, Maia JGS, Zoghbi MGB. 2000. Aroma volatile constituents of Brazilian varieties of mango fruit. *J Food Compost Anal* 13:27–33.

Aries RE, Gutteridge CS. 1987. Applications of pyrolysis mass spectrometry to food science. In Gilbert J (ed.), *Applications of Mass Spectrometry in Food Science*. London: Elsevier Applied Science, pp. 377–431.

Arvisenet G, Billy L, Royer G, Prost C. 2006. Role of mastication on the release of apple volatile compounds in a model mouth system. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 465–8.

Atanasova B, Thomas-Danguin T, Chabanet C, Langlois D, Nicklaus S, Etiévant PX. 2005. Perceptual interactions in odour mixtures: Odour quality in binary mixtures of woody and fruity wine odorants. *Chem Senses* 30:209–17.

Atanasova B, Thomas-Danguin T, Langlois D, Nicklaus S, Etiévant P. 2004. Perceptual interactions between fruity and woody notes of wine. *Flav Frag J* 19:476–82.

Augusto F, Valente ALP, Tada ED, Rivellino SR. 2000. Screening of Brazilian fruit aromas using solid-phase microextraction-gas chromatography-mass spectrometry. *J Chromatogr A* 873:117–27.

Baltussen E, Sandra P, David F, Cramers C. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. *J Microcolumn Sep* 11:737–47.

Beaulieu JC, Grimm CC. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J Agric Food Chem* 49:1345–52.

Benedetti S, Buratti S, Spinardi A, Mannino S, Mignani I. 2008. Electronic nose as a non-destructive tool to characterise peach cultivars and to monitor their ripening stage during shelf-life. *Postharvest Biol Technol* 47:181–8.

Berna AZ, Buysens S, Natale CD, Grun IU, Lammertyn J, Nicolai BM. 2005. Relating sensory analysis with electronic nose and headspace fingerprint MS for tomato aroma profiling. *Postharvest Biol Technol* 36:143–55.

Berna AZ, Lammertyn J, Saevels S, Natale CD, Nicolai BM. 2004. Electronic nose systems to study shelf life and cultivar effect on tomato aroma profile. *Sens Actuators B Chem* 97:324–33.

Bernet C. 2000. Contribution à la connaissance des composés d'arôme clés des vins du cépage gewurztraminer cultivé en Alsace. PhD thesis, University of Burgundy, Dijon.

Bernet C, Dirninger N, Etiévant P, Schaeffer A. 1999. Evaluation de la représentativité aromatique d'extraits de vins de gewurztraminer. *Sci Aliments* 19:701–9.

Bicchi C, Drigo S, Rubiolo P. 2000. Influence of fibre coating in headspace solid-phase microextraction-gas chromatographic analysis of aromatic and medicinal plants. *J Chromatogr A* 892:469–85.

Bicchi C, Iori C, Rubiolo P, Sandra P. 2002. Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted arabica coffee and coffee brew. *J Agric Food Chem* 50:449–59.

Blanch GP, Herraiz M, Reglero G, Tabera J. 1993. Preconcentration of samples by steam distillation-solvent extraction at low-temperature. *J Chromatogr* 655:141–9.

Blanch GP, Ibanez E, Herraiz M, Reglero G. 1994. Use of a programmed temperature vaporizer for off-line SFE/GC analysis in food composition studies. *Anal Chem* 66:888–92.

Blanch GP, Reglero G, Herraiz M. 1996. Rapid extraction of wine aroma compounds using a new simultaneous distillation-solvent extraction device. *Food Chem* 56:439–44.

Blank I. 1997. Gas chromatography-olfactometry in food aroma analysis. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 293–329.

Blank I, Devaud S, Matthey-Doret W, Pollien P, Robert F, Yeretzian C. 2003. Formation of odour-active compounds in Maillard model systems based on proline. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 458–63.

Blank I, Milo C, Lin J, Fay LB. 1999. Quantification of aroma-impact components by isotope dilution assay-recent developments. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry Thirty Years of Progress*. New York: Kluwer Academic/Plenum, pp. 63–74.

Bleibaum RN, Stone H, Tan T, Labreche S, Saint-Martin E, Isz S. 2002. Comparison of sensory and consumer results with electronic nose and tongue sensors for apple juices. *Food Qual Prefer* 13:409–22.

Boccorh RK, Paterson A, Piggott JR. 2002. Extraction of aroma components to quantify overall sensory character in a processed black currant (*Ribes nigrum* L.) concentrate. *Flav Frag J* 17:385–91.

Boulanger R, Crouzet J. 2000. Free and bound flavour components of Amazonian fruits: 2. Cupuaçu volatile compounds. *Flav Frag J* 15:251–7.

Brauss MS, Linforth RST, Taylor AJ. 1998. Effect of variety, time of eating, and fruit-to-fruit variation on volatile release during eating of tomato fruits (*Lycopersicon esculentum*). *J Agric Food Chem* 46:2287–92.

Bredie WLP, Petersen MA. 2006. *Flavour Science: Recent Advances and Trends. Developments in Food Science*, Vol. 43. Amsterdam: Elsevier.

Buettner A, Otto S, Beer A, Mestres M, Schieberle P, Hummel T. 2008. Dynamics of retronasal aroma perception during consumption: Cross-linking on-line breath analysis with medico-analytical tools to elucidate a complex process. *Food Chem* 108:1234–46.

Buettner A, Schieberle P. 2001a. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J Agric Food Chem* 49:1358–63.

Buettner A, Schieberle P. 2001b. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J Agric Food Chem* 49:2387–94.

Buttery RG. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree TE, Teranishi R (eds.), *Flavor Science Sensible Principles and Techniques*. Washington, DC: American Chemical Society, pp. 259–86.

Buttery RG, Ling LC. 1996. Methods for isolating food and plant volatiles. In Takeoka GR, Teranishi R, Williams PJ, Kobayishi A (eds.), *Biotechnology for Improved Foods and Flavours*. Washington, DC: American Chemical Society, p. 240.

Callement G, Bouchet M, Langlois D, Etiévant P, Salles C. 2001. Odor intensity measurements in gas chromatography-olfactometry using cross modality matching: Evaluation of training effects. In Leland JV, Schieberle P, Buettner A, Acree TE (eds.), *Gas Chromatography-Olfactometry The State of the Art*. Washington, DC: American Chemical Society, pp. 172–86.

Canac-Arteaga D, Viallon C, Berdagué JL. 2000. Analytical artifacts caused by the presence of water in the headspace of food products. *Analusis* 28:550–6.

Carasek E, Pawliszyn J. 2006. Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber. *J Agric Food Chem* 54:8688–96.

Cavalli JF, Fernandez X, Lizzani-Cuvelier L, Loiseau AM. 2003. Comparison of static headspace, headspace solid phase microextraction, headspace sorptive extraction, and direct thermal desorption techniques on chemical composition of French olive oils. *J Agric Food Chem* 51:7709–16.

Cavalli JF, Fernandez X, Lizzani-Cuvelier L, Loiseau AM. 2004. Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME: Identification of quality-freshness markers. *Food Chem* 88:151–7.

Chaintreau A, Begnaud F, Starkenmann C. 2006. Novel concept of multidimensional gas chromatography. New capabilities for chiral analysis and olfactometric detection. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Advances and Trends*. Amsterdam: Elsevier, pp. 559–64.

Charles M, Martin B, Giniès C, Etiévant P, Coste G, Guichard E. 2000. Potent aroma compounds of two red wine vinegars. *J Agric Food Chem* 48:70–7.

Chin HW, Bernhard RA, Rosenberg M. 1996. Solid phase microextraction for cheese volatile compound analysis. *J Food Sci* 61:1118–22.

Cook DJ, Davidson JM, Linforth RST, Taylor AJ. 2004. Measuring the sensory impact of flavor mixtures using controlled delivery. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 135–49.

d'Acampora Zellner B, Casilli A, Dugo P, Dugo G, Mondello L. 2007. Odour fingerprint acquisition by means of comprehensive two-dimensional gas chromatography-olfactometry and comprehensive two-dimensional gas chromatography/mass spectrometry. *J Chromatogr A* 1141:279–86.

d'Acampora Zellner B, Dugo P, Dugo G, Mondello L. 2008. Gas chromatography-olfactometry in food flavour analysis. *J Chromatogr A* 1186:123–43.

Da Porto C, Pizzale L, Bravin M, Conte LS. 2003. Analyses of orange spirit flavour by direct-injection gas chromatography-mass spectrometry and headspace solid-phase microextraction/GC-MC. *Flav Frag J* 18:66–72.

Deibler KD, Lavin EH, Linforth RST, Taylor AJ, Acree TE. 2001. Verification of a mouth simulator by in vivo measurements. *J Agric Food Chem* 49:1388–93.

Deibler KD, Delwiche J. 2004. *Handbook of Flavor Characterization*. New York: Marcel Dekker.

de Kok PMT, Smorenburg HE. 1999. Flavor release in the mouth. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry Thirty Years of Progress*. New York: Kluwer Academic/Plenum, pp. 397–407.

Delahunty CM, Piggott JR, Conner JM, Paterson A. 1996. Comparison of dynamic flavour release from hard cheeses and analysis of headspace volatiles from the mouth with flavour perception during consumption. *J Sci Food Agric* 71:273–81.

Diaz-Maroto CM, Perez-Coello MS, Cabezudo MD. 2002. Supercritical carbon dioxide extraction of volatiles from spices. Comparison with simultaneous distillation-extraction. *J Chromatogr A* 947:23–9.

Dirinck I, Van Leuven I, Dirinck P. 2006. ChemSensor classification of red wines. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 521–4.

Dirinck P, De Pooter H, Schamp N. 1989. Aroma development in ripening fruits. In Teranishi R, Buttery RG, Shahidi F (eds.), *Flavor Chemistry: Trends and Developments*. Washington, DC: American Chemical Society, pp. 23–34.

Doleschall F, Recseg K, Kemeny Z, Kovari K. 2003. Comparison of differently coated SPME fibres applied for monitoring volatile substances in vegetable oils. *Eur J Lipid Sci Technol* 105:333–8.

Dufour JP, Delbecq P, Perez Albela L. 2001. Solid-phase microextraction combined with gas chromatography-olfactometry for analysis of cheese aroma. In Leland JV, Schieberle P, Buettner A, Acree TE (eds.), *Gas Chromatography-Olfactometry The State of the Art*. Washington, DC: American Chemical Society, pp. 123–37.

Eiceman GA. 2000. Instrumentation in gas chromatography. In Meyers RA (ed.), *Encyclopedia of Analytical Chemistry*. Chichester: John Wiley & Sons, pp. 10671–9.

Eiceman GA, Clement RE, Hill HH. 1992. Gas chromatography. *Anal Chem* 64:170R–80R.

Elmaci Y, Altug T. 2002. Flavour evaluation of three black mulberry (*Morus nigra*) cultivars using GC/MS, chemical and sensory data. *J Sci Food Agric* 82:632–5.

Escudero A, Etiévant P. 1999. Effect of antioxidants on the flavor characteristics and the gas chromatograohy/olfactometry profiles of champagne extracts. *J Agric Food Chem* 47:3303–8.

Etiévant P, Chaintreau A. 2001. Les analyses olfactométriques: Synthèse critique et recommandations. In *Proc. XXèmes Journées Internationales Huiles Essentielles et Extraits*, September 5–7, Digne-les-Bains.

Etiévant PX, Callement G, Langlois D, Issanchou S, Coquibus N. 1999. Odor intensity evaluation in gas chromatography-olfactometry by finger span method. *J Agric Food Chem* 47:1673–80.

Etiévant PX, Langlois D. 1998. L'amélioration de la représentativité olfactive des extraits aromatiques dans la recherche des composés clefs de l'arôme des aliments. In Peyron L (ed.), *17 èmes Journées Internationales Huiles Essentielles et extraits*. Digne les Bains: APPAM, pp. 268–78.

Etiévant PX, Moio L, Guichard E, Langlois D, Lesschaeve I, Schlich P, Chambellant E. 1994. Aroma extract dilution analysis (AEDA) and the representativeness of the odour of food extracts. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research, Developments in Food Science*, Vol. 35. Amsterdam: Elsevier, pp. 179–91.

Eyres G, Marriott PJ, Dufour JP. 2007. The combination of gas chromatography-olfactometry and multidimensional gas chromatography for the characterisation of essential oils. *J Chromatogr A* 1150:70–7.

Ferreira ACS, de Pinho PG. 2003. Analytical method for determination of some aroma compounds on white wines by solid phase microextraction and gas chromatography. *J Food Sci* 68:2817–20.

Ferreira V, Rapp A, Cacho JF, Hastrich H, Yavas I. 1993. Fast and quantitative determination of wine flavor compounds using microextraction with Freon 113. *J Agric Food Chem* 41:1413–20.

Forss DA, Holloway GL. 1967. Recovery of volatile compounds from butter oil. *J Am Oil Chem Soc* 44:572–5.

Friel EN, Wang M, Taylor AJ, MacRae EA. 2007. In vitro and in vivo release of aroma compounds from yellow-fleshed kiwifruit. *J Agric Food Chem* 55:6664–73.

Geary MD, van Ruth SM, Delahunty CM, Lavin EH, Acree TE. 2004. Effects of oral physiological characteristics on the release of aroma compounds from oil-in-water emulsions using two mouth simulator systems. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 345–61.

Given P, Paredes D. 2002. *Chemistry of Taste. Mechanisms, Behaviors, and Mimics*. *ACS Symposium Series 825*. Washington, DC: American Chemical Society.

Godefroot M, Sandra P, Verzele M. 1981. New method for quantitative essential oil analysis. *J Chromatogr* 203:325–35.

Gomez AH, Wang J, Hu G, Pereira AG. 2006. Electronic nose technique potential monitoring mandarin maturity. *Sens Actuators B Chem* 113:347–53.

Gomez AH, Wang J, Hu G, Pereira AG. 2008. Monitoring storage shelf life of tomato using electronic nose technique. *J Food Eng* 85:625–31.

Goodner KL, Dreher JG, Rouseff RL. 2001. The dangers of creating false classifications due to noise in electronic nose and similar multivariate analyses. *Sens Actuators B Chem* 80:261–6.

Granvogl M, Schieberle P. 2006. Facts and "artefacts" in the flavour chemistry of onions. In Bredie WLP, Petersen MA (eds.), *Flavour Science Recent Developments and Trends*. Amsterdam: Elsevier, pp. 359–62.

Green BG. 1996. Chemesthesis: Pungency as a component of flavor. *Trends Food Sci Technol* 7:415–20.

Grob K, Müller E. 1987. Sample reconcentration by column-external solvent evaporation or injection of large volumes into gas chromatographic capillary columns? *J Chromatogr* 404:297–305.

Grosch W. 1994. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flav Frag J* 9:147–58.

Grosch W. 2001. Evaluation of the key odorants of foods by dilution experiments, aroma models and omission. *Chem Senses* 26:533–45.

Guichard H, Guichard E, Langlois D, Issanchou S, Abbott N. 1995. GC sniffing analysis: Olfactive intensity measurement by two methods. *Z Lebensm Unters Forsch* 201:344–50.

Guillot S, Boulanger R, Crouzet J, Bureau S, Lepoutre JP, Galindo S. 2003. Tracers of apricot aromatic quality. In Le Quéré JL, Etiévant P (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 606–9.

Güntert M, Krammer G, Sommer H, Werkhoff P. 1998. The importance of the vacuum headspace method for the analysis of fruit flavors. In Mussinan CJ, Morello MJ (eds.), *Flavor Analysis: Developments in Isolation and Characterization*. Washington, DC: American Chemical Society, pp. 38–60.

Guth H, Grosch W. 1993. Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J Am Oil Chem Soc* 70:513–18.

Haahr AM, Madsen H, Smedsgaard J, Bredie WLP, Stahnke LH, Refsgaard HHF. 2003. Flavor release measurement by atmospheric pressure chemical ionization ion trap mass spectrometry, construction of interface and mathematical modeling of release profiles. *Anal Chem* 75:655–62.

Hansel A, Dunkl J. 2009. Contributions—4th International Conference on Proton Transfer Reaction Mass Spectrometry and Its Applications. In Märk TD, Holzner B (eds.), *Conference Series*. Innsbruck: Innsbruck University Press, p. 325.

Harmon AD. 1997. Solid-phase microextraction for the analysis of flavors. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 81–112.

Hattori S, Takagaki H, Fujimori T. 2003. Evaluation of Japanese green tea extract using GC/O with original aroma simultaneously input to the sniffing port method (OASIS). *Food Sci Technol Res* 9:350–2.

Ho CT, Manley CH. 1993. *Flavor Measurement. IFT Basic Symposium Series*. New York: Marcel Dekker.

Hodgins D. 1997. The electronic nose: Sensor array-based instruments that emulate the Human nose. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 331–71.

Holt RU. 2001. Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. *J Chromatogr A* 937:107–14.

Ibañez C, Sola J. 2006. Optimisation of stir bar sorptive extraction (SBSE) for flavour analysis. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Developments and Trends*. Amsterdam: Elsevier, pp. 577–80.

Ibanez E, Lopez-Sebastian S, Ramos E, Tabera J, Reglero G. 1998. Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem* 63:281–6.

Ingham KE, Linforth RST, Taylor AJ. 1995. The effect of eating on aroma release from strawberries. *Food Chem* 54:283–8.

Ioffe BV. 1984. New applications of gas chromatographic headspace analysis. *J Chromatogr* 290:363–75.

Jaillais B, Bertrand V, Auger J. 1999. Cryo-trapping/SPME/GC analysis of cheese aroma. *Talanta* 48:747–53.

Jelen HH, Obuchowska M, ZawirskaWojtasiak R, Wasowicz E. 2000. Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J Agric Food Chem* 48:2360–7.

Jennings W. 1987. *Analytical Gas Chromatography*. Orlando, FL: Academic Press.

Jennings W, Shibamoto T. 1980. *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. New York: Academic Press.

Jia MY, Zhang QH, Min DB. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *J Agric Food Chem* 46:2744–7.

Kanavouras A, Kiritsakis A, Hernandez RJ. 2005. Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase micro-extraction. *Food Chem* 90:69–79.

Kataoka H, Lord HL, Pawliszyn J. 2000. Applications of solid-phase microextraction in food analysis. *J Chromatogr A* 880:35–62.

Keszler A, Heberger K, Gude M. 1998. Identification of volatile compounds in sunflower oil by headspace SPME and ion-trap GC/MS. *J High Resolut Chromatogr* 21:368–70.

Kieckbusch TG, King CJ. 1979. An improved method of determining vapor-liquid equilibria for dilute organics in aqueous solution. *J Chromatogr Sci* 17:273–6.

Kittel KM, Lavin EH, Deibler KD, Acree TE, Maillard A. 2004. Analysis of off-aromas in wines caused by aging. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 455–62.

Kondjoyan, N, Berdagué JL.1996. *Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*. Clermont-Ferrand: Laboratoire Flaveur Publisher, INRA.

Kovats E. 1958. Gas chromatographische Charakteriserung organischer Verbindungen. I. Retentions indices aliphatischer halogenide, alkohole, aldehyde und ketone. *Helv Chim Acta* 41:1915–32.

Kubatova A, Lagadec AJM, Miller DJ, Hawthorne SB. 2001. Selective extraction of oxydatives from savory and peppermint using subcritical water. *Flav Frag J* 16:64–73.

Kubickova J, Grosch W. 1998. Quantification of potent odorants in Camembert cheese and calculation of their odour activity values. *Int Dairy J* 8:17–23.

Lecanu L, Ducruet V, Jouquand C, Gratadoux JJ, Feigenbaum A. 2002. Optimization of headspace solid-phase microextraction (SPME) for the odor analysis of surface-ripened cheese. *J Agric Food Chem* 50:3810–17.

Lee S-J. 2003. Finding key odorants in foods: Gas chromatography olfactometry (GC/O). *Food Sci Biotechnol* 12:597–602.

Le Guen S, Prost C, Demaimay M. 2000. Critical comparison of three olfactometric methods for the identification of the most potent odorants in cooked mussels (*Mytilus edulis*). *J Agric Food Chem* 48:1307–14.

Leland JV, Schieberle P, Buettner A, Acree TE. 2001. *Gas Chromatography-Olfactometry. The State of the Art. ACS Symposium Series 782*. Washington, DC: American Chemical Society.

Le Pape MA, Grua-Priol J, Prost C, Demaimay M. 2004. Optimization of dynamic headspace extraction of the edible red algae *Palmaria palmata* and identification of the volatile components. *J Agric Food Chem* 52:550–6.

Le Quéré JL, Etiévant PX. 2003. *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc.

Le Quere JL, Gierczynski I, Langlois D, Sémon E. 2006. Nosespace with an ion trap mass spectrometer—Quantitative aspects. In Bredie WLP, Petersen MA (eds.), *Flavour Science: Recent Advances and Trends*. Amsterdam: Elsevier, pp. 589–92.

Le Quéré JL, Salles C. 2001. Goat cheese flavour compounds. In Freund G (ed.), *Recent Advances on Goat Milk Quality, Raw Material for Cheesemaking*. Surgères: ITPLC Editions, pp. 115–22.

Le Quéré JL, Septier C, Demaizières D, Salles C. 1996. Identification and sensory evaluation of the character-impact compounds of goat cheese flavour. In Taylor AJ, Mottram DS (eds.), *Flavour Science Recent Developments*. Cambridge: Royal Society of Chemistry, pp. 325–30.

Lestremau F, Andersson FAT, Desauziers V. 2004. Investigation of artefact formation during analysis of volatile sulphur compounds using solid phase microextraction (SPME). *Chromatographia* 59:607–13.

Lestremau F, Desauziers V, Fanlo JL. 2001. Formation of artifacts during air analysis of volatile amines by solid-phase microextraction. *Analyst* 126:1969–73.

Likens ST, Nickerson GB. 1964. Detection of certain hop oil constituents in brewing products. In Proceedings of the American Society of Brewing Chemists, pp. 5–13.

Lindinger W, Hirber J, Paretzke H. 1993. An ion/molecule-reaction mass spectrometer used for on-line trace gas analysis. *Int J Mass Spectrom Ion Process* 129:79–88.

Linforth R, Taylor A. 2006. The process of flavour release. In Voilley A, Etiévant P (eds.), *Flavour in Food*. Cambridge: Woodhead Publishing, pp. 287–307.

Linforth RST, Hollowood T, Taylor AJ. 2000. Analysis of correlation between temporal aspects of aroma release and perception. In Schieberle P, Engels KH (eds.), *Frontiers of Flavour Science*. Garching: Deutsche Forschungsanstalt für Lebensmittelchemie, pp. 275–8.

Linforth RST, Ingham KE, Taylor AJ. 1996. Time course profiling of volatile release from foods during the eating process. In Taylor AJ, Mottram DS (eds.), *Flavour Science: Recent Developments*. Cambridge: Royal Society of Chemistry, pp. 361–8.

Linforth RST, Savary I, Pattenden B, Taylor AJ. 1994. Volatile compounds found in expired air during eating of fresh tomatoes and in the headspace above tomatoes. *J Sci Food Agric* 65:241–7.

Linforth RST, Taylor AJ. 1993. Measurement of volatile release in the mouth. *Food Chem* 48:115–20.

Linssen JPH, Janssens JLGM, Roozen JP, Posthumus MA. 1993. Combined gas chromatography and sniffing port analysis of volatile compounds of mineral water packed in laminated packages. *Food Chem* 46:367–71.

Liu TT, Yang TS. 2002. Optimization of solid-phase microextraction analysis for studying change of headspace flavor compounds of banana during ripening. *J Agric Food Chem* 50:653–7.

Lopez EF, Gomez EF. 2000. Comparison of solvents for determination of monoterpenes in wine using liquid-liquid extraction. *Chromatographia* 52:798–802.

Lopez R, Aznar M, Cacho J, Ferreira V. 2002. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J Chromatogr A* 966:167–77.

Lord H, Pawliszyn J. 2000. Evolution of solid-phase microextraction technology. *J Chromatogr A* 885:153–93.

Macku C, Kallio H, Takeoka G, Flath R. 1988. Aspects on the use of headspace GC on-column injections in flavor research. *J Chromatogr Sci* 26:557–60.

Maia JGS, Andrade EHA, Zoghbi MdGB. 2004. Aroma volatiles from two fruit varieties of jackfruit (*Artocarpus heterophyllus* Lam.). *Food Chem* 85:195–7.

Marsili R. 1997. *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker.

Marsili RT. 1999. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J Agric Food Chem* 47:648–54.

Matich AJ, Rowan DD, Banks NH. 1996. Solid phase microextraction for quantitative headspace sampling of apple volatiles. *Anal Chem* 68:4114–8.

Mau J-L, Ko P-T, Chyau C-C. 2003. Aroma characterization and antioxidant activity of supercritical carbon dioxide extracts from *Terminalia catappa* leaves. *Food Res Int* 36:97–104.

Mayr D, van Ruth SM, Mark TD. 2003a. Evaluation of the influence of mastication on temporal aroma release of ripe and unripe bananas, using a model mouth system and gas chromatography-olfactometry. *Eur Food Res Technol* 217:291–5.

Mayr D, Hartungen E, Märk T, Margesin R, Schinner F. 2003b. Determination of the spoolage status of meat by aroma detection using proto-transfer-reaction mass-spectrometry. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 757–60.

Mayr D, Märk T, Lindinger W, Brevard H, Yeretzian C. 2003c. In-vivo analysis of banana aroma by proton transfer reaction—mass spectrometry. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 256–9.

McDaniel MR, Miranda-Lopez R, Watson BT, Michaels NJ, Libbey LM. 1990. Pinot noir aroma: A sensory/gas chromatographic approach. In Charalambous G (ed.), *Flavors and Off-Flavors*. Amsterdam: Elsevier, pp. 23–36.

McFadden W. 1973. *Techniques of Combined Gas Chromatography/Mass Spectrometry: Applications in Organic Analysis*. New York: John Wiley & Sons.

Mehinagic E, Prost C, Demaimay M. 2003. Representativeness of apple aroma extract obtained by vacuum hydrodistillation: Comparison of two concentration techniques. *J Food Sci* 68:2411–15.

Mielle P. 1996. "Electronic noses": Towards the objective instrumental characterization of food aroma. *Trends Food Sci Technol* 7:432–8.

Mielle P, Marquis F, Latrasse C. 2000. Electronic noses: Specify or disappear. *Sens Actuators B Chem* 69:287–94.

Mildner-Szkudlarz S, Jelen HH, Zawirska-Wojtasiak R, Wasowicz E. 2003. Application of headspace—solid phase microextraction and multivariate analysis for plant oils differentiation. *Food Chem* 83:515–22.

Miller ME, Stuart JD. 1999. Comparison of gas-sampled and SPME-sampled static headspace for the determination of volatile flavor components. *Anal Chem* 71:23–7.

Milo C, Blank I. 1998. Quantification of impact odorants in food by isotope dilution assay: Strength and limitations. In Mussinan CJ, Morello MJ (eds.), *Flavor Analysis Developments in Isolation and Characterization*. Washington, DC: American Chemical Society, pp. 250–9.

Mistry BS, Reineccius T, Olson LK. 1997. Gas chromatography-olfactometry for the determination of key odorants in foods. In Marsili R (ed.), *Techniques for Analyzing Food Aroma*. New York: Marcel Dekker, pp. 265–92.

Moio L, Chambellant E, Lesschaeve I, Issanchou S, Schlich P, Etiévant P. 1995. Production of representative wine extracts for chemical and olfactory analysis. *Ital J Food Sci* 7:265–78.

Mondy N, Duplat D, Christides JP, Arnault I, Auger J. 2002. Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction-liquid extraction and gas chromatography-mass spectrometry. *J Chromatogr A* 963:89–93.

Mussinan CJ, Morello MJ. 1998. *Flavor Analysis. Developments in Isolation and Characterization*. Washington, DC: American Chemical Society.

Noble AC. 1996. Taste-aroma interactions. *Trends Food Sci Technol* 7:439–44.

Ortega-Heras M, Gonzalez-SanJose ML, Beltran S. 2002. Aroma composition of wine studied by different extraction methods. *Anal Chim Acta* 458:85–93.

Overbosch P. 1987. Flavour release and perception. In Martens M, Dalen GA, Russwurm H (eds.), *Flavour Science and Technology*. Chichester: John Wiley & Sons, pp. 291–300.

Overbosch P, Afterof WGM, Haring PGM. 1991. Flavor release in the mouth. *Food Rev Int* 7:137–84.

Pathange LP, Mallikarjunan P, Marini RP, O'Keefe S, Vaughan D. 2006. Non-destructive evaluation of apple maturity using an electronic nose system. *J Food Eng* 77:1018–23.

Pelusio F, Nilsson T, Montanarella L, Tilio R, Larsen B, Facchetti S, Madsen JO. 1995. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J Agric Food Chem* 43:2138–43.

Pérès C, Begnaud F, Eveleigh L, Berdagué JL. 2003. Fast characterization of foodstuff by headspace mass spectrometry (HS-MS). *Trends Anal Chem* 22:858–66.

Pérès C, Viallon C, Berdagué JL. 2002. Curie point pyrolysis-mass spectrometry applied to rapid characterisation of cheeses. *J Anal Appl Pyrolysis* 65:161–71.

Petersen MA, Poll L, Larsen LM. 1998. Comparison of volatiles in raw and boiled potatoes using a mild extraction technique combined with GC odour profiling and GC-MS. *Food Chem* 61:461–6.

Piggott JR. 2000. Dynamism in flavour science and sensory methodology. *Food Res Int* 33:191–7.

Pillonel L, Bosset JO, Tabacchi R. 2002. Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review. *Lebensm Wiss Technol* 35:1–14.

Polesello S, Lovati F, Rizzolo A, Rovida C. 1993. Supercritical fluid extraction as a preparative tool for strawberry aroma analysis. *J High Resolut Chromatogr* 16:555–9.

Pollien P, Chaintreau A. 1997. Simultaneous distillation-extraction: Theoretical model and development of a preparative unit. *Anal Chem* 69:3285–92.

Pollien P, Ott A, Fay LB, Maignial L, Chaintreau A. 1998. Simultaneous distillation-extraction: Preparative recovery of volatiles under mild conditions in batch or continuous operations. *Flav Frag J* 13:413–23.

Pollien P, Ott A, Montigon F, Baumgartner M, Munoz Box R, Chaintreau A. 1997. Hyphenated headspace-gas chromatography-sniffing technique: Screening of impact odorants and quantitative aromagram comparisons. *J Agric Food Chem* 45:2630–7.

Preininger M, Warmke R, Grosch W. 1996. Identification of the character impact flavour compounds of Swiss cheese by sensory studies of models. *Z Lebensm Unters Forsch* 202:30–4.

Przyjazny A, Janicki W, Chrzanowski W, Staszewski R. 1983. Headspace gas chromatographic determination of distribution coefficients of selected organosulphur compounds and their dependence on some parameters. *J Chromatogr A* 280:249–60.

Rabe S, Krings U, Banavara DS, Berger RG. 2002. Computerized apparatus for measuring dynamic flavor release from liquid food matrices. *J Agric Food Chem* 50:6440–7.

Rega B, Fournier N, Guichard E. 2003. Solid phase microextraction (SPME) of orange juice flavor: Odor representativeness by direct gas chromatography olfactometry (D-GC-O). *J Agric Food Chem* 51:7092–9.

Reid LM, O'Donnell CP, Downey G. 2004. Potential of SPME-GC and chemometrics to detect adulteration of soft fruit purees. *J Agric Food Chem* 52:421–7.

Reineccius G. 2002. Instrumental methods of analysis. In Taylor AJ (ed.), *Food Flavour Technology*. Sheffield: Sheffield Academic Press, pp. 210–51.

Reineccius G. 2006. Choosing the correct analytical technique in aroma analysis. In Voilley A, Etiévant P (eds.), *Flavour in Food*. Cambridge: Woodhead Publishing, pp. 81–97.

Riu-Aumatell M, Castellari M, Lopez-Tamames E, Galassi S, Buxaderas S. 2004. Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GUMS. *Food Chem* 87:627–37.

Robbins LA, Cusack RW. 1997. Liquid-liquid extraction operations and equipment. In Perry RH, Green DW, Maloney JO (eds.), *Perry's Chemicals Engineers' Handbook*, 7th Ed. New York: McGraw-Hill, pp. 15/1–15/47.

Roberts DD, Acree TE. 1995. Simulation of retronasal aroma using a modified headspace technique: Investigating the effects of saliva, temperature, shearing, and oil on flavor release. *J Agric Food Chem* 43:2179–86.

Roberts DD, Pollien P, Antille N, Lindinger C, Yeretzian C. 2003. Comparison of nosespace, headspace, and sensory intensity ratings for the evaluation of flavor absorption by fat. *J Agric Food Chem* 51:3636–42.

Roberts DD, Pollien P, Yeretzian C, Lindinger C. 2004. Nosespace analysis with proton-transfer-reaction mass spectrometry: Intra- and interpersonal variability. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 151–62.

Roberts DD, Taylor AJ. 2000. *Flavor Release*. *ACS Symposium Series 763*, Washington, DC: American Chemical Society.

Rocha S, Coutinho P, Barros A, Coimbra MA, Delgadillo I, Dias Cardoso A. 2000. Aroma Potential of two Bairrada white grape varieties: Maria Gomes and Bical. *J Agric Food Chem* 48:4802–7.

Ruiz del Castillo ML, Dobson G. 2002. Varietal differences in terpene composition of black currant (*Ribes nigrum* L) berries by solid phase microextraction/gas chromatography. *J Sci Food Agric* 82:1510–15.

Saevels S, Lammertyn J, Berna AZ, Veraverbeke EA, Di Natale C, Nicolai BM. 2004. An electronic nose and a mass spectrometry-based electronic nose for assessing apple quality during shelf life. *Postharvest Biol Technol* 31:9–19.

Saito N, Ikushima Y, Hatakeda K, Ito S, Goto T. 1991. Fractional extraction of rice bran oil and their ester with supercritical carbon dioxide. *Nippon Nogeikagaku Kaishi (J Agric Chem Soc Jpn)* 65:153–61.

Salles C, Dalmas S, Septier C, Issanchou S, Noel Y, Etiévant P, Le Quéré JL. 1995. Production of a cheese model for sensory evaluation of flavour compounds. *Lait* 75:309–24.

Salles C, Hollowood TA, Linforth RST, Taylor AJ. 2003. Relating real time flavour release to sensory perception of soft cheeses. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 170–5.

Salles C, Le Quéré JL. 1998. Impact of small hydrophobic and hydrophilic molecules on the flavour of goat cheese. In Werner H (ed.), *25th International Dairy Congress*. Aarhus: The Danish National Committee of the IDF, pp. 165–74.

Salles C, Sommerer N, Septier C, Issanchou S, Chabanet C, Garem A, Le Quéré JL. 2002. Goat cheese flavor: Sensory evaluation of branched-chain fatty acids and small peptides. *J Food Sci* 67:835–41.

Salles C, Tarrega A, Mielle P, Maratray J, Gorria P, Liaboeuf J, Liodenot JJ. 2007. Development of a chewing simulator for food breakdown and the analysis of *in vitro* flavor compound release in a mouth environment. *J Food Eng* 82:189–98.

Saritas Y, Sonwa MM, Iznaguen H, Konig WA, Muhle H, Mues R. 2001. Volatile constituents in mosses (Musci). *Phytochemistry* 57:443–57.

Schaller E, Bosset JO, Escher F. 1998. "Electronic noses" and their application to food. *Lebensm Wiss Technol* 31:305–16.

Schaller E, Bosset JO, Escher F. 2000. Instability of conducting polymer sensors in an electronic nose system. *Analysis* 28:217–27.

Schieberle P. 1991. Primary odorants in popcorn. *J Agric Food Chem* 39:1141–4.

Schultz TH, Flath RA, Mon TR, Eggling SB, Teranishi R. 1977. Isolation of volatile components from a model system. *J Agric Food Chem* 25:446–9.

Schulz I, Ulrich D, Fischer C. 2003. Rapid differentiation of new apple cultivars by headspace solid-phase microextraction in combination with chemometrical data processing. *Nahr-Food* 47:136–9.

Sémon E, Gierczynski I, Langlois D, Le Quéré JL. 2003. Analysis of aroma compounds by atmospheric pressure chemical ionisation—ion trap mass spectrometry. Construction and validation of an interface for in vivo analysis of human breath volatile content. 16th International Mass Spectrometry Conference, August 31–September 5, Edinburgh.

Serot T, Prost C, Visan L, Burcea M. 2001. Identification of the main odor-active compounds in musts from French and Romanian hybrids by three olfactometric methods. *J Agric Food Chem* 49:1909–14.

Seto Y. 1994. Determination of volatile substances in biological samples by headspace gas chromatography. *J Chromatogr A* 674:25–62.

Shaw PE, Rouseff RL, Goodner KL, Bazemore R, Nordby HE, Widmer WW. 2000. Comparison of headspace GC and electronic sensor techniques for classification of processed orange juices. *Lebensm Wiss Technol* 33:331–4.

Sides A, Robards K, Helliwell S. 2000. Developments in extraction techniques and their application to analysis of volatiles in foods. *Trends Anal Chem* 19:322–9.

Silverstein RM, Bassler GC, Morill TC. 1974. *Spectrometric Identification of Organic Compounds*, 3rd Ed. New York: Wiley.

Soeting W, Heridema J. 1988. A mass spectrometric method for measuring flavour concentration/time profiles in human breath. *Chem Senses* 13:607–17.

Song J, Fan LH, Beaudry RM. 1998. Application of solid phase microextraction and gas chromatography time-of-flight mass spectrometry for rapid analysis of flavor volatiles in tomato and strawberry fruits. *J Agric Food Chem* 46:3721–6.

Sonsuzer S, Sahin S, Yilmaz L. 2004. Optimization of supercritical CO_2 extraction of *Thymbra spicata* oil. *J Supercrit Fluids* 30:189–99.

Steffen A, Pawliszyn J. 1996. Analysis of flavor volatiles using headspace solid-phase microextraction. *J Agric Food Chem* 44:2187–93.

Stephan A, Bücking M, Steinhart H. 2000. Novel analytical tools for food flavours. *Food Res Int* 33:199–209.

Stevenson RJ, Chen XD, Mills OE. 1996. Modern analyses and binding studies of flavour volatiles with particular reference to dairy protein products. *Food Res Int* 29:265–90.

Talon R, Walter D, Viallon C, Berdagué JL. 2002. Prediction of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* populations in yoghurt by Curie point pyrolysis-mass spectrometry. *J Microbiol Methods* 48:271–9.

Taylor AJ. 2002. Release and transport of flavors in vivo: Physicochemical, physiological, and perceptual considerations. *Compr Rev Food Sci Food Saf* 1:45–57.

Taylor AJ, Hort J. 2004. Measuring proximal stimuli involved in flavour perception. In Taylor AJ, Roberts DD (eds.), *Flavor Perception*. Oxford: Blackwell Publishing, pp. 1–38.

Taylor AJ, Linforth RST. 1994. Methodology for measuring volatile profiles in the mouth and nose during eating. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier, pp. 3–14.

Taylor AJ, Linforth RST. 1996. Flavour release in the mouth. *Trends Food Sci Technol* 7:444–8.

Taylor AJ, Linforth RST. 2003. Direct mass spectrometry of complex volatile and non-volatile flavour mixtures. *Int J Mass Spectrom* 223-224:179–91.

Taylor AJ, Linforth RST, Harvey BA, Blake A. 2000. Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chem* 71:327–38.

Teranishi R. 1998. Challenges in flavor chemistry: An overview. In Mussinan CJ, Morello MJ (eds.), *Flavor Analysis Developments in Isolation and Characterization*. Washington, DC: American Chemical Society, pp. 1–6.

Tienpont B, David F, Bicchi C, Sandra P. 2000. High capacity headspace sorptive extraction. *J Microcolumn Sep* 12:577–84.

Tranchida PQ, Dugo P, Dugo G, Mondello L. 2004. Comprehensive two-dimensional chromatography in food analysis. *J Chromatogr A* 1054:3–16.

Tredoux AGJ, Lauer HH, Heideman T, Sandra P. 2000. The determination of benzoic acid in lemon flavored beverages by stir bar sorptive extraction-CGC-MS. *J High Resolut Chromatogr* 23:644–6.

Ullrich F, Grosch W. 1987. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z Lebensm Unters Forsch* 184:277–82.

Ulrich D, Eunert S, Hoberg E, Rapp A. 1995. Analysis of strawberry aroma with solid phase microextraction. *Dtsch Lebensmitt Rundsch* 91:349–51.

Ulrich D, Krumbein A, Rapp A. 1997a. Analysis of aroma compounds in strawberry, sour cherry and tomato by gas chromatography after solid phase micro extraction. *Dtsch Lebensmitt Rundsch* 93:311–6.

Ulrich D, Hoberg E, Rapp A, Sandke G. 1997b. Flavour analysis in plant breeding—Solid phase micro extraction of strawberry aroma compounds. 5th Wartburg Aroma Symposium, March 17–20, Eisenach, p. 12.

Van den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr* 11:463–71.

van Loon WAM, Linssen JPH, Boelrijk AEM, Burgering MJM, Voragen AGJ. 2005. Real-time flavor release from French fries using atmospheric pressure chemical ionization-mass spectrometry. *J Agric Food Chem* 53:6438–42.

van Ruth S, Geary MD, Buhr K, Delahunty CM. 2004. Representative sampling of volatile flavor compounds: The model mouth combined with gas chromatogaphy and direct mass spectrometry. In Deibler KD, Delwiche J (eds.), *Handbook of Flavor Characterization*. New York: Marcel Dekker, pp. 335–44.

van Ruth SM. 2001a. Methods for gas chromatography-olfactometry: A review. *Biomol Eng* 17:121–8.

van Ruth SM. 2001b. Aroma measurement: Recent developments in isolation and characterisation. In De Cuyper M, Bulte JWM (eds.), *Physics and Chemistry Basis of Biotechnology*. Amsterdam: Kluwer Academic Publishers, pp. 305–28.

van Ruth SM, Buhr K, Delahunty CM. 2005. Strawberry flavour analysis by PTR-MS: Various low and high time resolution applications. In Hansel A, Märk TD (eds.), *2nd International Conference on Proton Transfer Reaction Mass Spectrometry and Its Applications*. Innsbruck: Innsbruck University Press, pp. 81–4.

van Ruth SM, Buhr K, Geary M, Dings L, Odake S. 2003. Effect of oral parameters on dynamic aroma release under mouth conditions. In Le Quéré JL, Etiévant PX (eds.), *Flavour Research at the Dawn of the Twenty-First Century*. Paris: Lavoisier, Tec & Doc, pp. 176–81.

van Ruth SM, Frasnelli J, Carbonell L. 2008. Volatile flavour retention in food technology and during consumption: Juice and custard examples. *Food Chem* 106:1385–92.

van Ruth SM, O'Connor CH. 2001. Evaluation of three gas chromatography-olfactometry methods: Comparison of odour intensity-concentration relationships of eight volatile compounds with sensory headspace data. *Food Chem* 74:341–7.

van Ruth SM, Roozen JP. 2000. Influence of mastication and saliva on aroma release in a model mouth system. *Food Chem* 71:339–45.

van Ruth SM, Roozen JP, Cozijnsen JL. 1994. Comparison of dynamic headspace mouth model systems for flavour release from rehydrated bell pepper cuttings. In Maarse H, Van der Heij DG (eds.), *Trends in Flavour Research*. Amsterdam: Elsevier, pp. 59–64.

van Ruth SM, Roozen JP, Cozijnsen JL. 1995. Volatile compounds of rehydrated French beans, bell peppers and leeks. Part 1. Flavour release in the mouth and in three mouth model systems. *Food Chem* 53:15–22.

Verhoeven H, Beuerle T, Schwab W. 1997. Solid-phase microextraction: Artefact formation and its avoidance. *Chromatographia* 46:63–6.

Vernat G, Berdagué JL. 1995. Dynamic headspace-mass spectrometry (DHS-MS): A new approach to real-time characterization of food products. In Etiévant P, Schreier P (eds.), *Bioflavour 95*. Versailles: INRA Editions, pp. 59–62.

Villen J, Senorans FJ, Reglero G, Herraiz M. 1995. Analysis of wine aroma by direct injection in gas chromatography without previous extraction. *J Agric Food Chem* 43:717–22.

Wagner R, Grosch W. 1997. Evaluation of potent odorants of French fries. *Lebensm Wiss Technol* 30:164–9.

Wampler TP. 1997. Analysis of food volatiles using headspace-gas chromatographic techniques. In Marsili R (ed.), *Techniques for Analysing Food Aroma*. New York: Marcel Dekker, pp. 27–58.

Wan XM, Stevenson RJ, Chen XD, Melton LD. 1999. Application of headspace solid-phase microextraction to volatile flavour profile development during storage and ripening of kiwifruit. *Food Res Int* 32:175–83.

Wang YM, Wyllie SG, Leach DN. 1996. Chemical changes during the development and ripening of the fruit of *Cucumis melo* (cv Makdimon). *J Agric Food Chem* 44:210–16.

Watson JT. 1985. *Introduction to Mass Spectrometry*, 2nd Ed. New York: Raven Press.

Yang X, Peppard T. 1994. Solid-phase microextraction for flavor analysis. *J Agric Food Chem* 42:1925–30.

Yeretzian C, Jordan A, Brevard H, Lindinger W. 2000a. On-line monitoring of coffee roasting by proton-transfer-reaction mass-spectrometry. In Roberts DD, Taylor AJ (eds.), *Flavor Release*. Washington, DC: American Chemical Society, pp. 112–23.

Yeretzian C, Jordan A, Brevard H, Lindinger W. 2000b. Time-resolved headspace analysis by proton-transfer-reaction mass-spectrometry. In Roberts DD, Taylor AJ (eds.), *Flavor Release*. Washington, DC: American Chemical Society, pp. 58–72.

Young CT, Hovis AR. 1990. A method for the rapid analysis of headspace volatiles of raw and roasted peanuts. A research note. *J Food Sci* 55:279–80.

Zhou Y, Riesen R, Gilpin CS. 1996. Comparison of Amberlite XAD-2/freon 11 extraction with liquid/liquid extraction for the determination of wine flavor components. *J Agric Food Chem* 44:818–22.

CHAPTER 34

Vegetable Flavors from Cell Culture

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INTRODUCTION

Nowadays, flavors represent over a quarter of the world market for food additives, and most of the flavoring compounds are produced via chemical synthesis or by extraction from natural materials. Flavor is usually the result of the presence, within complex matrices, of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties, which are used to give taste and/or smell to food. Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. Studies of flavor preferences and aversions suggest that flavor perception may be linked to the nutritional or health value associated with the perceived foods. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short-to-medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds, and sulfur compounds (Gatfield 1988; Longo and Sanroman 2006; Urbach 1997).

Many volatiles are produced in plant tissues at specific developmental stages, during flowering, ripening, or maturation. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the "odor-active compounds" that help animals and humans to recognize appropriate foods and to avoid poor or dangerous food choices.

Knowledge of the identities of the aroma components of food is important to increase its aroma properties. In order to give a certain odor to a product, it is necessary to be sure that only the compound that leads to the desired odor perception is added. This means that highly pure compounds have to be produced.

The volatile profiles of several vegetables have been determined in order to identify the odor-active compounds. Tomato is a model for fruit development, and more is known about the chemicals contributing to tomato flavor than for any other fruit or vegetable. Virtually all of the major tomato volatiles (Table 34.1) can be linked to compounds providing health benefits to humans. Thus, flavor volatiles can

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TABLE 34.1. Volatile Compounds and Their Odor Threshold of Tomato (Goff and Klee 2006)

Volatile Compound	Odor Threshold (ppb)
cis-3-Hexanal	0.25
β-Lonone	0.007
Hexanal	5.0
β-Damascenone	0.002
1-Penten-3-one	1.0
2-Methylbutanal	1.0
3- Methylbutanal	0.2
trans-2-Hexanal	17.0
Isobutylthiazole	3.5
1-Nitro-2-phenyletanhe	2.0
trans-2-Heptenal	13.0
Phenylacetaldehyde	4.0
6-Methyl-5-hepten-2-one	2000.0
cis-3-Hexanol	70.0
2-Phenyletahol	750.0
3-Methylbutanol	120.0
Methyl salicylate	40.0

be perceived as positive nutritional signals. Both its concentration and the odor threshold (our ability to sense it) determine the impact of a chemical on flavor perception. As described by Goff and Klee (2006), only a small number of the more than 400 volatiles detected in tomato have a positive impact on the flavor profile. These volatiles are listed in their approximate order of importance in Table 34.1.

Buckwheat honey, which has a distinct malty aroma, has found use as a natural preservative because of its high antioxidant content. Zhou and others (2002) determined that 3-methylbutanal, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), and (E)-β-damascenone were the most potent odorants in buckwheat honey, with 3-methylbutanal being primarily responsible for the distinct malty aroma. Other important aroma-active compounds included methylpropanal, 2,3-butanedione, phenylacetaldehyde, 3-methylbutyric acid, maltol, vanillin, methional, coumarin, and *p*-cresol.

The volatiles produced by raw and cooked potatoes have been extensively studied, and over 250 compounds have been identified in potato volatile fractions. Attempts have been made to discriminate which of those components are important for potato flavor, which are specific to the method of cooking, cultivar differences, the effects of agronomic conditions, and the effects of storage (Morris et al. 2007). Overall, there is no clear-cut identification of which volatiles (if any) are the key contributors to cooked potato flavor and taste. Duckham and others (2001) studied the volatile flavor components of baked potato flesh from 11 potato cultivars. They concluded that lipid oxidation and the Maillard reaction are the major sources of flavor compounds of baked potato flesh, and other components (sulfur compounds, methoxypyrazines, and terpenes) are also present at lower levels. 2-Isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, β-damascenone, dimethyl trisulfide, decanal, and 3-methylbutanal are major contributors to flavor in at least one

cultivar. Differences (some of which are significant) in levels of compounds among cultivars suggest the possibility of breeding potatoes that, following cooking, possess distinctive aromas.

Flavor is considered the single most critical quality trait in rice affecting consumer preference. Over 300 volatile compounds have been identified from various cultivars of aromatic and nonaromatic rice. Among the volatiles identified, there are a relatively small number of odor-active compounds and 2-acetyl-1-pyrroline has been determined as the main aroma compound (Buttery et al. 1983). Although aromatic types assessed to date contain 2-acetyl-1-pyrroline, they have very different aromas, indicating that other compounds contribute to their respective flavors, such as guaiacol, indole, and *p*-xylene (Dong et al. 2008).

Garlic contains allyl-S-cysteine sulfoxide (alliin) and an enzyme, allinase. By the action of allinase on alliin, S-(2-propenyl) 2-propene-1-sulfinothioate (allicin) is formed. This compound is the predominant thiosulfinate responsible for the typical odor of garlic (Brodnitz et al. 1971).

The flavor of onion is mainly due to sulfur-containing compounds, formed by the cleavage of three S-alk(en)yl-L-cysteine sulfoxides (ACSOs) by alliin alkylsulfenate-lyase (alliinase) (Griffiths et al. 2002). These three ACSOs are (+)-S-methyl-L-cysteine sulfoxide (MCSO, methiin), (+)-S-propyl-L-cysteine sulfoxide (PrCSO, propiin) and trans-(+)-S-(propen-1-yl)-L-cysteine sulfoxide (1-PeCSO, isoalliin). 1-PeCSO is by far the most abundant of the ACSOs, often being more than 80% of the total, and it is responsible for the majority of the flavor chemistry in onion. The major flavor compounds are generated by the spontaneous reactions undergone by the S-alk(en)yl sulfenic acids among themselves and other compounds. The result is a mixture of over 50 sulfur-containing compounds including thiosulfinates, thiosulfonates, mono-, di-, and trisulfides, as well as specific compounds such as the lachrymatory or tear factor, thiopropanal S-oxide. Moreover, Widder and others (2000) identified 3-mercapto-2-methylpentan-1-ol as an aroma compound of onions.

Since early times, flavor compounds ranging from single to complex substances have been extracted from plant sources. However, this method of obtaining essential oils and flavors presents some problems. First, the raw materials often contain low concentrations of the desired compounds, making the extraction expensive. Additionally, the availability and cost of the raw materials can be strongly influenced by difficult-to-control environmental factors, such as weather conditions and plant diseases.

Eventually, after the elucidation of their structure, synthetic flavors have been produced by chemical synthesis. Aldehydes, lactones, ketones, esters, alcohols, hydrocarbons, phenolic compounds, and sulfur compounds are the common groups of volatiles that have been detected and identified. However, the consumer has developed a "chemophobia" attitude toward chemical or synthetic (even nature-identical) compounds, especially when related to food and products used in the home (Vandamme and Soetaert 2002). In addition to the diversity in chemical structures, chirality is important. A large number of flavors are chiral, and often, the enantiomers have different sensory properties. Chemical synthesis often results in environmentally unfriendly production processes and lacks substrate selectivity, which may cause the formation of undesirable racemic mixtures, thus reducing process efficiency and increasing downstream costs.

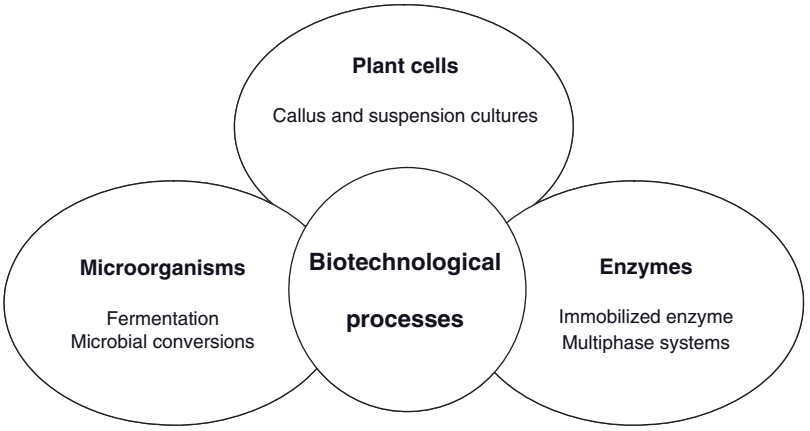

Figure 34.1. Biotechnological processes for the production of flavor compounds.

The disadvantages of traditional methods (extraction from natural materials and chemical synthesis) and the increasing interest in natural products have directed many investigations toward the search for other strategies to produce natural flavors.

An alternative route for flavor synthesis is based on microbial biosynthesis or bioconversion (Aguedo et al. 2004; Janssens et al. 1992; Krings and Berger 1998; Vandamme and Soetaert 2002). Biotechnological processes for the production of aroma compounds comprise biotransformation, de novo synthesis, and genetic engineering (Fig. 34.1). The products thus obtained may possess the legal status of a natural substance, with the advantage of a higher stereoselectivity in the catalytic enzyme reactions.

Although there are many investigations on the generation of flavor and aroma compounds during the manufacture of food products, only a few aroma components are specifically produced by biotechnological routes. Bio-aroma production can include the culture of plant cells or microorganisms and the use of enzymes. Consequently, aroma production by microorganisms, usually as the result of secondary metabolism, has been the focus of several studies. Microorganisms may produce aroma compounds during fermentation of certain foods and beverages such as cheeses and yogurts. In addition, several microorganisms, including bacteria and fungi, are currently known for their ability to synthesize different aroma compounds, which can be finally extracted from the fermentation broth and used in the food industry (Longo and Sanroman 2006).

The aim of this paper is to review the current state of the art on the biotechnological production of aroma compounds responsible for the characteristics of vegetable food, with a special focus on plant cell and microbial flavor production, as well as flavor formation by enzymes.

PLANT CELL CULTURES

The first experiment with plant tissue cultures was reported 102 years ago by the Austrian scientist Gottlieb Haberland. During the 100 years since this first report,

TABLE 34.2. Flavors from Plant Cell Cultures

Products	Plant Species	References
2,3-Butanedione, (E,Z)-2,6-nonadienal, and (E,Z)-2,6-nonadien-1-ol	*Agastache rugosa*	Kim and others (2001)
Apple aroma	*Malus silvestris*	Drawert and others (1984)
Cinnamic acid	*Nicotiana tabacum*	
Caryophyllen	*Lindera strychnifolia*	
Limonene, linalool	*Perilla fructescens*	
Basmati flavor	*Oryza sativa*	Suvarnalatha and others (1994)
Cocoa flavor	*Theobromo cacao*	Townsley (1972)
Flavanol	*Polygonum hydropiper*	Nakao and others (1999)
Garlic	*Allium sativum*	Ohsumi and others (1993)
Monoterpenes	*Perrilla frutiscens*	Nabeta and others (1983)
Onion	*Allium cepa*	Prince and others (1997)
Triterpenoid	*Glycyrrhiza glabra glandulifera*	Ayabe and others (1990)
Vanillin	*Vanilla planifolia*	Dornenburg and Knorr (1996)

the use of cell cultures increased rapidly. Cell and tissue cultures today are not only used for the propagation of plants but are also evaluated for the commercial production of natural products, including flavors and fragrances (Hrazdina 2006). Thus, plant cell cultures appear to be an attractive alternative source to produce a wide range of flavors and aroma compounds characteristic of their plant origin (Table 34.2). The rationale for the production of flavor compounds with plant cell cultures is based on the unique biochemical and genetic capacity and on the totipotency of plant cells (Harlander 1994; Sahai 1994; Scragg 1997). Every cell of a plant culture contains the genetic information necessary to produce numerous chemical components (or their precursors) that constitute natural flavor. The best results toward the production of flavor compounds by cell cultures have been achieved in cases when the characteristic aroma of the fruit or plant is caused mainly or entirely by the synthesis and accumulation of a single compound or only a few compounds with similar structures and properties. This is the case with vanilla, for which the major flavor component is vanillin; with raspberries, for which raspberry ketone gives the characteristic flavor; and with onions and garlic, for which the characteristic flavor derives from alliin derivatives.

Feeding intermediates of the biosynthetic pathway can enhance the production of flavor metabolites by precursor biotransformation. Some authors (Mulabagal and Tsay 2004; Rao and Ravishankar 2002) summarized the advantages of the use of plant cell culture technology over the conventional agricultural production, pointing out the following aspects:

- independence of geographical and seasonal variations, political interference, and other environmental factors; it also offers a defined production system, which ensures a continuous product supply, as well as uniform quality and yield;

- possibility to produce novel compounds that are not normally found in parent plants, either directly or through stereo- and regiospecific biotransformations of cheap precursors;
- selection of the species of high productivity;
- costs can be decreased and productivity increased by automatization of cell growth control and metabolic process regulation; and
- low cost due to efficient downstream recovery and rapid production.

Nevertheless, tissue or cell cultures pose some problems, which need to be solved before plant cell cultures are extensively used for plant metabolite production. The first requirement for establishing a cell culture is the development of a friable callus requiring tissue regeneration over a number of transfers, which may take time. Also, the cultures have to be kept under sterile conditions at all times, and this requires special handling and equipment. Moreover, the technology for large-scale suspension cultures should be further developed since it differs from that commonly employed for microbial systems. Sensitivity to shear stress, relatively long growth cycles, low yields, progressive loss of biosynthetic activity, and rare product secretion are some of the features of plant cell cultures that need special attention. However, some strategies have been developed to stimulate the biosynthetic activities of cultured plant cells by optimization of culture conditions, selection of high-producing strains, precursor feeding, and elicitation. Also, cell immobilization techniques could help to prolong viability periods, to maintain high cell density in the bioreactors, and to reduce shear stress, among other advantages. Besides, regulation of plant secondary metabolism at the biochemical and genetic levels could lead to improved production systems (Hrazdina 2006; Mulabagal and Tsay 2004).

As for specific efforts related to flavor production by plant cell cultures, several researchers have investigated the synthesis of vanillin, a much sought-after flavor compound. The natural vanilla flavor is a mixture of compounds in which vanillin is the major component (86%), followed by *p*-hydroxybenzaldehyde (8.6%), vanillic acid (4.3%), and *p*-hydroxybenzyl methyl ether (0.9%) (Rao and Ravishankar 2000). Vanilla is the most universally used flavoring ingredient in the world. Its history and agronomic production have been reviewed recently by Havkin-Frenkel and Dorn (1997). Vanilla is produced by the plant *Vanilla planifolia*, an orchid native to the southern region of Mexico. Harvested pods or beans are exposed to a lengthy and laborious curing process, during which time they turn black and develop the characteristic vanilla flavor. The cured pods are marketed under the name vanilla. Plant cell cultures of *V. planifolia* have been initiated from various plant cells and tissues (Davidonis and Knorr 1991), and the convenience of using elicitors to induce vanillic acid synthesis has been assessed (Funk and Brodelius 1992). Also, feeding of the precursor ferulic acid resulted in an increase in vanillin accumulation (Funk and Brodelius 1990; Romagnoli and Knorr 1988). Furthermore, the production of vanillin from ferulic acid with vanilla aerial roots on charcoal as a product reservoir has been described (Westcott et al. 1994). *Capsicum frutescens* root cultures have also been used for the bioconversion of ferulic acid to vanillin (Suresh et al. 2003).

Some other works involve the production of monoterpenes (i.e., limonene and linalool) in callus tissues and cell suspensions of *Perilla frutescens* (Nabeta et al. 1983) and of Basmati rice volatile flavor components in callus cultures of *Oryza*

sativa (Suvarnalatha et al. 1994). In some cases, the flavor profiles obtained in plant cell cultures differ from those encountered in the parent plants. Such was the case in suspension cultures of *Agastache rugosa* Kuntze (Korean mint), which had a marked cucumber/wine-like aroma, and produced some interesting flavor-related alcohols (i.e., 2-phenylethanol) (Kim et al. 2001). This alteration of the original flavor profiles can be deliberately induced by the addition of precursors, as demonstrated in root cultures of *Allium cepa* L. (onion) (Prince et al. 1997).

MICROBIAL CULTURES

Microorganisms have historically played an integral role in the elaboration of the flavor components of many different foods. Products such as wine, vinegar, beer, fermented vegetables, milk, soya, and meat have been preserved, modified, and flavored by means of microbial strains. Bioprocess with microorganisms or enzymes can be used in the production of natural flavors:

- *Fermentation.* The microorganisms are able to produce flavors by fermentation from nutrients such as sugars and amino acids.
- *Microbial Conversion.* The microorganisms are able to catalyze specific conversions of added precursors or intermediates. The possibility for regio- and stereospecific bioconversions, whether or not complementary to chemical synthesis, is important for the resolution of optical isomers.
- *Enzyme-Catalyzed Reactions.* A number of enzymes may directly produce flavor molecules by regio- and stereospecific bioconversions of larger progenitors. Also, recent developments on biocatalysis in unconventional media have made possible the utilization of hydrolytic enzymes to specifically catalyze the synthesis of a number of valuable compounds. This strategy can be applied for the production of food aromas, as is the case of ester synthesis by lipases in low-water-content media.

There are various families of aroma compounds, and the differences used to classify them can be based on not only chemical structures, physicochemical properties, or sensorial properties of the compounds but also, and in fact more commonly, on the chemical family of the substrate. On this latter basis, lipid-derived aroma compounds constitute one of the most important families, which include volatile fatty acids or esters, lactones, aldehydes, alcohols, and ketones. Detailed information on the production of some commonly used food aroma compounds by microorganisms or enzymes is presented below.

Lactones

Lactones are cyclic esters of primarily γ- and δ-hydroxy acids. Lactones are ubiquitous in food, contributing taste and flavor nuances. These are associated with odor impressions such as fruity, coconut-like, buttery, creamy, sweet, or nutty. The possibility of producing a lactone using a biotechnological route was discovered in the 1960s by the group of Okui and others (1963a,b) during the investigation of hydroxyacid

catabolism by several organisms. Dimick and others (1969) stated in their review that raw milk does not contain free lactones, which only appear after heating. The milky, buttery, and coconut-like flavor notes provided by these compounds are generally considered as desirable in dairy products. However, the presence of lactones may contribute to the stale flavor of heated milk, although to a lesser extent than ketones.

A coconut aroma is highly desired by flavorists, and 6-pentyl-2-pyrone possesses this aroma. This compound was found in cultures of the fungus *Trichoderma viride* as the major volatile constituent (Collin and Halim 1972). Other fungi such as *Tyromyces sambuceus* and *Cladosporium suaveolens* efficiently generate the coconut-flavored lactones γ-decalactone and δ-dodecalactone from ricinoleic acid and linoleic acid, respectively (Allegrone et al. 1991; Kapfer et al. 1989).

Some yeasts such as *Candida tropicalis* or *Yarrowia lipolytica* degraded ricinoleic acid to C16, C14, and C12 acids and, interestingly, accumulated δ-decalactone, which exhibits fruity and oily notes important in the formulation of peach, apricot, or strawberry aromas. However, the yields of this biotransformation are commonly poor, and they rarely reach concentrations over 4–5 g/L in the fermentation broth (Gatfield 1999). Wache and others (2001) investigated the enzymes involved in γ-decalactone production by *Y. lipolytica* and encountered the reasons for low yields.

Esters

Esters are commonly used flavoring agents, very appreciated for the fruity and vegetable aromas they provide. These esters are responsible for fruity flavors that can be regarded either as a defect or as an attribute by the consumer. Acetate esters, such as ethyl acetate, hexyl acetate, isoamyl acetate, and 2-phenylethyl acetate, are recognized as important flavor compounds in wine and in other grape-derived alcoholic beverages. Rojas and others (2001) studied several so-called non-*Saccharomyces* wine yeasts as the producers of acetate ester. Among them, the yeasts *Hanseniaspora guilliermondii* and *Pichia anomala* were found to be potent 2-phenylethyl acetate and isoamyl acetate producers, respectively.

Ethyl or methyl esters of short-chain fatty acids generally bring about fruity and vegetable flavors, while thioesters derived from thiols are associated with cabbage or sulfur aromas (Liu et al. 2004). The capacity of lactic acid bacteria to synthesize both ethyl esters and thioesters has been reported. The role of a unique esterase from *Lactococcus lactis* in the formation of these aroma compounds has been investigated and ascertained as at least partially responsible for the esterification reactions leading to the production of aroma ester compounds. This was undertaken by using an esterase-negative mutant of *L. lactis* (Nardi et al. 2002).

One of the most promising applications of enzyme technology in the food aroma field is the use of reversed lipolysis in low-water-content systems (Dordick 1989) in order to carry out esterification or transesterification reactions for the production of esters from inexpensive raw materials (i.e., fatty acids and alcohols). A number of lipases have been tested for their ability to promote ester synthesis in low-water-content media, such as those from *Candida cylindracea*, *Pseudomonas fluorescens*, *Mucor miehei*, *Aspergillus* sp., *Rhizopus arrhizus*, and *Candida rugosa*, among others.

Lipases are usually highly specific, which makes esterification between carboxylic acids and alcohols dependent on alcohol and/or acid chain length. Kumar and others (2005) studied the esterification of fatty acids of different chain lengths and isoamyl alcohol with three different commercial lipases. *Candida antarctica* lipase fraction B showed substrate specificity involving both acids (short-chain fatty acids having linear and branched-chain structures, as well as unsaturated fatty acids) and alcohols (n-butyl, isopentyl, 2-phenylethyl, and geraniol) when the synthesis of esters in n-hexane was considered (Larios et al. 2004).

Terpenes

Terpenes are one of the most widespread groups of natural products. They have many different functions in plants and animals, but for food, they are mainly important as aroma components. They are composed of isoprene units and can be cyclic, open chained, saturated, unsaturated, oxidized, and so on. The biotransformation of these compounds is potentially of considerable interest for application in the food flavor industry. The aroma of citrus, cinnamon, and many other spices, for example, is characterized by several terpenes. Common terpenes are limonene and citral (both in lemons), camphor, pinene (pine trees), eugenol (cloves), anethol (fennel and anise), thymol (thyme and oregano), geraniol (roses), and menthol.

Most of the terpenes obtained in microbial cultures are produced by fungi that belong to the ascomycete and basidiomycete species. Schindeler and Bruns (1980) demonstrated that terpene yields in *Ceratocystis variospora* cultures could be improved when toxic end products were removed using ion exchange resins. The fungus *Ceratocystis moniliformis* produces several aroma products such as ethyl acetate, propyl acetate, isobutyl acetate, isoamyl acetate, citronellol, and geraniol. In order to avoid the inhibitory effects detected in these cultures, it is necessary to decrease product concentrations in the bioreactor. Bluemke and Schrader (2001) developed an integrated bioprocess to enhance the production of natural flavors by *C. moniliformis*. The total yield of aroma compounds produced in the integrated bioprocess, with *in situ* product removal using pervaporation, is higher than in conventional batch cultivation. In addition, permeates obtained from pervaporation consist of highly enriched mixtures of flavors and fragrances. On the other hand, microbial transformation of terpenes has received considerable attention. Many microorganisms are able to break down terpenes or to carry out specific conversions, creating products with an added value. Dhavlikar and Albroscheit (1973) demonstrated that sesquiterpene valencene could be converted by some bacteria to sesquiterpene nootkatone, thus transforming a cheap compound into an important aroma.

Recently, significant research effort has focused on the enzymes related to terpene biosynthesis. The nucleic acid sequence of a monoterpene synthase from sweet basil, a key enzyme for the production of geraniol, has been determined in order to allow the production of a recombinant geraniol synthase (Pichersky et al. 2005). Also, a geraniol synthase from the evergreen camphor tree *Cinnamomum tenuipilum* was cloned and expressed in *Escherichia coli* (Yang et al. 2005). Functional genomics has also been applied to identify the genes for monoterpene synthases from *Vitis vinifera* grapes in order to characterize the enzymes by expression in *E. coli* and by subsequent analysis (Martin and Bohlmann 2004).

Alcohols

Alcohol compounds and their derived esters have interesting organoleptic properties. It is known that several yeasts produce long-chain and complex alcohols by fermentation. Some authors have proposed strategies for promoting this kind of flavor compounds during alcoholic beverage production. Mallouchos and others (2002) utilized *Saccharomyces cerevisiae* immobilized on delignified cellulosic material and gluten pellets. The former produced higher amounts of esters, whereas the latter gave higher amounts of alcohols. Kana and others (1992) found an increase in the concentration of amyl alcohols, total volatiles, and ethyl acetate when yeast immobilizated on γ-alumina and kissiris was employed in the fermentation process.

2-Phenylethanol is one of the most relevant aroma-related alcohols. Nowadays, it is predominantly synthesized by petrochemical routes from toluene, benzene, styrene, or methylphenylacetate (Nomura et al. 2001), while the natural 2-phenylethanol is mainly extracted from rose petals through a high-cost process (Fabre et al. 1998). However, it has been reported that different yeast strains such as *Hansenula anomala*, *Kluyveromyces marxianus*, or *S. cerevisiae* show a high potential for 2-phenylethanol production, which is derived from 2-phenylalanine by bioconversion (Fabre et al. 1998; Stark et al. 2002). Stark and others (2003) reported that the synergistic inhibition due to the presence of ethanol and 2-phenylethanol in the medium reduced the tolerance of *S. cerevisiae* to 2-phenylethanol, and thus its final concentration. To enhance the productivity of the bioconversion of 2-phenylalanine by *S. cerevisiae*, a novel *in situ* product recovery strategy was proposed by Serp and others (2003). An organic solvent (dibutyl sebacate) was entrapped within a polyethylene matrix in order to form a highly absorbent, chemically and mechanically stable composite resin. The use of this technique increased twofold the volumetric productivity of 2-phenylethanol and significantly facilitated downstream processing. Fabre and others (1997) screened 21 yeast strains for 2-phenylethanol production. Among the different 2-phenylethanol producers, *K. marxianus* was outstanding, which makes this strain a promising candidate to be applied in an industrial process.

de Temiño and others (2005) investigated the use of an immobilized alcohol dehydrogenase from *Lactobacillus kefir* to synthesize (R)-phenylethanol from acetophenone in an organic solvent (hexane). The enzyme and its cofactor were entrapped in polyvinyl alcohol gel beads in order to enhance their stability in organic solvents and to enable both cofactor diffusion and *in situ* regeneration.

Vanillin

As indicated above, vanillin (4-hydroxy-3-methoxybenzaldehyde) is a universally appreciated flavor chemical widely used in foods, beverages, perfumes, pharmaceuticals, and in various medical industries. Conventional production of vanillin by cultivating *Vanilla* plants is tedious and expensive. As for the use of plant cell culture, some further developments are still needed before extensive application. Though vanilla flavor has its own utility as flavoring, the use of vanillin obtained through biotechnology assumes significance since there is worldwide demand for natural compounds. In this context, vanillin and its other flavor metabolites are being produced through biotransformation as an alternative method of producing biovanillin

from cheaper substrates. Vanillin is an intermediate in the microbial degradation of several substrates, such as ferulic acid, phenolic stilbenes, lignin, eugenol, and isoeugenol. The conversion of natural eugenol and isoeugenol from essential oils into vanillin has been investigated using microbial and enzymatic biotransformations (Overhage et al. 1999; Rao and Ravishankar 1999; Shimoni et al. 2000, 2003; Washisu et al. 1993). Moreover, enzymatic synthesis of vanillin from vanillylamine using amine oxidase from *Aspergillus niger* has been reported and a continuous production process using immobilized enzyme assessed (Yoshida et al. 1997). Vanillylamine can be isolated from capsaicin, a natural ingredient of peppers and capsicums.

Strains including *Pseudomonas putida*, *A. niger*, *Corynebacterium glutamicum*, *Corynebacterium* sp., *Arthrobacter globiformis*, and *Serratia marcescens* (Priefert et al. 2001) can also convert eugenol or isoeugenol to vanillin.

A two-step bioconversion process using filamentous fungi was developed by Lesage-Meessen and others (1996, 2002) to transform ferulic acid into vanillin. First, *A. niger* transformed ferulic acid to vanillic acid, and then vanillic acid was reduced to vanillin by *Pycnoporus cinnabarinus*. Bonnin and others (2000) showed that the yield of vanillin may be significantly increased by adding cellobiose to *P. cinnabarinus* culture medium due to the decrease in oxidative decarboxylation of vanillic acid.

The importance of ferulic acid as precursor of vanillin has brought about a number of efforts in the investigation of its production. Feruloyl esterase has been identified as the key enzyme in the biosynthesis of ferulic acid, and some researchers have studied the production of this enzyme in microbial cultures of several fungi grown on different pretreated cereal brans, such as wheat, maize, rice bran, and sugarcane bagasse (Mathew and Abraham 2005). The metabolism of ferulic acid in some microorganisms has also been investigated (Falconnier et al. 1994).

Benzaldehyde

Benzaldehyde is the second most important molecule after vanillin for its use in cherry and other natural fruit and vegetable flavors. The world consumption of benzaldehyde amounts to approximately 7000 tons/year (Clark 1995). Natural benzaldehyde is generally extracted from fruit kernels such as apricots, leading to the undesirable formation of the toxic hydrocyanic acid. Nowadays, the fermentation of natural substrates is an alternative route to the production of benzaldehyde without harmful by-products. However, benzaldehyde is toxic toward microbial metabolism, and its accumulation in the culture medium may strongly inhibit cell growth (Lomascolo et al. 1999). For this reason, only a few microorganisms have been reported as benzaldehyde producers. Among them, the bacterium *P. putida* (Wilcocks et al. 1992) and the white rot fungi *Trametes suaveolens*, *Polyporus tuberaster*, *Bjerkandera adusta*, and *Phanerochaete chrysosporium* (Jensen et al. 1994; Kawabe and Morita 1994; Lapadatescu et al. 1999; Lomascolo et al. 2001) are mentioned as biocatalysts in the biosynthesis of benzaldehyde from phenylalanine. Park and Jung (2002) proposed the use of calcium alginate-encapsulated whole-cell enzymes from *P. putida* for the production of benzaldehyde from benzoylformate. This allowed the accumulation of benzaldehyde in the capsule core, minimizing its subsequent transformation to benzyl alcohol by the action of alcohol dehydrogenase and thus providing continuous production of benzaldehyde until reactant exhaustion.

SOLID-STATE FERMENTATION (SSF)

SSF has been mentioned as a potentially useful culture method for the production of flavors (Berger 1995; Feron et al. 1996; Soccol and Vandenberghe 2003) since it may lead to higher yields or better product characteristics than submerged fermentation (SmF). In addition, costs are much lower due to the efficient utilization and value addition of wastes. SSF is defined as any fermentation process performed on a nonsoluble material that acts both as physical support and source of nutrients in the absence of free-flowing liquid. This technique reproduces natural microbiological processes like composting and ensiling. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used (Pandey 1992; Pandey et al. 2000).

Several researchers have studied SSF production of aroma compounds by several microorganisms, such as *Neurospora* sp. (Pastore et al. 1994), *Zygosaccharomyces rouxii* (Sugawara et al. 1994), and *Aspergillus* sp. (Ito et al. 1990), using pregelatinized rice, miso, and cellulose fibers, respectively. Bramorski and others (1998b) compared fruity aroma production by *Ceratocystis fimbriata* in solid-state cultures using several agroindustrial wastes (cassava bagasse, apple pomace, amaranth, and soybean) and found that the medium with cassava bagasse, apple pomace, or soybean produced a strong, fruity aroma. Soares and others (2000) also reported the production of strong pineapple aroma when SSF was carried out using coffee husk as a substrate by this strain. Compounds such as acetaldehyde, ethanol, ethyl acetate (the major compound produced), ethyl isobutyrate, isobutyl acetate, isoamyl acetate, and ethyl-3-hexanoate were identified in the headspace of the cultures. The addition of leucine increased ethyl acetate and isoamyl acetate production, and then a strong odor of banana was detected. Bramorski and others (1998a) and Christen and others (2000) described the production of volatile compounds such as acetaldehyde and 3-methylbutanol by the edible fungus *Rhizopus oryzae* during SSF on tropical agroindustrial substrates.

The production of 6-pentyl-α-pyrone (6-PP), an unsaturated lactone with a strong coconut-like aroma, was studied using liquid and solid substrates by de Araujo and others (2002). Sugarcane bagasse was adequate for growth and aroma production; it has been demonstrated that, by the SSF process, it is possible to produce 6-PP at a higher concentration than that reported in literature for the submerged process.

K. marxianus produced fruity aroma compounds in SSF using cassava bagasse or giant palm bran (*Opuntia ficus indica*) as a substrate (Medeiros et al. 2000). In this report, several parameters were studied such as initial substrate pH, addition of glucose, cultivation temperature, initial substrate moisture, and inoculum size. The analysis showed the production of 9 and 11 compounds from palm bran and cassava bagasse substrate, respectively, including alcohols, esters, and aldehydes. In both cases, two species remained unidentified, and ethyl acetate, ethanol, and acetaldehyde were the major compounds produced.

It is known that several methylketones such as 2-undecanone, 2-nonanone, and 2-heptanone are produced at commercial scale by SSF from *A. niger* using coconut fat as substrate with a yield of 40% (Janssens et al. 1992). Several methods have been developed in order to enable vanillin and furanone or pyranone derivatives of natural origin to be produced from agricultural wastes (Fig. 34.2). The basic

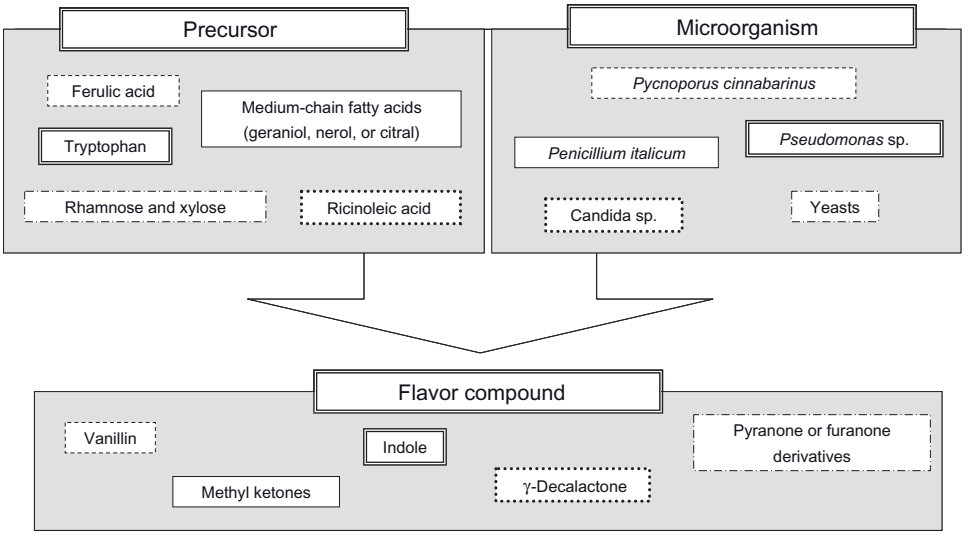

Figure 34.2. Examples of flavors produced by the microbial bioconversion of the precursors.

process combines enzyme degradation of plant cell walls and fungal fermentations. Ferulic acid (precursor) is released from agricultural wastes using polysaccharide-degrading enzymes and specific ferulic acid esterases. Then, ferulic acid is directly converted to vanillin by a selected basidiomycete, *P. cinnabarinus*, or by a two-step process using, first, *A. niger* to transform the ferulic acid into vanillic acid, then *P. cinnabarinus* to obtain vanillin from vanillic acid. Several wastes such as beet pulp and cereal bran (maize and wheat) have been examined (Bonnin et al. 2001; Mathew and Abraham 2005).

CONCLUSIONS

A brief survey on vegetable aroma compounds and the methods for their production has been presented. Although chemical synthesis or extraction from plants is still the prevailing manufacturing strategy, biotechnological production routes are progressively gaining relevance in food industries. Thus, a considerable amount of recent research has focused on the utilization of microbial cultures, both submerged and solid state, and enzymatic conversions for the production of flavors.

REFERENCES

Aguedo M, Ly MH, Belo I, Teixeira JA, Belin JM, Waché Y. 2004. The use of enzymes and microorganisms for the production of aroma compounds from lipids. *Food Technol Biotechnol* 42:327–36.

Allegrone G, Barbeni M, Cardillo R, Fuganti C, Grasselli P, Miele A, Pisciotta A. 1991. On the steric course of the microbial generation of (Z6)-gamma-dodecenolactone from (10R,S) 10-hydroxyoctadeca-(E8,Z12)-dienoic acid. *Biotechnol Lett* 13:765–8.

Ayabe S, Takano H, Fujita T, Furuya T, Hirota H, Takahashi T. 1990. Studies on plant tissue cultures. 72. Triterpenoid biosynthesis in tissue cultures of *Glycyrrhiza glabra* var. *glandulifera*. *Plant Cell Rep* 9:181–4.

Berger RG. 1995. *Aroma Biotechnology*. Berlin: Springer.

Bluemke W, Schrader J. 2001. Integrated bioprocess for enhanced production of natural flavors and fragrances by *Ceratocystis moniliformis*. *Biomol Eng* 17:137–42.

Bonnin E, Brunel M, Gouy Y, Lesage-Meessen L, Asther M, Thibault JF. 2001. *Aspergillus niger* I-1472 and *Pycnoporus cinnabarinus* MUCL39533, selected for the biotransformation of ferulic acid to vanillin, are also able to produce cell wall polysaccharide-degrading enzymes and feruloyl esterases. *Enzyme Microb Technol* 28:70–80.

Bonnin E, Grange H, Lesage-Meessen L, Asther M, Thibault JF. 2000. Enzymic release of cellobiose from sugar beet pulp, and its use to flavour vanillin production in *Pycnoporus cinnabarinus* from vanillic acid. *Carbohydr Polym* 41:143–51.

Bramorski A, Christen P, Ramirez M, Soccol CR, Revah S. 1998a. Production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates. *Biotechnol Lett* 20:359–62.

Bramorski A, Soccol CR, Christen P, Revah S. 1998b. Fruit aroma production by *Ceratocystis fimbriata* in static cultures from solid agro-industrial wastes. *Rev Microbiol* 28:208–12.

Brodnitz MH, Pascale JV, Van Derslice L. 1971. Flavor components of garlic extract. *J Agric Food Chem* 19(2):273–5.

Buttery RG, Ling LC, Juliano BO, Turnbaugh JG. 1983. Cooked rice aroma and 2-acetyl-1-pyrroline. *J Agric Food Chem* 31(4):823–6.

Christen P, Bramorski A, Revah S, Soccol CR. 2000. Characterization of volatile compounds produced by *Rhizopus* strains grown on agro-industrial solid wastes. *Bioresour Technol* 71:211–15.

Clark GS. 1995. Benzaldehyde. An aroma chemical profile. *Perfum Flavor* 20:53–60.

Collin RP, Halim AF. 1972. Characterization of the major aroma constituent of the fungus *Trichoderma viride* (Pers.). *J Agric Food Chem* 20:437–8.

Davidonis G, Knorr D. 1991. Callus formation and shoot regeneration in *Vanilla planifolia*. *Food Biotechnol* 5:59–66.

de Araujo A, Pastore GM, Berger RG. 2002. Production of coconut aroma by fungi cultivation in solid-state fermentation. *Appl Biochem Biotechnol* 98–100:747–51.

de Temiño D, Hartmeier W, Ansorge-Schumacher MB. 2005. Entrapment of the alcohol dehydrogenase from *Lactobacillus kefir* in polyvinyl alcohol for the synthesis of chiral hydrophobic alcohols in organic solvents. *Enzyme Microb Technol* 36:3–9.

Dhavlikar RS, Albroscheit G. 1973. Microbial transformation of terpenoids: Valencene. *Dragoco Report* 20:251–8.

Dimick PS, Walker NJ, Patton S. 1969. Occurrence and biochemical origin of aliphatic lactones in milk fat—A review. *J Agric Food Chem* 17649–55.

Dong SY, Lee KS, Jeong OY, Kim KJ, Kays SJ. 2008. Characterization of volatile aroma compounds in cooked black rice. *J Agric Food Chem* 56(1):235–40.

Dordick JS. 1989. Enzymic catalysis in monophasic organic solvents. *Enzyme Microb Technol* 11:194–211.

Dornenburg H, Knorr D. 1996. Production of the phenolic flavour compounds with cultured cells and tissues of *Vanilla planifolia* species. *Food Biotechnol* 10:75–92.

Drawert F, Berger RG, Godelmann R. 1984. Regioselective biotransformation of valencene in cell suspension cultures of *Citrus* spp. *Plant Cell Rep* 3:37–40.

Duckham SC, Dodson AT, Bakker J, Ames JM. 2001. Volatile flavour components of baked potato flesh. A comparison of eleven potato cultivars. *Nahrung* 45(5):317–23.

Fabre CE, Blanc PJ, Goma G. 1997. Screening of yeasts producing 2-phenylethanol. *Biotechnol Technol* 11:523–5.

Fabre CE, Blanc PJ, Goma G. 1998. Production of 2-phenylethyl alcohol by *Kluyveromyces marxianus*. *Biotechnol Prog* 14:270–4.

Falconnier B, Lapierre C, Lesage-Meessen L, Yonnet G, Brunerie P, Colonna-Ceccaldi B, Corrieu G, Asther M. 1994. Vanillin as a product of ferulic acid biotransformation by the white-rot fungus *Pycnoporus cinnabarinus* I-937: Identification of metabolic pathways. *J Biotechnol* 37:123–32.

Feron G, Bonnarme P, Durand A. 1996. Prospects of the microbial production of food flavours. *Trends Food Sci Technol* 7:285–93.

Funk C, Brodelius P. 1990. Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. II. Effects of precursor feeding and metabolic inhibitors. *Plant Physiol* 94:95–101.

Funk C, Brodelius P. 1992. Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. IV. Induction of vanillic acid formation. *Plant Physiol* 99:256–62.

Gatfield IL. 1988. Production of flavour and aroma compounds by biotechnology. *Food Technol* 10:110–22.

Gatfield IL. 1999. Biotechnological production of natural flavor materials. In Teranishi R, Wick EL, Hornstein I (eds.), *Flavor Chemistry, Thirty Years of Progress*. New York: Plenum Press, pp. 211–27.

Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311(5762):815–19.

Griffiths G, Trueman L, Crowther T, Thomas B, Smith B. 2002. Onions—A global benefit to health. *Phytother Res* 16(7):603–15.

Harlander S. 1994. Biotechnology for the production of flavouring materials. In Reineccius G (ed.), *Source Book of Flavours*. New York: Chapman and Hall, pp. 151–75.

Havkin-Frenkel D, Dorn R. 1997. Vanilla. In Risch SJ, Chi-Tang Ho C-T (eds.), *Spices: Flavor Chemistry and Antioxidant Properties. ACS Symposium Series 660*. Washington, DC: American Chemical Society, pp. 29–40.

Hrazdina G. 2006. Aroma production by tissue cultures. *J Agric Food Chem* 54(4):1116–23.

Ito K, Yoshida K, Ishikawa T, Kobayashi S. 1990. Volatile compounds produced by fungus *Aspergillus oryzae* in rice koji and their changes during cultivation. *J Ferment Bioeng* 70:169–72.

Janssens L, de Pooter HL, Vandamme EJ, Schamp NM. 1992. Production of flavours by microorganisms. *Process Biochem* 27:195–215.

Jensen KA, Evans KMC, Kirk TK, Hammel KE. 1994. Biosynthetic pathway for veratryl alcohol in the ligninolytic fungus *Phanerochaete chrysosporium*. *Appl Environ Microbiol* 60:709–14.

Kana K, Kanellaki M, Koutinas AA. 1992. Volatile by-products formed in batch alcoholic fermentations: Effect of γ-alumina and kissiris supported biocatalysts. *Food Biotechnol* 6:65–74.

Kapfer GF, Berger RG, Draweti F. 1989. Production of 4-decanolide by semicontinuous fermentation of *Tyromyces sambuceus*. *Biotechnol Lett* 11:561–6.

Kawabe T, Morita H. 1994. Production of benzaldehyde and benzyl alcohol by the mushroom *Polyporus tuberaster* K2606. *J Agric Food Chem* 42:2556–60.

Kim TH, Shin JH, Baek HH, Lee HJ. 2001. Volatile flavour compounds in suspension culture of *Agastache rugosa* Kuntze (Korean mint). *J Sci Food Agric* 81:569–75.

Krings U, Berger RG. 1998. Biotechnological production of flavours and fragrances. *Appl Microbiol Biotechnol* 49:1–8.

Kumar R, Modak J, Madras G. 2005. Effect of chain length of the acid on the enzymatic synthesis of flavours in supercritical carbon dioxide. *Biochem Eng J* 23:199–202.

Lapadatescu C, Ginies C, Djian A, Spinnler HE, Le Quéré JL, Bonnarme P. 1999. Regulation of the synthesis of aryl metabolites by phospholipids sources in the white-rot fungus *Bjerkandera adusta*. *Arch Microbiol* 171:151–8.

Larios A, Garcia HS, Oliart MR, Valerio-Alfaro G. 2004. Synthesis of flavour and fragrance esters using *Candida antarctica* lipase. *Appl Microbiol Biotechnol* 65:373–6.

Lesage-Meessen L, Delattre M, Haon M, Thibault JF, Colonna Ceccaldi B, Brunerie P, Asther M. 1996. A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. *J Biotechnol* 50:107–13.

Lesage-Meessen L, Lomascolo A, Bonnin E, Thibault JF, Buleon A, Roller M, Asther M, Record E, Ceccaldi BC, Asther M. 2002. A biotechnological process involving filamentous fungi to produce natural crystalline vanillin from maize bran. *Appl Biochem Biotechnol* 102-103:141–53.

Liu SQ, Holland R, Crow VL. 2004. Esters and their biosynthesis in fermented dairy products: A review. *Int Dairy J* 14:923–45.

Lomascolo A, Asther M, Navarro D, Antona C, Delattre M, Lesage-Meessen L. 2001. Shifting the biotransformation pathways of L-phenylalanine into benzaldehyde by *Trametes suaveolens* CBS 334.85 using HP20 resin. *Lett Appl Microbiol* 32:262–7.

Lomascolo A, Lesage-Meessen L, Labat M, Navarro D, Delattre M, Asther M. 1999. Enhanced benzaldehyde formation by a monokaryotic strain of *Pycnoporus cinnabarinus* using a selective solid adsorbent in the culture medium. *Can J Microbiol* 45:653–7.

Longo MA, Sanroman MA. 2006. Production of food aroma compounds: Microbial and enzymatic methodologies. *Food Technol Biotechnol* 44:335–53.

Mallouchos A, Komaitis M, Koutinas A, Kanellaki M. 2002. Wine fermentations by immobilized and free cells at different temperatures. Effect of immobilization and temperature on volatile by-products. *Food Chem* 80:109–13.

Martin DM, Bohlmann J. 2004. Identification of *Vitis vinifera* (-)-α-terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. *Phytochemistry* 65:1223–9.

Mathew S, Abraham TE. 2005. Studies on the production of feruloyl esterase from cereal brans and sugarcane bagasse by microbial fermentation. *Enzyme Microb Technol* 36:565–70.

Medeiros ABP, Pandey A, Freitas RJS, Christen P, Soccol CR. 2000. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. *Biochem Eng J* 6:33–9.

Morris WL, Ross HA, Ducreux LJM, Bradshaw JE, Bryan GJ, Taylor MA. 2007. Umami compounds are a determinant of the flavor of potato (*Solanum tuberosum* L.). *J Agric Food Chem* 55:9627–33.

Mulabagal V, Tsay HS. 2004. Plant cell cultures—An alternative and efficient source for the production of biologically important secondary metabolites. *Int J Appl Sci Eng* 2:29–48.

Nabeta K, Ohnishi Y, Hirose T, Sugisawa H. 1983. Monoterpene biosynthesis by callus tissues and suspension cells from *Perilla* species. *Phytochemistry* 22:423–5.

Nakao M, Ono K, Takio S. 1999. The effect of calcium on flavanol production in cell suspension culture of *Polygonum hydropiper*. *Plant Cell Rep* 18:759–63.

Nardi M, Fiez-Vandal C, Tailliez P, Monnet V. 2002. The EstA esterase is responsible for the main capacity of *Lactococcus lactis* to synthesize short chain fatty acid esters *in vitro*. *J Appl Microbiol* 93:994–1002.

Nomura K, Ogura H, Imanishi Y. 2001. Direct synthesis of 2-phenylethanol by hydrogenation of methyl phenylacetate using homogeneous ruthenium-phosphine catalysis under low hydrogen pressure. *J Mol Catal A: Chem* 166:345–9.

Ohsumi C, Hayashi T, Sano K. 1993. Formation of allin in the culture tissues of *Allium sativum* oxidation of S-allyl-L-cysteine. *Phytochemistry* 33:107–11.

Okui S, Uchiyama M, Mizugaki M. 1963a. Metabolism of hydroxy fatty acids. I. Metabolic conversion of ricinoleic acid by a certain microorganism to (+)-D-8-hydroxy-*cis*-5-tetradecenoic acid. *J Biochem* 53:265–70.

Okui S, Uchiyama M, Mizugaki M. 1963b. Metabolism of hydroxy fatty acids. II. Intermediates of the oxidative breakdown of ricinoleic acid by genus *Candida*. *J Biochem* 54:536–40.

Overhage J, Priefert H, Rabenhorst J, Steinbuchel A. 1999. Biotransformation of eugenol to vanillin by a mutant of *Pseudomonas* sp. strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh) gene. *Appl Microbiol Biotechnol* 52:820–8.

Pandey A. 1992. Recent process developments in solid-state fermentation. *Process Biochem* 27:109–17.

Pandey A, Soccol CR, Mitchell D. 2000. New developments in solid state fermentation: I Bioprocesses and products. *Process Biochem* 35:1153–69.

Park JK, Jung JY. 2002. Production of benzaldehyde by encapsulated whole-cell benzoylformate decarboxylase. *Enzyme Microb Technol* 30:726–33.

Pastore GM, Park YK, Min DB. 1994. Production of a fruity aroma by *Neurospora* from beiju. *Mycol Res* 98:25–35.

Pichersky E, Iijima Y, Lewinsohn E, Gang DR, Simon J. 2005. Cloning and sequence of geraniol synthase from sweet basil and use in the production of geraniol and metabolites. International Patent PCT WO 2005060553.

Priefert H, Babenhorst J, Steinbuchel A. 2001. Biotechnological production of vanillin. *Appl Microbiol Biotechnol* 56:296–314.

Prince CL, Shuler ML, Yamada Y. 1997. Altering flavour profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis. *Biotechnol Prog* 13:506–10.

Rao SR, Ravishankar GA. 1999. Biotransformation of isoeugenol to vanilla flavour metabolites and capsaicin in suspended and immobilized cell cultures of *Capsicum frutescens*: Study of the influence of β-cyclodextrin and fungal elicitor. *Process Biochem* 35:341–8.

Rao SR, Ravishankar GA. 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol Adv* 20:101–53.

Rao SR, Ravishankar GA. 2000. Vanilla flavour: Production by conventional and biotechnological routes. *J Sci Food Agric* 80:289–304.

Rojas V, Gil JV, Pinaga F, Manzanares P. 2001. Studies on acetate ester production by non-*Saccharomyces* wine yeasts. *Int J Food Microbiol* 70:283–9.

Romagnoli LG, Knorr D. 1988. Effects of ferulic acid treatment on growth and flavour development of cultured *Vanilla planifolia* cells. *Food Biotechnol* 2:93–104.

Sahai OM. 1994. Plant tissue culture. In Gabelman A (ed.), *Bioprocess Production of Flavour, Fragrances and Color Ingredients*. New York: Wiley, pp. 239–75.

Schindeler J, Bruns K. 1980. Process for producing monoterpene containing aroma by fermentation. Germany Patent 2840143.

Scragg AH. 1997. The production of aromas by plant cell cultures. In Scheper P (ed.), *Advances in Biochemical Engineering Biotechnology*, Vol. 55. Berlin: Springer, pp. 239–63.

Serp D, von Stockar U, Marison IW. 2003. Enhancement of 2-phenylethanol productivity by *Saccharomyces cerevisiae* in two-phase fed-batch fermentations using solvent immobilization. *Biotechnol Bioeng* 82:103–10.

Shimoni E, Baasov T, Ravid U, Shoham Y. 2003. Biotransformations of propenylbenzenes by an *Arthrobacter* sp. and its *t*-anethole blocked mutants. *J Biotechnol* 105:61–70.

Shimoni E, Ravid U, Shoham Y. 2000. Isolation of a *Bacillus* sp. capable of transforming isoeugenol to vanillin. *J Biotechnol* 78:1–9.

Soares M, Christen P, Pandey A, Soccol CR. 2000. Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid state fermentation. *Process Biochem* 35:857–61.

Soccol CR, Vandenberghe LPS. 2003. Overview of applied solid-state fermentation in Brazil. *Biochem Eng J* 13:1205–18.

Stark D, Münch T, Sonnleitner B, Marison IW, von Stockar U. 2002. Extractive bioconversion of 2-phenylethanol from L-phenylalanine by *Saccharomyces cerevisiae*. *Biotechnol Prog* 18:514–23.

Stark D, Zala D, Münch T, Sonnleitner B, Marison IW, von Stockar U. 2003. Inhibition aspects of the bioconversion of L-phenylalanine to 2-phenylethanol by *Saccharomyces cerevisiae*. *Enzyme Microb Technol* 32:212–23.

Sugawara E, Hashimoto S, Sakurai Y, Kobayashi A. 1994. Formation by yeast of the HEMF (4-hydroxy-2(or5)-ethyl-5(or2)-methyl-3(2H)-furanone) aroma components in miso with aging. *Biosci Biotechnol Biochem* 58:1134–5.

Suresh B, Ritu T, Ravishankar GA. 2003. Vanilla flavour production through biotransformation using *Capsicum frutescens* root cultures. *Biocatal Biotransformation* 21:333–40.

Suvarnalatha G, Narayan MS, Ravishankar GA, Venkataraman LV. 1994. Flavour production in plant cell cultures of Basmati rice (*Oryza sativa* L.). *J Sci Food Agric* 66:439–42.

Townsley PM. 1972. Chocolate from plant cells. *J Inst Can Sci Technol Aliment* 7:76–8.

Urbach G. 1997. The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. *Int J Dairy Technol* 50:79–89.

Vandamme EJ, Soetaert W. 2002. Bioflavours and fragrances *via* fermentation and biocatalysis. *J Chem Technol Biotechnol* 77:1323–32.

Wache Y, Aguedo M, Choquet A, Gatfield IL, Nicaud JM, Belin JM. 2001. Role of β-oxidation enzymes in γ–decalactone production by the yeast *Yarrowia lipolytica*. *Appl Environ Microbiol* 67:5700–4.

Washisu Y, Tetsushi A, Hashimoto N, Kanisawa T. 1993. Manufacture of vanillin and related compounds with *Pseudomonas*. Japanese Patent JP52279.

Westcott RJ, Cheethan PSJ, Barraclough AJ. 1994. Use of organized viable vanilla plant aerial roots for the production of natural vanillin. *Phytochemistry* 35:135–8.

Widder S, Lüntzel CS, Dittner T, Pickenhagen W. 2000. 3-Mercapto-2-methylpentan-1-ol, a new powerful aroma compound. *J Agric Food Chem* 48(2):418–23.

Wilcocks R, Ward OP, Scott C, Nolan JD, Yaping H, Elizabeth P. 1992. Acyloin formation by benzoylformate decarboxylase from *Pseudomonas putida*. *Appl Environ Microbiol* 58:1699–704.

Yang T, Li J, Wang HX, Zeng Y. 2005. A geraniol synthase gene from *Cinnamomum tenuipilum*. *Phytochemistry* 66:285–93.

Yoshida A, Takenaka Y, Tamaki H, Frebort I, Adachi O, Kumagai H. 1997. Vanillin formation by microbial amine oxidases from vanillylamine. *J Ferment Bioeng* 84:603–5.

Zhou Q, Wintersteen CL, Cadwallader KR. 2002. Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. *J Agric Food Chem* 50(7):2016–21.

CHAPTER 35

Flavor from Transgenic Vegetables

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INTRODUCTION

Vegetables play an important role in human nutrition and health by providing minerals, vitamins, micronutrients, antioxidants, dietary fiber, and phytosterols. Because of the trend toward a healthy way of living, the consumption of vegetables is constantly increasing. There is an increasing demand from consumers for produce with improved flavor and aroma and other nutritional characteristics. Flavor and aroma are the most subjective quality attributes. Flavor is composed of sweetness and sourness, which are perceived at the taste level, and aroma corresponds to those perceived by smell such as sugars, acids, and volatiles. Flavor also includes bitterness (sesquiterpene lactones, saltiness, and astringency related to flavonoids, tannins, and other factors) (DeRovira 1997; Peters and Amerongen 1998; Taylor 1993).

The quality of fresh produce has been based on physicochemical parameters of which most emphasis was given to the external characteristics such as absence of defects, size, and color. The crop plant breeders mostly focused on disease resistance, yield, color, size, and other easily quantifiable parameters. Flavor and texture of crops were not a part of the selection process, and improvements in these quality attributes were delayed. Conventional breeding in the past has significantly contributed to the improvement of vegetable yields, postharvest life, quality, and resistance to biotic and abiotic stresses. The primary determinant of flavor is genetics. Other factors such as preharvest environment, cultural practices, harvesting maturity, and postharvest handling play a lesser role (Baldwin et al. 1991a,b; Cunningham et al. 1985; Fellman and Mattheis 1999; Mattheis et al. 1995; Maul et al. 1998; Romani et al. 1983; Wright and Harris 1985). There exist constraints in traditional breeding, which can be addressed through advancement in biology, such as genetic engineering. In the last decade, various quality and physicochemical traits have been successfully engineered into vegetable crops. The purpose of this chapter is to evaluate research related to the flavor of transgenic vegetables.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

TRANSGENIC VEGETABLES—DEFINITION

Transgenic crops in general are defined as plants that contain a gene or genes that have been artificially inserted. The inserted gene sequence, also called transgene, may come from an unrelated plant or from a completely different species. A gene from bacterium *Bacillus thuringiensis* (Bt) inserted in corn designated as Bt corn made the product resistant to chewing insects. Plants containing transgenes are often called genetically modified (GM) crops. Transgenic technology enables plant breeders to bring together useful genes from a wide range of living sources in a single plant species. Biotechnology approaches also provide the means for identifying and isolating genes controlling specific characteristics in one kind of organism and for moving copies of these genes into another quite different organism, which will then have the same phenotypical characteristics. This powerful tool helps plant breeders in designing more useful and productive crop varieties containing new combinations of genes. The years 1996–1997 was historic for American agriculture with the introduction of large-scale GM crops for agricultural use. Examples of GM crops that have been approved for commercialization are discussed as follows:

- *Soybean.* Transgenic soybean with enhanced oleic acid content from DuPont and soybean with glyphosate (round up) and synchrony tolerance from Monsanto and Dekalb Genetics, respectively, were developed.
- *Corn.* Mostly insect resistant as well as glyphosate and glufosinate tolerance corns were developed.
- *Canola.* Transgenic canola with enhanced laurate oil content and tolerance to glyphosate and glufosinate were developed.
- *Potato.* Genetically engineered potato resistant to Colorado potato beetle was also developed.
- *Squash and Tomato.* Among the vegetables approved for commercialization were virus-resistant squash and tomato with delayed ripening and enhanced flavor, to name a few.

The flowchart for the development of GM crops is depicted in Figure 35.1.

More studies are being conducted on various crops including field crops such as barley, rice, wheat, and tobacco; flowers (gladiolus, petunia, and chrysanthemum); trees (poplar, spruce, and sweet gum); oil (soybean, sunflower, and peanut); nut

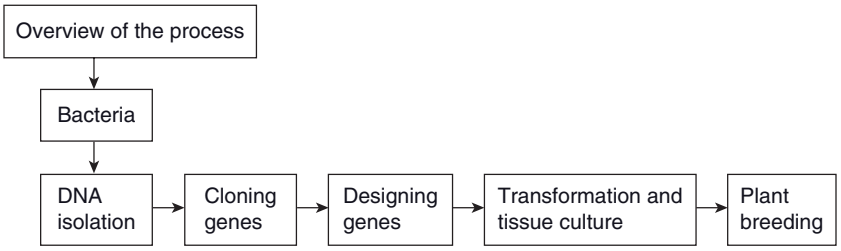

Figure 35.1. Flowchart for GM crops. *Source*: Adapted from http://cls.casa.colostate.edu/transgenicCrops/what.html (accessed April 12, 2008).

(walnut); grasses (creeping bent grass and bluegrass); sugar content (beet and sugarcane); fruits (apple, cranberry, grape, melon, plum, raspberry, strawberry, and watermelon); vegetable (broccoli, carrot, cucumber, eggplant, lettuce, pea, pepper, squash, and tomato).

RESEARCH TRENDS IN TRANSGENIC VEGETABLE CROPS

Vegetables play an important role in human health and nutrition. Cultivation of vegetable crops is an integral part of the agricultural economy of many countries. The production and quality of vegetables is often compromised by several biotic and abiotic stresses, which destabilize rural economies in many countries. Also, the absence of postharvest storage and processing facilities leads to qualitative ad quantitative losses. Conventional breeding had contributed significantly for the improvement of vegetable yields, quality, postharvest life, and resistance to diseases. However, there are many constraints in conventional breeding, which can only be overcome by advancements made in modern biology. In the past decades, various traits such as biotic stress resistance, quality, and storage life have been successfully engineered into vegetable crops, and some of them have been commercialized. Recently, significant progress has been made to manipulate vegetable crops for abiotic stress tolerance, for quality improvement, and for pharmaceutical and industrial applications. Since the introduction in 1996 of the first large-scale insect-resistant transgenic crops, the global area of cultivation of these crops had increased 47-fold, from 1.7 million to 81.0 million hectares in 2004 (Dalal et al. 2006; Ghosh et al. 2004; James 2004). The most dominant trait among the transgenic crop plants is herbicide tolerance, followed by Bt-based insect resistance. The four major transgenic crops are herbicide-resistant soybean and canola, Bt maize, and cotton (James 2004). The two major commercialized transgenic vegetables are insect- and virus-resistant potato and tomato with delayed fruit ripening. The goal of plant biotechnology is not only to improve the agronomic traits of crop plants but also to improve the nutritional content and organoleptic qualities such as taste and aroma of fruits and vegetables.

TRANSGENIC VEGETABLES AND NUTRITION

Crop plants are known to be a great source of nutrients, minerals, vitamins, proteins, carotenoids, and flavonoids. These nutritional compounds produced by plants can be enhanced through genetic modifications. Some vegetables are known to be deficient in amino acids such as methionine and lysine. The nutritive value of potato proteins, for example, is reduced because of the deficiency in essential amino acids tyrosine, lysine, methionine, and cysteine. In order to improve the nutritive value of potato, an *Amaranthus* seed albumin gene *AmA1* has been expressed in transgenic potato tubers (Dalal et al. 2006). Similarly, a 292-bp artificial gene (*asp-1*) encoding a storage protein composed of essential amino acids was introduced in sweet potato, resulting in a fourfold increase in the expression of this protein in transgenic potato compared to the untransformed parent (Egnin and Prakash 1997). Zeh and others (2001) reported a 30-fold increase in methionine content in transgenic potato tubers

engineered with antisense threonine synthase (TS) gene. The amino acids of these vegetables were enhanced by expression of the synthetic protein, overexpression of homologus proteins, modifying the amino acid sequence of specific proteins, or through metabolic engineering. The levels of antioxidants such as precursors of vitamin A (β-carotene) and lycopene can also be increased through genetic engineering. These compounds are normally synthetized through the isoprenoid biosynthetic pathway and therefore, genetic manipulation of this pathway led to the improvement of the nutritional and organoleptic qualities of vegetables. Roemer and others (2000) manipulated a bacterial gene encoding for the enzyme phytoene desaturase, which converts phytoene into lycopene, resulting in a threefold increase in the level of β-carotene in transgenic tomato. Similarly, Romer and others (2002) reported a sixfold increase in carotenoid content and a two- to threefold increase in α-tocopherol content in transgenic potato plants by antisense or by co-suppression of the zeaxanthin peroxidase gene that results in the inhibition of zeaxanthin conversion to violaxanthin. Ducreux and others (2005) also reported an increase of 11- to 19-fold in the levels of carotene and lutein in transgenic potato tubers obtained through the introduction of the *Erwinia uredovora ertB* gene encoding phytoene synthase in potatoes. As well, the levels of flavonoid content in tomato can be increased by overexpression of either the transcription factors that regulate the genes of this pathway or the enzymes involved in flavonoid biosynthesis. Le Gall and others (2003) reported an increase of 10-fold in flavonoid content that has been achieved by ectopic expression of the maize transcription factors LC and Cl in transgenic tomato. Muir and others (2001) also reported a significant increase in the level of flavonoids in transgenic tomato plants expressing petunia CHI-A gene encoding chalcone isomerase. The above are just a few examples of the application of genetic engineering to design vegetables with improved nutritional content and organoleptic qualities.

IMPROVEMENT OF AROMA

Aroma and flavors strongly influence people's choice of foods. The aroma of crops is a property contributed by a complex mixture of metabolites such as alcohols, ketone, phenols, aldehydes, and ethers. Some of the alcohols and aldehydes that contribute to flavor derived from the actions of enzymes such as alcohol dehydrogenase, lipases, and hydroperoxide lyases. Wang and others (1996) expressed yeast Δ-9 desaturase gene in tomato, leading to transgenic plants with changes in important flavors such as *cis*-3-hexenol, 1-hexanol, hexanal, and *cis*-3-hexenal. Speirs and others (1998) in another study showed that overexpressing nonspecific alcohol dehydrogenase gene in tomato altered the levels of aroma determining short-chain aldehydes and alcohols, resulting in an intense "ripe fruit" flavor in tomato. One of the main compounds that influence the flavor quality of tomato and tomato products is linalool, an acyclic monoterpene alcohol. Linalool imparts a sweet, floral, alcoholic note to fresh tomato. Lewinsohn and others (2001) altered linalool levels through genetic engineering of the S-linalool synthase enzyme. The latter catalyzes the formation of linalool and therefore increases the levels of linalool and linalool derivatives in the transgenic tomato. The following are some of the limited examples of transgenic vegetables and flavor.

Potato

Loss of the flavor compound methional and browning in potato during processing is a major concern for the quality of the product. To overcome these deficiencies, Coetzer and others (2001) have engineered transgenic potatoes by antisense inhibition of polyphenol oxidase, resulting in potatoes with improved flavor and excellent quality attributes. In another study, Di and others (2003) increased the level of soluble methionine in potato by introducing *Arabidopsis thaliana* CGS cDNA into Russet Burbank potato, under transcriptional control of the cauliflower mosaic virus 35S promoter. This strategy increases the methional level in tubers of field-grown transgenic potato lines 2.4- to 4.4-fold. There was a correlation between the methional level and the level of soluble methionine in the tubers from the same lines before processing.

Soybean

Soybean is one of the most important cash crops in America and is used for animal feed and human food. One way to improve the stability of soy oil is to modify the polyunsaturated fatty acid composition of soybean. This means increasing the oleic acid content and reducing the linolenic acid content of soybeans. High oleic soybeans are stably integrated and the line is phenotypically and genetically stable over several generations and in various environments. None of the genes are expressed and therefore do not give rise to any new proteins in the transgenic high oleic soybeans. The chemical composition of the transgenic soybeans is similar to commodity soybean lines within the ranges of natural variation except for the fatty acid composition. High oleic soybean oil compares favorably to the many commonly used frying oils and fats with regard to the absence of trans-fatty acids, the reduction of saturated fatty acids to about 10%, and the elimination of off-flavors usually associated with a hydrogenated fat or oil (Table 35.1). The feeding and allergenic studies did not show any significant difference between the transgenic high oleic and the commodity soybeans. The possibility of horizontal gene transfer of the β-lactamase

TABLE 35.1. A Comparison of the Fatty Acid Compositions (% w/w) of Existing Commercial Shortenings from German Grocery Stores with High Oleic Soybean Oil

Description	SFA[a]	MUFA[b]	Others[c]
Partially hydrogenated sunflower and palm oil	38.1	39.4	1.4
Vegetable shortening	50.1	38.5	1.4
Sunflower, palm, and palm kernel oil	29.0	18.2	0.0
Partially hydrogenated coconut oil	78.3	16.1	0.0
Sunflower and palm coconut oil	37.7	36.6	1.2
Fry shortening	50.2	39.1	0.9
Butterfat	59.0	26.6	10.6
High oleic soybean oil (transgenic)	10.0	83.8	0.0

Source: http://www.agbios.com/docroot/decdocs/HOS1.pdf (accessed June 22, 2008).

[a]Saturated fatty acid, predominantly C16:0 and C18:0.

[b]Monounsaturated fatty acid, exclusively C18:1 n–9.

[c]Includes trans-fatty acids, positional isomers, and other fatty acids.

TABLE 35.2. Comparison of the Fatty Acid Composition of the Transgenic to the Parent and the Commodity Soybeans

Oil	C16:0 Palmitic Acid	C18:0 Stearic Acid	C18:1 Oleic Acid	C18:2 (9, 12) Linoleic Acid	C18:2 (9, 15) Linoleic Acid Isomer	C18:3 Linolenic Acid	Others
Transgenic soybean oil	6.2	3.1	82.3	2.2	0.5	3.5	2.2
Parent soybean oil	9.7	3.7	22.7	54.2	0.0	7.2	2.5
Edible soybean oil	7.14	1.4–5.5	19.0–30.0	44.0–62.0	0.0	4.0–11.0	—

Source: DuPont. 1997. Occurrence of linoleic acid (9–15) isomer in commonly used oils and fats for frying and baking in Europe. http://www.agbios.com/docroot/decdocs/HOS1.pdf (accessed June 22, 2008).

gene from the transgenic soybeans to intestinal bacteria in humans and in animals is remote, and its presence in the transgenic soybeans would not increase the risk of spreading ampicillin resistance genes nor would it compromise human and animal therapy. The high oleic phenotype does not affect the agronomic performance of high oleic soybeans, nor the susceptibility to diseases and insects, and would be expected to have no effect on the weediness potential, impact on nontarget organisms, and biodiversity. The comparison of the fatty acid composition of the transgenic, the parent, and the commodity soybeans are presented in Table 35.2. The oleic acid content of the transgenic soybean is between 80% and 85% compared to the commodity ones around 25% (Table 35.2). The results of the risk assessment by the Canadian Food Inspection Agency (CFIA) proved that the transgenic high oleic soybeans would not have any substantial negative effect on the environment. As well, there was no difference in the nutritional composition and feed safety attributes of the transgenic high oleic soybeans compared to the conventional ones and therefore were allowed to be grown in Canada and to be used as an ingredient in livestock feed (CFIA 2001).

Tomato

Tomato is the most studied vegetable for flavor and aroma. Buttery (1993) identified over 400 volatile compounds in tomato of which 16 have odor thresholds that would indicate their contribution to the flavor of tomato. The combination of *cis*-3-hexenal, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β-ionone in the right proportion produces the aroma of fresh ripe tomato (Buttery 1993; Oke et al. 2003). Oke and others (2003) identified several flavor components in the flavor profile of transgenic tomato, GM with an antisense phospholipase D cDNA, and found no major qualitative differences in the profiles of the control and the transgenic tomato (Table 35.3). Membrane phospholipid degradation mediated by phospholipase D is a key step in the catabolism and subsequent generation of fatty acids, the precursors of volatile short-chain fatty acids, aldehydes, and alcohols during fruit ripening. The

TABLE 35.3. Relative Amounts of Volatile Compounds of Transgenic and Control Tomato Fruits Analyzed by SPME–GC/MS

	Total Ion Counts $\times 10^{-4}$	
Volatiles	Control	Transgenic
Pentenal (E)	126 ± 153	2785 ± 657
2-Hexenal	392 ± 109	2046 ± 109
6-Methyl-5-hepten-2-One	805 ± 40	849 ± 202
2-Isobutyl-thiazole	321 ± 124	88 ± 19
2-Octenal(E)	336 ± 48	243 ± 74
Geranylacetone	361 ± 41	302 ± 6

The transgenic tomato was obtained through transformation with antisense phospholipase D cDNA. Adapted from Oke and others (2003). SPME, solid phase microextraction; GC/MS, gas chromatography/mass spectrometry.

volatile composition in control and transformed tomato fruits primarily consisted of hydrocarbons, aldehydes, esters, ketones, and sulfur- and nitrogen-containing heterocyclic compounds. Most of them were highly volatile (C5–C9) carbon chains, and a few were less volatile such as methyl salycilate, β-ionone, and geranylacetone. There were quantitative differences in certain volatiles such as pentenal and hexenal, which were several folds higher in the transgenic tomatoes and were noticed in certain seasons (Oke et al. 2003). Among these volatile compounds, *cis*-3-hexenal and β-ionone have the highest odor units, and 2-isobutylthiazole is found only in tomato. The production of volatiles occurs in conjunction with the climacteric increase in respiration and ethylene evolution as well as carotenoid breakdown (Baldwin 1991a). The important enzymes in the synthesis of volatiles from lipids include lipoxygenase, hydroperoxide lyase, and alcohol dehydrogenase (Galliard et al. 1977; Riley et al. 1996). Buttery and Ling (1993) found amino acids alanine, isoleucine, leucine, phenylalanine, and valine as precursors for tomato volatiles. Krammer and others (1994) also proved that some volatiles have glycosides as precursors. Transgenic vegetables with up- or downregulated alcohol dehydrogenase expression exhibited altered levels of some related volatiles (Speirs et al. 1998). Lewinsohn and others (2001) and Sangwan and Sangwan (2007), enhanced the levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits (Fig. 35.2). The genetic transformation of tomato with antisense phospholipase D cDNA resulted in improved quality characteristics of fruits and their processed products including the volatile compounds (Oke et al. 2003; Pinhero et al. 2003). There are three major pathways in the biosynthesis of the aroma component in plants (Croteau et al. 2000):

1. degradation pathway of lipids for the formation of short-chain alcohols and aldehydes, such as n-hexanol or *cis*-3-hexenol (compounds imparting fresh and green notes);
2. the shikimic acid pathway by which eugenol (cloves), t-anethole (anise), and estragole (basil) biosynthesize; and
3. the terpenoid pathway by which geraniol (rose), 1, 8-cineole (eucalyptus), and menthol peppermint are synthesized.

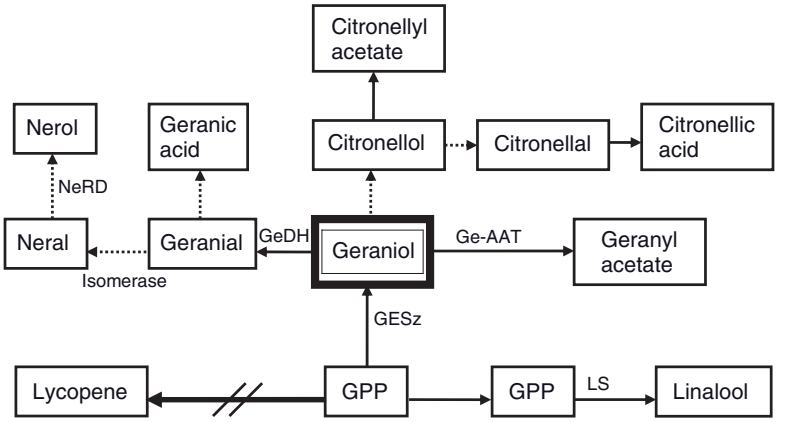

Figure 35.2. Pathway showing geraniol metabolism in tomato (and other acyclic monoterpenoid-bearing aromatic plants) and diversion of precursors from lycopene synthesis to monoterpenoid flavors. Geraniol molecule, after being synthesized, is successively transformed into geranial (by geraniol dehydrogenase [GeDH]), geranyl acetate (by geraniol alcohol CoA transferase [Ge-AAT]); citronellol (by putative geraniol reductase), and citronellyl acetate (by citronellyl-alcohol acetyl transferase). Adapted from Sangwan and Sangwan (2007). CoA, coenzyme A; NeRD, neral reductase; GESz, geranyl pyrophosphate synthase; LS, linalool synthase.

While many volatile components have been identified in vegetables, especially in tomato, many of the enzymes and genes involved in their biosynthesis have not been characterized. Modification of vegetable aroma by genetic engineering is dependent on the availability of identified genes, which encode the enzymes of key reactions that influence or divert biosynthetic pathways of known aroma components. Several approaches aimed at isolating and characterizing genes involved in fragrance formation have been successful. These methodologies have made available dozens of genes inferred to be involved in the formation of particular aromas. A few attempts to modify aroma by genetic engineering technology have been reported. Wang and others (1996) successfully increased the levels of some short-chain alcohols and aldehydes by overexpressing a yeast desaturase in tomato fruits. Other two research groups have successfully changed the aroma volatile content in tomato fruit by overexpressing an alcohol dehydrogenase and thereby altering the ratio of short-chain alcohols to aldehydes (Prestage et al. 1999; Speirs et al. 1998).

METABOLIC ENGINEERING OF THE TERPENOID PATHWAY

Genetic engineering to improve vegetable aroma through modification of the early steps of the terpenoid pathway has been demonstrated. The metabolic engineering effort to enhance tomato flavor by diverting the plastidial precursors resulted in a several-fold increase in the pathway leading to novel aroma constituents in tomatoes. Lewinsohn and others (2001) successfully attempted to divert the metabolic flow from the biosynthesis of the red pigment lycopene to aroma compounds (Fig. 35.2). The drawback of the method was that the obtained transgenic tomato contained less lycopene compared to the parents. Nonetheless, the experiment

showed the possibility of diverting the carbon flux in carotenoid-containing fruits and vegetables that display the same effect if a foreign gene is expressed in them. Linalool, an acyclic monoterpene alcohol that has an aroma with a sweet, floral alcoholic note, is a major component of the scent of many flowers and of edible fruits such as peach, plum, and pineapple. Linalool naturally appears in two forms: S- and R-linalool with different aroma specificities. The enzyme linalool synthase that catalyzes the formation of S-linalool from the monoterpene precursor geranyl pyrophosphate (GPP) has been purifed (Pichersky et al. 1995) and its gene (*LIS*) has been cloned from the flowers of the California annual plant *Clarkia breweri*. This gene has been suggested as a key candidate for metabolic engineering to modify the aroma of the modern tomato varieties that have lost some of their volatiles such as linalool. Since GPP is also an intermediate in the pathway to the tetraterpenoid carotenoid synthesis during fruit ripening, it was hypothesized that expressing the *C. breweri* LIS in the fruits during ripening would divert a portion of the GPP pool to the production of the volatile S-linalool and possibly would improve the fruit aroma without having a significant influence on the accumulation of carotenoids (Lewinsohn et al. 2001). Tomato varieties have been transformed with the Clarkial LIS transgene under the control of the late-ripening specific E8 promoter, and the accumulation of S-linalool was observed in the ripening fruits. Linalool that accumulated in the LIS-transgenic plants was virtually enantiomerically pure S-linalool. The apparent lack of racemization might reflect a process of compartmentalization that separates linalool from the acid environment in the fruit tissues. Transgenic fruits also accumulated low levels of 8-hydroxylinalool, possibly due to allylic hydroxylation, a common reaction in monoterpene metabolism. The results suggest that such a hydroxylating activity is present in ripening tomato fruits, although the endogenous substrate is presently unknown. This finding is an example of one of the possible drawbacks of metabolic engineering, where introducing one trait, such as the production of S-linalool, can unintentionally cause the appearance of an unexpected novel metabolite, such as 8-hydroxylinalool, due to the genetic and biochemical background of the transformed plants. The researchers also investigated the possibility of having a negative effect on the accumulation of related terpenoind compounds resulting from the manipulation of the terpenoids, and tocopherols were unaffected in the linalool-accumulating transgenic fruits as compared to the controls. These results indicate that it is possible to significantly increase the levels of aroma components in ripening fruits (Lewinsohn et al. 2001). With the advent of other genes encoding enzymes and other biosynthetic pathways leading to the reduction of various volatile aroma chemicals, the potential for flavor and aroma improvement in a variety of fruits, flowers, and vegetables by genetic manipulation is very promising (Fig. 35.2).

CONCLUSIONS

Aroma and flavor compounds are essential parameters in defining the quality of vegetables. Flavor volatiles are formed during the ripening stage or when the tissue disrupts mechanically or physiologically. Conventional breeding has contributed significantly to the improvement of vegetable crop yields, quality (color and size), postharvest life, and resistance to biotic and abiotic stresses. The aroma and flavor of vegetables, although important quality attributes, were not a focus of scientific

research studies. Very few vegetables were studied with the objectives of improving their flavor profiles. Successes in the flavor improvement of tomato and potato are encouraging examples for the future development of more varieties of transgenic vegetables with enhanced flavor profiles.

REFERENCES

Baldwin EA, Nisperos MO, Moshonas MG. 1991a. Quantitative analysis of flavor and other volatiles and for other constituents of two tomato varieties during ripening. *J Am Soc Hortic Sci* 116:265–9.

Baldwin EA, Nisperos MO, Moshonas MG. 1991b. Quantitative analysis of flavor parameters in six Florida tomato varieties (*Lycopersicon esculentum* Mill.). *J Agric Food Chem* 39:1135–40.

Buttery RG. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetable and fruits. In Acree TE, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: ACS Publications, pp. 259–86.

Buttery RG, Ling LC. 1993. Volatiles of tomato fruit and plant parts: Relationship and biogenesis. In Tarnish R, Buttery R, Sugisawa H (eds.), *Bioactive Volatile Compounds from Plants*. Washington, DC: ACS Books, pp. 23–34.

CFIA. 2001. CFIA's assessment of high oleic soybean for environmental and feed safety (From Decision Document 2001-34). http://www.inspection.gc.ca/english/sci/biotech/dd/dd0134e.shtml (accessed July 5, 2008).

Coetzer C, Corsini D, Love S, Pavek J, Tumer N. 2001. Control of enzymatic browning in tomato (*Solanum tuberosum* L.) by sense and antisense RNA from tomato polyphenol oxidase. *J Agric Food Chem* 49:652–7.

Croteau R, Kutchan TM, Lewis NG. 2000. Natural products (secondary metabolites). In Buchanan B, Guissem W, Joneas R (eds.), *Biochemistry and Molecular Biology of Plants*. Rockville, MD: American Society of Plant Biologists, pp. 1250–68.

Cunningham DG, Acree TE, Barnard J, Butts RM, Braell PA. 1985. Charm analysis of apple volatiles. *Food Chem* 19:137–47.

Dalal M, Dani RG, Kumar PA. 2006. Current trends in the genetic engineering of vegetable crops. *Sci Hortic* 107:215–25.

DeRovira D. 1997. *Manual—Flavor Nomenclature Workshop: An Odor Description and Sensory Evaluation Workshop*. Somerset, NJ: Flavor Dynamics.

Di R, Kim J, Martin MN, Leustek T, Jhoo J, Ho CT, Tumer NE. 2003. Enhancement of the primary flavor compound methional in potato by increasing the level of soluble methionine. *J Agric Food Chem* 51:5695–702.

Ducreux LM, Morris WL, Hedley PE, Shepherd T, Davies HVJ, Millam S, Taylor MA. 2005. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β-carotene and lutein. *J Exp Bot* 56:81–9.

DuPont. 1997. Occurrence of linoleic acid (9-15) isomer in commonly used oils and fats for frying and baking in Europe. http://www.agbios.com/docroot/decdocs/HOS1.pdf (accessed June 22, 2008).

Egnin M, Prakash CS. 1997. Transgenic sweet potato (*Ipomoea batatas*) expressing a synthetic storage protein gene exhibits high levels of total protein and essential amino acids. *In Vitro* 33(Pt. 2):52A.

Fellman JK, Mattheis JP. 1999. Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *J Agric Food Chem* 47:2847–53.

Galliard T, Mathew JA, Wright AJ, Fishwick MJ. 1977. The enzymic breakdown of lipids to volatile and non volatile carbonyl fragments in disrupted tomato fruits. *J Sci Food Agric* 28:863–8.

Ghosh N, Dani RG, Smith DW, Chalterjee A, Hannigan KP, Allen RD. 2004. Current aspects of biotechnology in agriculture and medicine. *Perspect Cytol Genet* 11:121–32.

James C. 2004. *Preview: Global Status of Commercialized Biotech/GM Crops, 2004: ISAAA Briefs No. 30*. Ithaca, NY: ISAAA.

Krammer GE, Takeoka GR, Buttery RG. 1994. Isolation and identification of 2, 5-dimethyl-4-hydroxy-3(2H)-furanone glucoside from tomatoes. *J Agric Food Chem* 42:1595–7.

Le Gall G, DuPont MS, Mellon FA, Davis AL, Collins GJ, Verhoeyen ME, Colquhoun IJ. 2003. Characterization and content of flavonoid glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruits. *J Agric Food Chem* 51:2438–46.

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam KH, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E. 2001. Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 127:1256–65.

Mattheis JP, Buchanan DA, Fellman JK. 1995. Volatile compound production by Brisbee Delicious apples after sequential atmosphere storage. *J Agric Food Chem* 43:194–9.

Maul F, Sargent SA, Balaban MO, Baldwin EA, Huber DJ, Sims CA 1998. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: An application for electronic nose technology. *J Am Soc Hortic Sci* 123:1094–101.

Muir SR, Collins GJ, Robinson S, Huges S, Bovy A, Ric De vos CH, van Tunen AJ, Verhoeyen ME. 2001. Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat Biotech* 19:470–4.

Oke M, Pinhero RG, Paliyath G. 2003. The effects of genetic transformation of tomato with antisense phospholipase D cDNA on the quality of characteristics of fruits and their processed products. *Food Biotechnol* 17(3):163–82.

Peters AM, Van Amerongen A. 1998. Relationship between levels of sesquiterpene lactones in chicory and sensory evaluation. *J Am Soc Hortic Sci* 123:326–9.

Pichersky E, Lewinsohn E, Croteau R. 1995. Purification and characterization of S-linalool, an enzyme involved in the production of floral scent in *Clarkia breweri*. *Arch Biochem Biophys* 316:803–7.

Pinhero RG, Almquist KC, Novotna Z, Paliyath G. 2003. Developmental regulation of phospholipase D in tomato fruit. *Plant Physiol Biochem* 41:223–40.

Prestage S, Linforth RST, Taylor AJ, Lee E, Speirs J, Schuch W. 1999. Volatile production in tomato fruit with modified alcohol dehydrogenase activity. *J Sci Food Agric* 79:131–6.

Riley JCM, Willemot C, Thompson JE. 1996. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biol Technol* 7:97–107.

Roemer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM. 2000. Elevation of the provitamin A content of transgenic tomato plants. *Nat Biotech* 18:666–9.

Romani R, Labavitch J, Yamashita T, Hess B, Rae H. 1983. Preharvest AVG treatment of "Bartlett" pear fruit: Effects on ripening, color change and volatiles. *J Am Soc Hortic Sci* 108:1046–9.

Romer S, Lubeck J, Kauder F, Steiger S, Adomat C, Sandmann G. 2002. Genetic engineering of a zeaxanthin-rich potato by antisence inactivation and co-suppression of carotenoid epoxidation. *Metab Eng* 4:263–72.

Sangwan NS, Sangwan RS. 2007. Metabolic engineering for flavor enhancement in tomato—Path setting for opportunities and strategies. *Curr Sci* 93(7):899–901.

Speirs J, Lee E, Holt K, Yong-Duk K, Steel N, loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117:1047–58.

Taylor JE. 1993. Exotics. In Seymour GB, Taylor JE, Tucker TA (eds.), *Biochemistry of Fruit Ripening*. London: Chapman and Hall, pp. 151–87.

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE. 1996. Changes of fatty acid and fatty acid derivatives flavor compounds by expressing the yeast ?-9 desaturase gene, in tomato. *J Agric Food Chem* 44:3399–402.

Wright DH, Harris ND. 1985. Effect of nitrogen and potassium fertilization on tomato flavor. *J Agric Food Chem* 33:355–8.

Zeh M, Casazza AP, Kreft O, Roessner U, Bieberich K, Wilmitzer L, Hoefgen R, Hesse H. 2001. Antisense inhibition of threonine synthase leads to high methionine content in transgenic potato plants. *Plant Physiol* 127:792–802.

CHAPTER 36

Genetic Engineering of Fruit Flavors

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INTRODUCTION

A fruit is the ripened ovary of a flowering plant that contains the seeds. The primary biological function of a fleshy fruit is to facilitate seed dispersal. To do so, fruits have been selected during evolution for the manifestation of a number of characters, one of which is flavor. Fruit flavor affects animals' perception of a specific type of fruit. It is therefore important for the reproductive success of flowering plants. Fruit flavor has also played an important role in human culture. Indeed, fruit crops have been cultivated by humans for both their nutritional values and diverse flavors.

Human perception of fruit flavor is generally considered to be a combination of taste and aroma (Baldwin et al. 2000). There are five primary taste sensations: sweetness, sourness, saltiness, bitterness, and, recently discovered, umaminess, each of which is determined by the contact of the mouth and tongue with sugars, acids, several amino acids, nucleotides, and a number of bitter compounds (Kinnamonll and Margolskeea 1996). Aroma is determined by volatile compounds sensed by the olfactory system (Goff and Klee 2006). Because flavor is an important attribute affecting human selection and enjoyment of fruits, the genetic improvement of flavors of fresh fruits has been an important endeavor in agriculture. With the advent of molecular biology and plant transformation, much attention has been paid to using genetic engineering for fruit flavor improvement.

CHEMISTRY AND BIOCHEMISTRY OF FRUIT FLAVORS

Fruit flavors are the sum of the interaction between sugars, acids, lipids, and a blend of volatile compounds (Acree 1993). The content of sugars, mainly glucose and fructose, and its ratio to the content of acids, such as citric acid and malic acid, determine the sweetness of fruits. In contrast, the volatile compounds involved in

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aroma determination are often much more complex. Many fruits produce a large number of volatile compounds. The fruits of fresh tomato (*Solanum lycopersicum*), for example, produce more than 400 different volatile constituents (Buttery and Ling 1993). These volatile components include acyclic, cyclic, and heterocyclic hydrocarbons, alcohols, phenols, ethers, aldehydes, ketones, carboxylic acids, esters, and lactones, as well as nitrogen, sulfur, and halogen-containing compounds (Buttery et al. 1987, 1988, 1990; Lewinsohn et al. 2001; Linforth et al. 1994; Maul et al. 1998; Petro-Turza 1986). The contribution of individual volatiles to human perception of fruit flavor is not equal. For some fruits, flavor is determined by a few dominant volatile compounds. Banana (*Musa acuminata* Colla) flavor, for example, is mainly determined by 3-methybutyl acetate (Berger 1991). For Polynesian pineapple (*Ananas comosus*), esters, lactones, furanoids, and sulfur compounds act as potent odor components (Tokitomo et al. 2005). In contrast, approximately 30 volatiles were determined to contribute significantly to the fruit flavor of fresh tomato (Buttery 1993; Buttery and Ling 1993).

Although chemically complex, the majority of flavor volatiles from fruits are synthesized from three major biochemical pathways: the terpenoid pathway, the lipid degradation pathway, and the phenylpropanoid/benzenoid pathway (Dudareva et al. 2006; Hoffmann et al. 2003; Walker and Croteau 2000; Wang et al. 2001). The terpenoids compose the largest class of plant secondary metabolites. All terpenoids are synthesized from the universal five carbon precursors, isopentenyl diphosphate, and its allylic isomer dimethylallyl diphosphate. Based on the number of five carbon units they contain, terpenoids can be grouped into hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), homoterpenes (C11 and C16), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes (>C40). Mainly monoterpenes and sesquiterpenes have been identified at varying levels in the flavor profiles of most fruits. Monoterpenes are derived from acetyl-CoA by the mevalonic acid pathway localized in the cytosol (McCaskill and Croteau 1995). Sesquiterpenes are formed from pyruvate and glyceraldehyde-3-phosphate via the methylerythritol phosphate pathway localized in plastids (Rodriguez-Concepcion and Boronat 2002). The enzymes that catalyze the formation of monoterpenes and sesquiterpenes from their corresponding immediate universal substrates, geranyl diphosphate and farnesyl diphosphate, respectively, are called terpene synthases (TPSs). A large number of genes encoding TPSs have been isolated and characterized from a variety of plant species (Chen et al. 2003; Tholl 2006). This provides an important repository of genes for the genetic engineering of fruit flavors. In addition to terpenes synthesized by the action of TPSs, some volatiles are formed from the degradation of carotenoids, which are usually red, orange, or yellow pigments synthesized from the terpenoid pathway. For example, carotenoid-derived volatile norisoprenoids are important flavor compounds of fresh tomato fruits (Simkin et al. 2004).

A large number of volatiles such as aldehydes, alcohols, and esters are formed from the lipid degradation pathway (Feussner and Wasternack 2002). Unsaturated fatty acids, especially linoleic acid (18:2) and linolenic acid (18:3), are formed from lipids by the action of acyl hydrolase enzymes. Unsaturated fatty acids undergo dioxygenation in a reaction catalyzed by lipoxygenases (LOXs). LOXs can catalyze the oxygenation of polyenoic fatty acids at C9 or C13 positions to produce two groups of compounds: the 9-hydroperoxy and the 13-hydroperoxy derivatives of polyenoic fatty acids. Two branches of the LOX pathway, the hydroperoxide lyase

(HPL) branch and the allene oxide synthase (AOS) branch, are responsible for the formation of volatile compounds. In the HPL branch pathway, HPL catalyzes the oxidative cleavage of hydroperoxy fatty acids that leads to the formation of short-chain C6- or C9-volatile aldehydes and the corresponding C12- or C9-ω fatty acids. The C6-aldehyde products of HPLs can be further converted to their isomers by spontaneous rearrangement or by alkenal isomerases, or they can be reduced to alcohols by the action of alcohol dehydrogenases (ADHs) (Prestage et al. 1999). The AOS branch of the LOX pathway leads to the formation of jasmonic acid, an important defense signaling and plant hormone (Liechti and Farmer 2006). The methyl ester of jasmonic acid, methyl jasmonate, is a volatile compound. The conversion from jasmonic acid to methyl jasmonate is catalyzed from jasmonic acid methyltransferase (Seo et al. 2001). The alcohols produced from fatty acid degradation can be used as substrates to form esters catalyzed by alcohol acyltransferases (AATs) (Beekwilder et al. 2004).

Phenylpropanoid/benzenoid compounds are derived from L-phenylalanine (Phe). Shared with the lignin biosynthetic pathway, a variety of hydroxycinnamic acids, aldehydes, and alcohols are formed from *trans*-cinnamic acid, which is an immediate product from Phe by the action of phenylalanine ammonia lyase (PAL), via a series of hydroxylation and methylation reactions (Humphreys and Chapple 2002). Some of these intermediates could be converted to volatile compounds. For example, coniferyl acetate can be converted to eugenol by the action of eugenol synthase (Koeduka et al. 2006). Eugenol can be further modified by methylation to form methyl eugenol (Gang et al. 2002), which is also a volatile compound. Benzenoid compounds also originate from *trans*-cinnamic acid. As a side branch of the general phenylpropanoid pathway, benzoic acid biosynthesis first involves the shortening of *trans*-cinnamic side chain by a C2 unit. The subsequent biochemical pathways leading to benzoic acid has not been solved. The biosynthesis of C6-C2 volatile compounds, such as phenylacetaldehyde, does not go through *trans*-cinnamic acid (Boatright et al. 2004). One recent study suggested that phenylacetaldehyde is formed directly from Phe via an unusual combined decarboxylation–amine oxidation reaction catalyzed by phenylacetaldehyde synthase (Kaminaga et al. 2006). The phenylpropanoid/benzenoid pathway also provides the precursors of acyl-CoAs, which are important for volatile ester formation catalyzed by AATs. In addition to Phe, other amino acids, such as isoleucine (Dickinson et al. 2000), may also serve as precursors for plant volatile formation.

REGULATION OF FLAVOR BIOGENESIS

In addition to biochemical pathways, the site of flavor compound production is also important for fruit flavor formation. Generally, many volatile and nonvolatile aroma compounds are produced in fruit skin, while organic acids and sugars are produced in pulp. For example, the skin and pulp of Queen Anne's pocket melon (*Cucumis melo*) showed difference in flavor profiles. The levels of volatiles in skin were significantly higher than those in pulp (Aubert and Pitrat 2006). After being synthesized, some volatile compounds could be released from the fruit surface immediately. Others may accumulate in the fruit and will not be released until the split opening. Some flavor compounds are accumulated in glycosidically conjugated forms (Parada

et al. 2000). (1S, 2S)-1-Phenylpropane-1,2-diol 2-O-beta-D-glucopyranoside and p-menth-4(8)-ene-1,2-diol1-O-alpha-L-arabinopyranosyl-(1-6)-beta-D-glucopyranoside, for example, are immediate precursors of 1-phenylpropane-1,2-diol and p-menth-4(8)-ene-1,2-diol, respectively. The latter two are typical volatiles found in the fruit of cape gooseberry (*Physalis peruviana*) (Mayorga et al. 2001). In some fruits, the levels of bound compounds increase significantly with maturation (Aubert et al. 2003), suggesting that both the biogenesis and accumulation of such bound compounds play important roles in the determination of fruit flavors.

In general, fruit flavor releasing is a dynamic process. In the mature process of fruits, the volatile composition of fruits changes along with the different stages of maturity. At the fully mature stage, key flavor compounds increase significantly, correlating with skin color development (Menager et al. 2004). During the maturation of snake fruit (*Salacca edulis* Reinw.) Pondoh, the contents of sucrose, glucose, fructose, and volatile compounds change drastically. While the contents of glucose, fructose, and volatile compounds achieve their maximal levels at the end of maturation, the contents of sucrose decrease (Supriyadi et al. 2002). Storage can also have an effect on fruit flavor. Some aroma-related volatile components increase during storage, while others, especially esters, may decrease (Chen et al. 2006).

Ethylene plays an important role in fruit flavor biogenesis. Due to its regulatory role in fruit ripening, ethylene is recognized as a "ripening hormone" (Abeles et al. 1992). The factors that regulate the biosynthesis and action of ethylene may impact fruit flavor formation. 1-Methylcyclopropene (MCP) is an ethylene inhibitor. MCP-treated apples (*Malus domestica*) were found to retain more alcohols, aldehydes, and β-damascenone volatiles than untreated apples (Lurie et al. 2002). In transgenic apples in which the ethylene signaling pathway is suppressed, the composition and accumulation of sugars, acids, and the aldehydes and alcohol precursors of volatile esters were not affected. However, the synthesis of ester volatiles was significantly suppressed (Dandekari et al. 2004). In addition to plant hormones, stress factors such as high levels of CO_2 can also have an additive effect on fruit flavor formation (Perez and Sanz 2001).

GENETIC ENGINEERING OF FRUIT FLAVORS: CASE STUDIES

Selection for important agricultural traits, such as overall yield and disease resistance, often results in the loss of aroma and taste in fruits and horticultural crops (Galili et al. 2002). Restoration of appealing flavors in fruits can be realized through classical breeding. For example, the distinct aromatic fragrance of *Lycopersicon peruvianum* LA 1554 has been introduced into the cultivated tomato, *Lycopersicon esculentum* (Kamal et al. 2001). These two species are distantly related. Ovule selection and culture method were used to circumvent the strong breeding barrier. From this breeding study, a large BC1F1 population was developed. Among the plants that were self-compatible and yielded fruits, fruits of some of these selected plants displayed an enriched sweet aromatic flavor. In sensory evaluation, panel opinion on flavor desirability significantly favored the BC1F1 fruits of some selected plants over the cultivar "Momotaro," one of the best consumer-rated Japanese commercial tomato cultivars.

Recently, Sams and Pantalone (2007) utilized molecular breeding to develop and release the edible vegetable soybean (*Glycine max*) "NUTRIVEG Soy6407," which

significantly exhibited superior flavor than commercial edamame soybean varieties based on 2 years of sensory panel data. Those researchers are now utilizing microarray analyses and analytical chemistry to identify specific flavor compounds in order to breed superior new varieties of better-tasting vegetable soybean.

Despite the above-described successful examples, classical breeding programs are often labor-intensive and time-consuming. In addition, the traits of fruit flavors are genetically complex and difficult to be quantified (Galili et al. 2002), making them difficult targets for conventional breeding. With the advent of molecular biology and plant transformation, people become interested in using genetic engineering to improve fruit flavors. Genetic engineering may reduce some of the drawbacks of classical breeding. For example, it can introduce a single gene at a time, making the progress less complex. In addition, genetic engineering can introduce foreign genes to produce exotic flavors.

During the last decade, a number of genetic engineering studies were conducted with an objective to alter fruit flavors (Aharoni et al. 2005). These studies either modified existing pathways (e.g., up- or downregulation of one specific gene or redirection of flux to a desirable compound by blockage of competing pathways) or introduced novel genes not existing in the host plant. Most of these studies used the cauliflower mosaic virus 35S promoter (35S promoter), a constitutive promoter, to drive the expression of the transgene. Others used fruit-specific promoters. In the following, we will describe case by case the genetic engineering studies of fruit flavors using tomato as a model (Table 36.1). These studies are categorized based on the target biochemical pathway to be manipulated.

Manipulating the Fatty Acid Degradation Pathway

A successful attempt to modify fruit flavor is through the introduction of a yeast Δ-9 desaturase gene into tomato. In transgenic tomato fruits, the levels of palmitoleic acid, 9, 12-hexadienoic acid, and linoleic acid increased, accompanied with a reduction in palmitic acid and stearic acid. Alteration of the profile of fatty acids was associated with changes in certain flavor compounds derived from fatty acids, most notably *cis*-3-hexenol, 1-hexanol, hexanal, and *cis*-3-hexenal. Some flavor compounds that are not derived from fatty acids, including 6-methyl-5-hepten-2-one and 2-isobutylthiazole, also showed increases in transgenic fruits (Wang et al. 2001).

LOX is another important target for genetic modification of the fatty acid degradation pathway. Five *LOX* genes (*TomloxA*, *TomloxB*, *TomloxC*, *TomloxD*, and *TomloxE*) have been identified in tomato. When the expression of *TomloxA* and *TomloxB* was suppressed in tomato fruit via an antisense strategy, no significant alterations in the production of the known tomato flavor volatiles were detected (Griffiths et al. 1999). In contrast, depletion of the expression of *TomloxC* via cosuppression or antisense inhibition led to major decreases in the flavor volatiles in both fruit and leaf (Chen et al. 2004), suggesting that *TomloxC* is the major *LOX* gene responsible for the production of fatty acid-derived volatiles in tomato fruits.

In another study, a cucumber (*Cucumis sativus*) *HPL* gene was introduced into tomato plants (Matsui et al. 2001). Though high activity of the introduced HPL could be detected in the fruits of transgenic tomatoes, the composition of volatile short-chain aldehydes and alcohols was little changed. The failure to alter volatile profiles in HPL transgenic fruits may be due to the high levels of endogenous HPL activity that are responsible for the formation of 9-hydroperoxides (Matsui et al. 2001).

TABLE 36.1. Genetic Engineering of Fruit Flavors in Tomato

Pathway to Be Altered	Transgene	Origin of Transgene	Means of Genetic Engineering	Promoter Used	Changes of Volatile Profile	References
Lipid degradation pathway	Acyl-CoA Δ-9 desaturase gene	Yeast	OE	35S	*cis*-3-Hexenol ↑, 1-hexanol ↑, hexanal ↑, *cis*-hexenal ↑, 6-methyl-5-hepten-2-one ↑, 2-isobutylthiazole ↑	Wang and others (1996)
Lipid degradation pathway	*LOX*	Tomato	OE, AS, co-suppression	35S	Hexanal ↓, hexenal ↓, hexenol ↓	Chen and others (2004)
Lipid degradation pathway	*ADH*	Tomato	OE	35S, PG	Hexanol ↑, (Z)-3-hexenol ↑	Speirs and others (1998)
Terpenoid pathway	*LIS*	*Clarkia breweri*	OE	E8	(S)-Linalool ↑, 8-hydroxylinalool ↑	Lewinsohn and others (2001)
Terpenoid pathway	*GES*	Basil	OE	PG	Geraniol ↑ and its derivatives ↑	Davidovich-Rikanati and others (2007)
Carotenoid degradation pathway	*CCD*	Tomato	AS	35S	β-Ionone ↓, pseudoionone ↓, geranylacetone ↓	Simkin and others (2004)
Phenylpropanoid/ benzenoid pathway	*AADC*	Tomato	OE	35S	Phenylacetaldehyde ↑, 2-phenylethanl↑,1-nitro-2-phenylathane ↑	Tieman and others (2006)

AADC, aromatic L-amino acid decarboxylase gene; *AAT*, alcohol acyltransferase gene; *ADH*, alcohol dehydrogenase gene; AS, antisense; *CCD*, carotenoid cleavage dioxygenase gene; E8, a tomato fruit-specific promoter; *GES*, geraniol synthase gene; *LIS*, *S*-linalool synthase gene; *LOX*, lipoxygenase gene; OE, overexpression; PG, a tomato polygalacturonase promoter that is fruit specific; 35S, cauliflower mosaic virus 35S promoter.

The transformed tomatoes have also been obtained by altering the expression of *ADH* genes driven by either the 35S promoter or the tomato fruit-specific tomato polygalacturonase (PG) promoter. In the ripening transgenetic tomatoes, modified ADH levels were found to have an important role on the balance between some of the aldehydes and the corresponding alcohols associated with flavor production. These phenotypes were transmitted to second-generation plants. In a preliminary taste trial, fruits with increased ADH activity and higher levels of alcohols were perceived as having a more intense "ripe fruit" flavor (Speirs et al. 1998).

Manipulating the Terpenoid Pathway

Because of the importance of the terpenoid pathway, genetic engineering has been used to manipulate this pathway, which resulted in the generation of transgenic plants containing high concentrations of provitamin A (β-carotene), such as "Golden Rice" (*Oryza sativa*) (Ye et al. 2000). The study in tomato with a gene encoding *S*-linalool synthase from *Clarkia breweri* flowers represents the first attempt to manipulate fruit flavor via the modification of the terpenoid pathway. In this study, transgenic tomato plants were generated by the introduction of a heterologous *C. breweri S*-linalool synthase gene (*LIS*), driven by a tomato late-ripening-specific *E8* promoter. The transgenic tomato synthesized and accumulated *S*-linalool and 8-hydroxylinalool in ripening fruits, which are absent in the control fruit. This study also showed that no other phenotypic alterations were observed despite the difference in fruit volatiles (Lewinsohn et al. 2001).

The flavor and aroma of tomato has recently been modified through the introduction of another monoterpenoid biosynthetic gene, a geraniol synthase gene (*GES*) from basil (*Ocimum basilicum*) (Davidovich-Rikanati et al. 2007). Transgene *GES* was under the control of a tomato ripening-specific PG promoter. The expression of *GES* in ripening tomatoes caused marked changes in fruit volatiles. The levels of some existing volatiles, such as neral plus geranial, were sixfold higher in *GES*-expressing fruits than those in control fruits. Some volatiles, such as geraniol, nerol, citronellol, and citronellic acid, which are absent in control fruits, are present in high concentration in transgenic fruits. The increases in the levels of key volatiles resulted in a marked impact on the organoleptic attributes of tomato fruits. In a flavor panel study, >90% of the untrained panelists reported differences in their perception of the smell. A majority of the untrained taste panelists preferred the transgenic fruits over controls (Davidovich-Rikanati et al. 2007).

Manipulating the Carotenoid Degradation Pathway

Geranylacetone, β-ionone, and pseudoionone are among a group of related terpenoid flavor volatile compounds that are generally present at relatively low levels but possess strong effects on the overall human perception of fruit flavors (Simkin et al. 2004). Recent biological studies demonstrated that these volatiles are synthesized from the degradation of carotenoids, of which carotenoid cleavage dioxygenases (CCDs) play an essential role (Simkin et al. 2004). *LeCCD1B*, a *CCD* gene isolated from tomato, can cleave multiple carotenoid substrates at 9, 10 (9′, 10′) bonds to produce a C14 dialdehyde and two C13 cyclohexones depending on the substrate. Transgenic tomato was generated in which the *LeCCD1B* gene was

constitutively expressed in a reverse orientation (antisense). Transgenic fruits did not show significant modification in the carotenoid content of fruit tissue. However, when the volatile profile of tomato fruits was analyzed, a ≥50% decrease in β-ionone (a β-carotene-derived C13 cyclohexone) and a ≥60% decrease in geranylacetone (a C13 acyclic product likely derived from a lycopene precursor) were observed in selected transgenic lines (Simkin et al. 2004).

Manipulating the Phenylpropanoid/Benzenoid Pathway

Phenylethanol is an important flavor compound important for the flavor of many foods and fruits (Tieman et al. 2006). A recent study in tomato showed that 2-phenylethanol is derived from Phe through the action of a small family of decarboxylases that can mediate that pathway's first step. In *in vitro* studies, these enzymes catalyze the conversion of Phe to phenethylamine and tyrosine to tyramine with tyrosine as the preferred substrate. In tomato fruits, however, the levels of Phe are much higher than those of tyrosine, indicating that Phe is the *in planta* substrate. When either *LeAADC1A* or *LeAADC2*, two tomato decarboxylase genes, was overexpressed in transgenic tomato plants, fruits exhibited 10-fold increases in the emission of the products of the pathway, including phenylethanol and phenylacetaldehyde. In contrast, reduction of *LeAADC2* expression using antisense significantly reduced emissions of these volatiles (Tieman et al. 2006).

CONSIDERATIONS FOR FUTURE STUDIES

Flavor is a valuable character of the fruits and has an important role in people's choice of fruits. The flavors of many fresh commercially produced fruits, such as tomato, are generally considered to be poor. It is therefore highly desirable to develop new fruits with better flavors. Because of the complex nature of the flavor trait, genetic engineering theoretically offers an ideal solution to improving fruit flavors. In reality, the impact of this technique on fruit flavor improvement thus far has been only limited (Simkin et al. 2004; Speirs et al. 1998). Much basic and applied research is still needed to achieve this goal.

Fruit flavors are often controlled by a complex mixture of plant metabolites. The molecular and biochemical mechanisms underlying the biosynthesis of these large numbers of compounds are still far from being fully elucidated. Many previous studies dealt with a single gene/enzyme at a time. If an overexpression strategy is used, the availability of appropriate substrates for the introduced enzyme will be critical for the development of an expected flavor. In this case, a thorough understanding of the metabolic pathway leading to the formation of a specific substrate will be important. Therefore, gene and pathway identification is a key area in the study of fruit flavor biogenesis. In addition to biosynthetic genes, the mechanisms that regulate the biosynthesis of flavor compounds from multiple pathways also need to be characterized. In light of the fact that a large number of compounds are involved in fruit flavor, it is important to use novel strategies for experimentation. Various novel genomic tools will be proved useful for elucidation of biosynthetic pathways and regulatory networks that govern flavor biogenesis. Identification of such key elements will be essential for success of genetic engineering.

The alteration of one metabolic pathway can have profound impacts on other metabolic pathways. For example, successful enrichment of monoterpene flavor compounds in transgenic tomato was at the expense of reduced lycopene accumulation (Davidovich-Rikanati et al. 2007). Such pleiotropic effects need to be evaluated. As a complex trait, fruit flavor can be influenced by many environmental factors, such as light intensity, atmospheric CO_2 concentration, temperature, relative humidity, and nutrient status. A deeper understanding of the environmental effects on the formation of fruit flavors of transgenic fruits will be important for the commercial success of such novel fruits. Many previous studies of the genetic engineering of fruit flavors lack a component of sensory evaluation. We have to bear in mind that alteration of fruit flavor does not necessarily lead to flavor improvement. Sensory evaluations will also be critical for the successful development of commercial cultivars. In addition, public perception of genetically modified foods also needs to be taken into consideration.

We are at an exciting time of conducting plant biological research and genetic engineering. At this genomics era, systems biology that integrates transcriptome, proteome, and metabolome will provide novel knowledge on the biosynthetic pathways, enzymes, genes, and regulatory mechanisms that control fruit flavor formation. With such information and better tools of genetic engineering becoming available, researchers have promising avenues to pursue the improvement of fruit flavor.

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REFERENCES

Abeles FB, Morgan PW, Saltveit ME. 1992. *Ethylene in Plant Biology*, 2nd Ed. New York: Academic Press.

Acree TE. 1993. Bioassays for flavor. In Acree TE, Teranishi R (eds.), *Flavor Science. Sensible Principles and Techniques*. ACS Professional Reference Book. Washington, DC: American Chemical Society, pp. 1–20.

Aharoni A, Jongsma MA, Bouwmeester HJ. 2005. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci* 10(12):594–602.

Aubert C, Ambid C, Baumes R, Gunata Z. 2003. Investigation of bound aroma constituents of yellow-fleshed nectarines (*Prunus persica* L. cv. Springbright) changes in bound aroma profile during maturation. *J Agric Food Chem* 51(21):6280–6.

Aubert C, Pitrat M. 2006. Volatile compounds in the skin and pulp of Queen Anne's pocket melon. *J Agric Food Chem* 54(21):8177–82.

Baldwin EA, Scott JW, Shewmaker CK, Schuch W. 2000. Flavor trivia and tomato aroma: Biochemistry and possible mechanism for control of important aroma components. *HortScience* 35(6):1013–22.

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135(4):1865–78.

Berger RG. 1991. Fruits I. In Mares H (ed.), *Volatile Compounds in Food and Beverages*. New York: Marcel Dekker, pp. 283–304.

Boatright J, Negre F, Chen XL, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N. 2004. Understanding in vivo benzenoid metabolism in petunia petal tissue. *Plant Physiol* 135(4):1993–2011.

Buttery RG. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree T, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: American Chemical Society, pp. 259–86.

Buttery RG, Ling LC. 1993. Enzymatic production of volatiles in tomatoes. In Schreier P, Winterhalter P (eds.), *Flavor Precursors*. Wheaton, IL: Allured Publishing, pp. 137–46.

Buttery RG, Teranishi R, Ling LC. 1987. Fresh tomato aroma volatiles: A quantitative study. *J Agric Food Chem* 35:540–4.

Buttery RG, Teranishi R, Ling LC, Flath RA, Stern DJ. 1988. Quantitative studies on origins of fresh tomato aroma volatiles. *J Agric Food Chem* 36(6):1247–50.

Buttery RG, Teranishi R, Ling LC, Turnbaugh JG. 1990. Quantitative and sensory studies on tomato paste volatiles. *J Agric Food Chem* 38:336–40.

Chen F, Tholl D, D'Auria JC, Farooq A, Pichersky E, Gershenzon J. 2003. Biosynthesis and emission of terpenoid volatiles from Arabidopsis flowers. *Plant Cell* 15(2):481–94.

Chen G, Hackett R, Walker D, Taylor A, Lin Z, Grierson D. 2004. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiol* 136(1):2641–51.

Chen JL, Wu JH, Wang Q, Deng H, Hu XS. 2006. Changes in the volatile compounds and chemical and physical properties of Kuerle fragrant pear (*Pyrus serotina* Reld) during storage. *J Agric Food Chem* 54(23):8842–7.

Dandekari AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ. 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. *Transgenic Res* 13(4):373–84.

Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E. 2007. Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. *Nat Biotechnol* 25(8):899–901.

Dickinson JR, Harrison SJ, Dickinson JA, Hewlins MJE. 2000. An investigation of the metabolism of isoleucine to active amyl alcohol in *Saccharomyces cerevisiae*. *J Biol Chem* 275(15):10937–42.

Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: Recent advances and future perspectives. *Crit Rev Plant Sci* 25(5):417–40.

Feussner I, Wasternack C. 2002. The lipoxygenase pathway. *Annu Rev Plant Biol* 53:275–97.

Galili G, Galili S, Lewinsohn E, Tadmor Y. 2002. Genetics, molecular and genomic approaches to improve the value of plant foods and feeds. *Crit Rev Plant Sci* 21(3):167–204.

Gang DR, Lavid N, Zubieta C, Chen F, Beuerle T, Lewinsohn E, Noel JP, Pichersky E. 2002. Characterization of phenylpropene O-methyltransferases from sweet basil: Facile change of substrate specificity and convergent evolution within a plant OMT family. *Plant Cell* 14(2):505–19.

Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311(5762):815–19.

Griffiths A, Prestage S, Linforth R, Zhang J, Taylor A, Grierson D. 1999. Fruit-specific lipoxygenase suppression in antisense-transgenic tomatoes. *Postharvest Biol Tech* 17(3): 163–173.

Hoffmann L, Maury S, Martz F, Geoffroy P, Legrand M. 2003. Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. *J Biol Chem* 278(1):95–103.

Humphreys JM, Chapple C. 2002. Rewriting the lignin roadmap. *Curr Opin Plant Biol* 5(3):224–9.

Kamal AHM, Takashina T, Egashira H, Satoh H, Imanishi S. 2001. Introduction of aromatic fragrance into cultivated tomato from the "*peruvianum complex*". *Plant Breed* 120(2):179–81.

Kaminaga Y, Schnepp J, Peel G, Kish CM, Ben-Nissan G, Weiss D, Orlova I, Lavies O, Rhodes D, Wood K, Porterfield DM, Cooper AJL, Schloss JV, Pichersky E, Vainstein A, Dudareva N. 2006. Plant phenylacetaldehyde synthase is a bifunctional homotetrameric enzyme that catalyzes phenylalanine decarboxylation and oxidation. *J Biol Chem* 281(33):23357–66.

Kinnamonll SC, Margolskeea RF. 1996. Mechanisms of taste transduction. *Curr Opin Neurobiol* 6(4):506–13.

Koeduka T, Fridman E, Gang DR, Vassão DG, Jackson BL, Kish CM, Orlova I, Spassova SM, Lewis NG, Noel JP, Baiga TJ, Dudareva N, Pichersky E. 2006. Eugenol and isoeugenol, charecteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc Natl Acad Sci U S A* 103(26):10128–33.

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam K, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E. 2001. Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 127(3):1256–65.

Liechti R, Farmer EE. 2006. Jasmonate biochemical pathway. *Sci STKE* 322:cm3.

Linforth RST, Savary I, Pattenden B, Taylor AJ. 1994. Volatile compounds found in expired air during eating of fresh tomatoes and in the headspace above tomatoes. *J Sci Food Agric* 65(2):241–7.

Lurie S, Pre-Aymard C, Ravid U, Larkov O, Fallik E. 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. *J Agric Food Chem* 50(15):4251–6.

Matsui K, Fukutomi S, Wilkinson J, Hiatt B, Knauf V, Kajwara T. 2001. Effect of overexpression of fatty acid 9-hydroperoxide lyase in tomatoes (*Lycopersicon esculentum* Mill.). *J Agric Food Chem* 49(11):5418–24.

Maul F, Sargent SA, Balaban MO, Baldwin EA, Huber DJ, Sims CA. 1998. Aroma volatile profiles from ripe tomatoes are influenced by physiological maturity at harvest: An application for electronic nose technology. *J Am Soc Hortic Sci* 123(6):1094–101.

Mayorga H, Knapp H, Winterhalter P, Duque C. 2001. Glycosidically bound flavor compounds of cape gooseberry (*Physalis peruviana* L.). *J Agric Food Chem* 49(4):1904–8.

McCaskill D, Croteau R. 1995. Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (*Mentha* × *piperita*) rely exclusively on plastid-derived isopentenyl diphosphate. *Planta* 197(1):49–56.

Menager I, Jost M, Aubert C. 2004. Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation. *J Agric Food Chem* 52(5):1248–54.

Parada F, Duque C, Fujimoto Y. 2000. Free and bound volatile composition and characterization of some glucoconjugates as aroma precursors in melon de olor fruit pulp (*Sicana odorifera*). *J Agric Food Chem* 48(12):6200–4.

Perez AG, Sanz C. 2001. Effect of high-oxygen and high-carbon-dioxide atmospheres on strawberry flavor and other quality traits. *J Agric Food Chem* 49(5):2370–5.

Petro-Turza M. 1986. Flavor of tomato and tomato products. *Food Rev Int* 2(3):309–51.

Prestage S, Linforth RST, Taylor AJ, Lee E, Speirs J, Schuch W. 1999. Volatile production in tomato fruit with modified alcohol dehydrogenase activity. *J Sci Food Agric* 79(1):131–16.

Rodriguez-Concepcion M, Boronat A. 2002. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. Ametabolic milestone achieved through genomics. *Plant Physiol* 130(3):1079–89.

Sams CE, Pantalone VR. 2007. *Release of NUTRI VEG Soy6407*. ••: Tennessee Agricultural Experiment Station.

Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD. 2001. Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. *Proc Natl Acad Sci U S A* 98(8):4788–93.

Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ. 2004. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β-ionone, pseudoionone, and geranylacetone. *Plant J* 40(6):882–92.

Speirs J, Lee E, Holt K, Yong-Duk K, Steele Scott N, Loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117(3):1047–58.

Suhardi S, Suzuki M Yoshida K, Muto T, Fujita A, Watanabe N. 2002. Changes in the volatile compounds and in the chemical and physical properties of snake fruit (*Salacca edulis* Reinw.) cv. Pondoh during maturation. *J Agric Food Chem* 50(26):7627–33.

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr Opin Plant Biol* 9(3):297–304.

Tieman DM, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ. 2006. Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci U S A* 103(21):8287–92.

Tokitomo Y, Steinhaus M, Buttner A, Schieberle P. 2005. Odor-active constituents in fresh pineapple (*Ananas comosus* [L.] Merr.) by quantitative and sensory evaluation. *Biosci Biotechnol Biochem* 69(7):1323–30.

Walker K, Croteau R. 2000. Molecular cloning of a 10-deacetylbaccatin III-10-O-acetyl transferase cDNA from Taxus and functional expression in *Escherichia coli*. *Proc Natl Acad Sci U S A* 97(2):583–7.

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE. 1996. Changes of fatty acids and fatty acid-derived flavor compounds by expressing the yeast D-9 desaturase gene in tomato. *J Agric Food Chem* 44:3399–402.

Wang C, Xing J, Chin CK, Ho CT, Martin CE. 2001. Modification of fatty acids changes the flavor volatiles in tomato leaves. *Phytochemistry* 58(2):227–32.

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I. 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 287(5451):303–5.

CHAPTER 37

Metabolic Factory for Flavors in Fruits and Vegetables

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INTRODUCTION

When we choose a food, its flavor, as well as its texture, has a direct effect on its palatability. Especially for food plants, such as vegetables, fruits, and herbs, the strength and character of the flavor are important factors for consumers to recognize their freshness, maturity, and preference. Therefore, in breeding programs, controlling or improving flavor has become one of the main purposes for the development of new cultivars and for decisions on cultivating conditions and environment.

Aroma, which is composed of various volatile hydrophobic compounds, is also one of the characteristics humans recognize in food. More than 1000 compounds have been detected in plant volatiles. Generally, the composition of volatile compounds, even in a single plant material, is complicated. For example, 322 volatile compounds were detected in the fruits of 94 tomato genotypes (Tikunov et al. 2005), and 139 compounds were found in cantaloupe fruits (Beaulieu and Grimm 2001). Since each compound has a different odor characteristic and threshold level for recognition (Buttery 1993), the compositional differences of aroma compounds may enable to discriminate a food plant from different species, cultivars, and, sometimes, different harvested areas. Moreover, understanding the biosynthesis mechanism of each aroma compound will facilitate the regulation or modification of flavor by breeding or bioengineering, resulting in the production of more valuable and high-quality plant materials. However, to achieve this, it is basically necessary to isolate and functionally characterize the gene encoding the enzyme involved in the biosynthesis of each compound.

In the recent decade, the molecular mechanisms for production and storage of many plant volatiles have been gradually clarified by biological, biochemical, and physiological research (Dudareva et al. 2004; Pichersky and Niyogi 2006; Pichersky

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

et al. 2006). In particular, the recent progress of functional genomics approaches, using the large resources of genome sequence database (*Arabidopsis* and rice) and expression sequence tag (EST) databases constructed from various plant materials, has facilitated an extensive determination of the functions of the genes involved in the biosynthesis or metabolism of aroma compounds (Dudareva et al. 2004; Gang 2005; Schwab et al. 2008).

In this chapter, recent advancements in our understanding of the volatile compounds responsible for aromas in food plants are reviewed by focusing on their biosynthetic mechanisms. In addition, some achievements in metabolic engineering for the improvement, suppression, or heterogonous production of aroma compounds in fruits and vegetables are introduced.

Biosynthesis of Volatile Compounds Responsible for Aroma in Plant Foods

The structures of volatile compounds found in plants are diverse. However, aroma compounds can be categorized by their structural similarities and their relationships in biosynthesis pathways. The main aroma groups in plant foods are (1) lipoxygenase (LOX)-catalyzed fatty acid degradation compounds, (2) short/medium aliphatic compounds, (3) terpenes (mainly mono- and sesquiterpenes), (4) phenolics (benzenoid/phenylpropanoid), and (5) sulfur- and nitrogen-containing compounds (Fig. 37.1). Yet, which metabolite group dominates in a plant material is dependent on the plant species and cultivars, as well as on the selected parts and the stage of maturity/ripening.

LOX-Catalyzed Aliphatic Compounds Dependent on LOX are the compounds derived from fatty acid degradation, including aliphatic aldehydes and alcohols, such as C_6-compounds (hexanal, (*Z*)-3-hexenal, hexanol, etc.) and C_9-compounds ((*Z*)-3-nonenal, (*E*)-2-nonenal, etc.). These are biosynthesized by LOX and hydroperoxide lyase (HPL) from fatty acids (Matsui 2006) (Fig. 37.2). The structures of these products are determined by the fatty acid precursors. For example, (*Z*)-3-hexenal is synthesized from the enzymatic degradation of linolenic acid. In contrast, hexanal is formed as the main compound from linoleic acid. The genes encoding LOX and HPL were isolated from various foods and were characterized in tomatoes, cucumbers, soybeans, rice, and almond seeds (Chen et al. 2004; Hornung et al. 1999; Howe et al. 2000; Matsui 2006; Matsui et al. 2000b,c).

C_6-Compounds are called "green leaf volatiles" and are well-known to be produced after wounding of plant leaves (D'Auria et al. 2007; Matsui et al. 2000a, 2006). Furthermore, C_6-compounds have been recently reported not only to produce a "green leaf" aroma but also to improve a plant's resistance to both herbivores and pathogens (Shiojiri et al. 2006).

The threshold levels of these fatty acid-derived compounds are relatively low (Buttery 1993). Thus, although they sometimes contribute positively to a "fresh green" flavor, in many cases, they are recognized as an off-flavor in plant foods, such as soybeans, rice, and tomatoes (Kobayashi et al. 1995; Suzuki et al. 1999; Torres-Penaranda and Reitmeier 2001; Vara-Ubol et al. 2004). LOX-deficient soybean cultivars, which lack three kinds of LOX isozymes, have already been

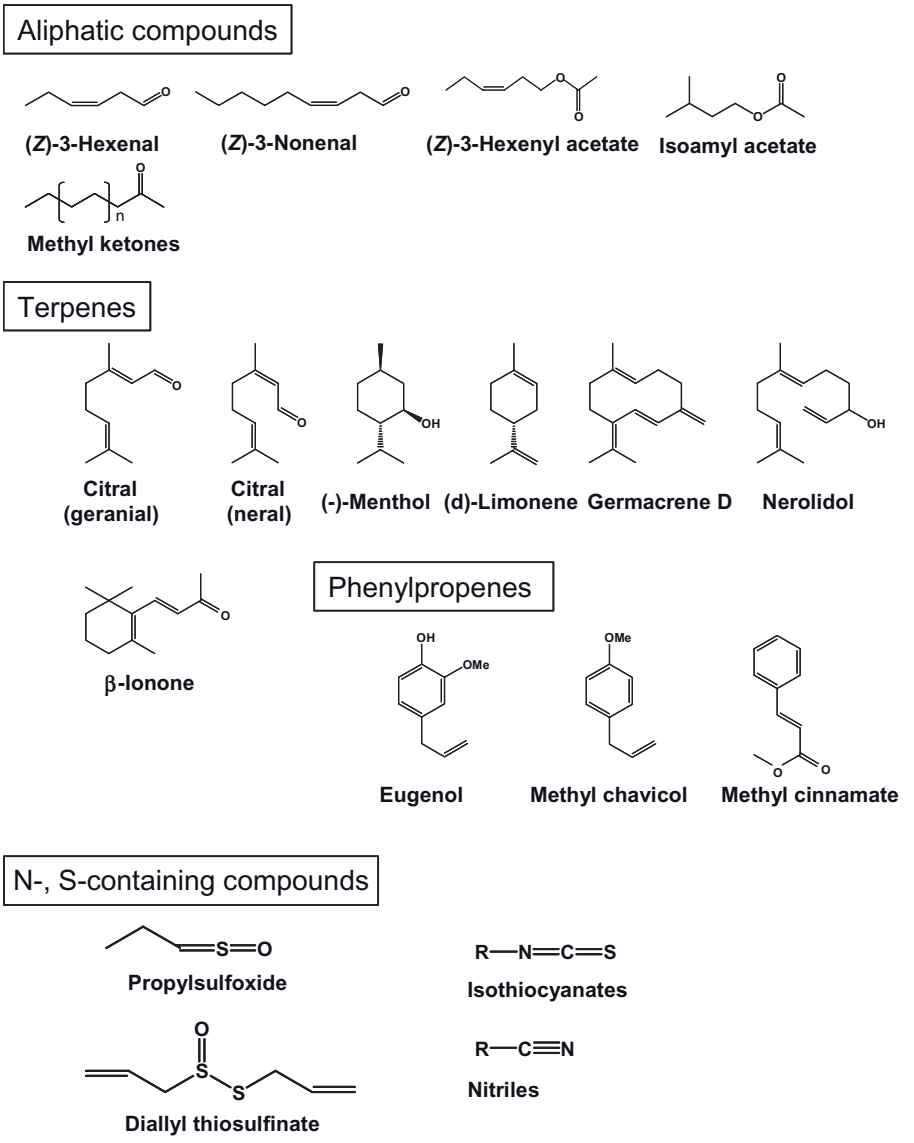

Figure 37.1. Various aroma (flavor) compounds in plant foods.

bred and are available in the market. In these cultivars, the flavor and synthesis of their corresponding various alcohols and aldehydes derived from fatty acids, except 1-octen-3-ol, have been greatly reduced compared with the wild type. Especially in the soybean milk prepared from all LOX-free soybeans, the yields of hexanal and hexanol, the main compounds, were decreased to 2.7% and 3.7%, respectively, compared to soybean milk from the wild types (Kobayashi et al. 1995). These results indicate that the expression of LOX directly affects production of green aroma compounds, although HPL activity was not referred to here.

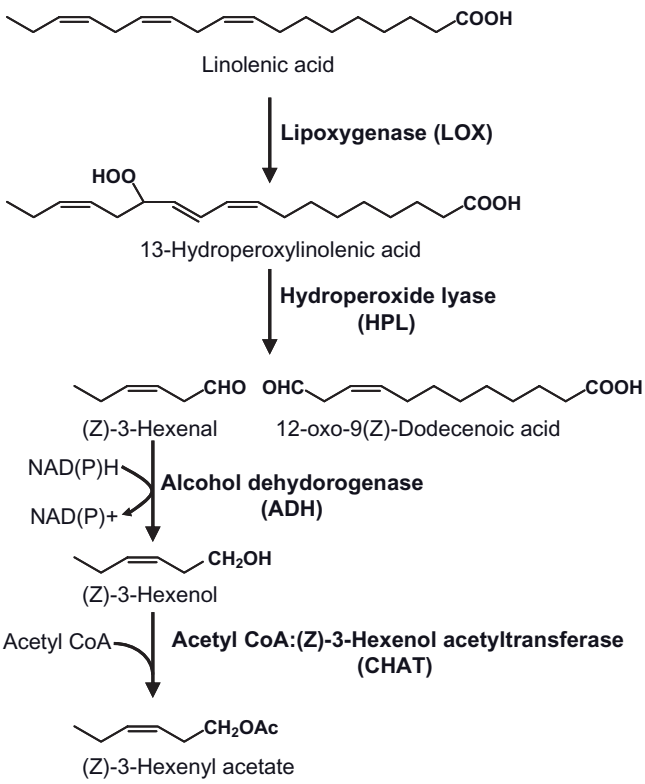

Figure 37.2. Biosynthesis pathway for lipoxygenase-catalyzed aliphatic compounds.

Aliphatic Aldehydes, Ketones, and Esters In addition to the C_6- and C_9-compounds, other short/medium-chain aliphatic aldehydes, ketones, and esters also play important roles as major aroma compounds in various plant foods. They are produced by the degradation of both fatty acids and amino acids (Fig. 37.3).

Straight-chain aliphatic compounds are produced mainly from acyl-coenzyme A (acyl-CoA) or acyl–acyl carrier protein (acyl-ACP) (Fig. 37.3A,B). Acyl-CoAs are formed by the successive β-oxidation of fatty acids (Fig. 37.3A). The mechanisms for the synthesis of acyl-CoA from fatty acids have been well investigated in peroxisomes, where five enzymes participate in their synthesis (Goepfert and Poirier 2007). The produced short/medium-chain acyl-CoAs react with acyl-CoA hydrolase to generate short/medium-chain fatty acids. It has been suggested that aliphatic aldehyde and alcohol are converted from free fatty acids or CoA esters by aldehyde dehydrogenase and alcohol dehydrogenases (Manriquez et al. 2006), but a detailed mechanism has not yet been made clear. Acyl-ACPs are the possible precursors for the short/medium-chain methylketones and secondary alcohols. Recently, methylketone synthase was isolated from tomato trichomes (Fridman et al. 2005) (Fig. 37.3B). It reacts with C_{12}, C_{14}, and C_{16} β-ketoacyl ACPs to produce C_{11}, C_{13}, and C_{15} methylketones, respectively. Further reduction of methylketone suggested the formation of secondary alcohol. Amino acid degradation by aminotransferase or decarboxylase also leads to the formation of aliphatic compounds (Fig. 37.3C). In particular, it has been suggested that branched-chain aliphatic compounds, such as 3-methylbutanol,

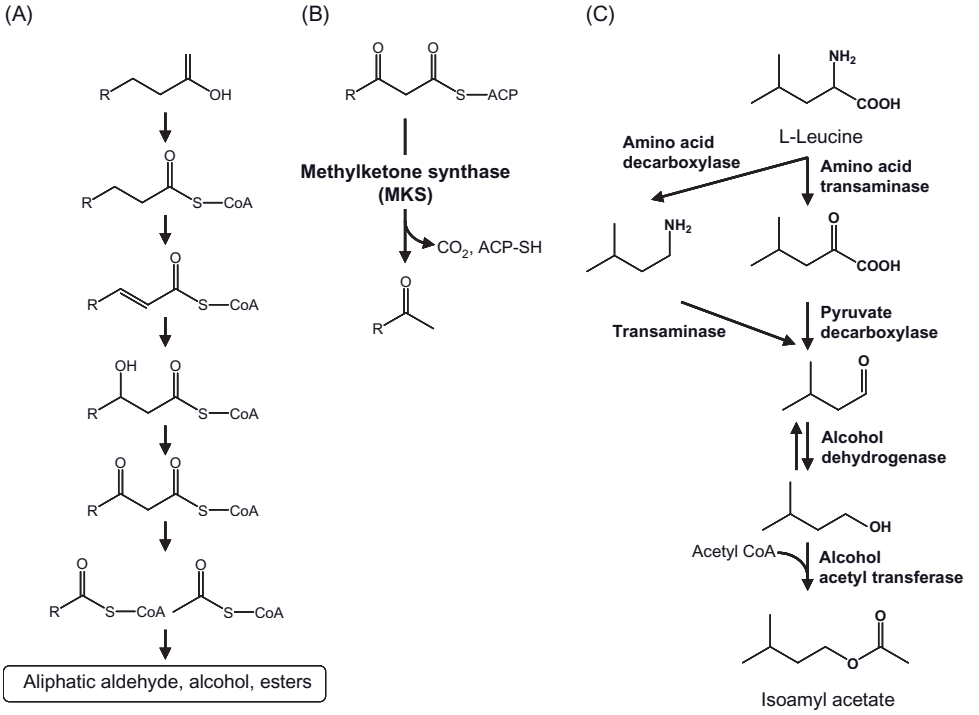

Figure 37.3. Biosynthesis pathway for the synthesis of aliphatic compounds from fatty acid and amino acid. (A) β-Oxidation dependent pathway. (B) Acyl-ACP-dependent pathway. (C) The putative pathway of isoamyl acetate biosynthesis from L-leucine.

are derived directly from branched-chain amino acids (Fig. 37.3C). However, so far, the details of their molecular mechanisms have not been clarified.

Aliphatic esters are the responsible compounds for the "fruity" character in many fruits, like melons, bananas, strawberries, and apples. They are synthesized from acyl-CoAs and alcohols by alcohol acyltransferase (AAT). AAT genes have been isolated and characterized from various fruits and vegetables (Aharoni et al. 2000; Beekwilder et al. 2004; El-Sharkawy et al. 2005; Li et al. 2006; Yahyaoui et al. 2002). In general, AATs show weak substrate specificity, and combinations of different acyl-CoAs and alcohols result in the formation of diverse characteristic esters. Strawberry AAT was induced with fruit maturation, and its recombinant enzyme showed stronger activity on the medium-chain alcohols, heptanol, octanol, nonanol, and geraniol in combination with acetyl-CoA, butyryl-CoA, and hexanoyl-CoA (Aharoni et al. 2000). The enzymatic activity derived from the banana AAT gene revealed narrower substrate specificity than strawberry, preferring to utilize cinnamyl alcohol and geraniol (Beekwilder et al. 2004). In Charentais melon, four AAT genes (CmAAT1–AAT4) were isolated, three of which (CmAAT1, 3, and 4) induced active AAT proteins but with different substrate preferences (El-Sharkawy et al. 2005). Although all three CmAATs accepted a wide range of substrates, CmAAT-1 recombinant protein showed a high preference to (*E*)-2-hexenol with acetyl-CoA, and hexanol with hexanoyl-CoA. For CmAAT-3, benzyl alcohol with acetyl-CoA

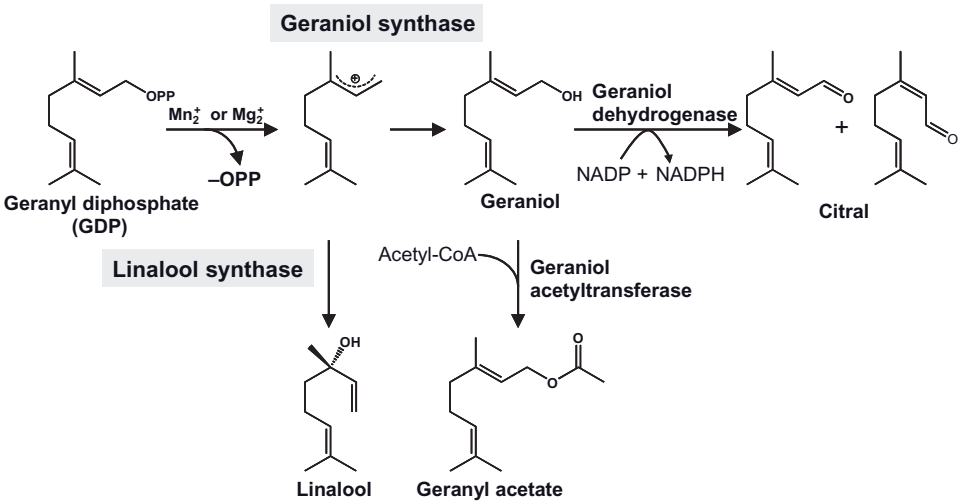

Figure 37.4. An example of terpene synthase and other related enzymatic reactions for terpene aroma compounds.

was preferred to produce benzylacetate. CmAAT-4 utilized a narrower range of substrates than the others but had the highest activity on cinnamyl alcohol with acetyl-CoA. In addition, the expressions of all the CmAAT genes were upregulated during ripening and were correlated with ethylene production (El-Sharkawy et al. 2005).

Terpenes Terpenoids compose the largest group of natural products in plants, with at least approximately 40,000 structures having been reported (Bohlmann and Keeling 2008). Although terpenoids are widespread in plants, monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are dominant in the volatiles of plant. Generally, monoterpenes show stronger aroma than sesquiterpenes, and they are the key compounds contributing to the flavor of many plant foods. Apart from the flavor property, volatile terpenes have also been reported to show various activities as toxins and deterrents to animals, insects, and microorganisms, or as advertisements to attract pollinators (Gershenzon and Dudareva 2007).

The common key step for the biosynthesis of all terpenes is the reaction of terpene synthases on corresponding diphosphate esters (Fig. 37.4). Monoterpenes and sesquiterpenes are produced from geranyl diphosphate (GDP) and farnesyl diphosphate (FDP), and diterpenes are produced from geranylgeranyl diphospate (GGDP). Terpene synthases comprise a large family, and each terpene synthase produces specific terpenes, which results in the generation of the structural diversity of terpenes (Bohlmann et al. 1998; Tholl 2006). The general mechanism of terpene synthases induces the removal of the diphosphate group and the generation of an intermediate with carbocation as the first step. In the various terpene synthases, such intermediates further rearrange to generate the high number of terpene skeletons observed in nature (Gershenzon and Croteau 1993; McGarvey and Croteau 1995). The simplest terpene synthase is the geraniol synthase isolated from lemon basil, which just removes –OPP and the attached –OH from water (Iijima et al. 2004b)

(Fig. 37.4). In the case of linalool synthase, the removal of –OPP occurs as in geraniol synthase; however, the generated carbocation resides at the C3 position and –OH is attached to the C3 position (Iijima et al. 2004a). Other than acyclic terpenes, cyclic terpenes such as limonene, β-pinene, 1,8-cineole, and zingiberene are also directly produced by each specific terpene synthase. Furthermore, some terpene synthases are known to generate multiple products by a single reaction. For example, the β-pinene synthase in citrus lemon produces three other monoterpenes in addition to β-pinene (Lucker et al. 2002).

Terpene synthase genes have been isolated from the leaves, flowers, and roots of many plants (Tholl 2006). The terpene synthases in the leaves of the Lamiaceae family (mint, sage, sweet basil) and the peel of citrus fruits, in which terpenes are the main aroma compounds, have been examined extensively. In sweet basil leaf, we compared the metabolic volatile profiles, enzymatic activities of terpene synthases, and transcriptions of the genes encoding eight distinct mono- and sesquiterpene synthases in three different chemotypes of basil (Iijima et al. 2004a). As a result, the total amounts of terpenes were correlated with the total levels of terpene synthase activity. In addition, the relative levels of distinct terpenes were correlated with the transcript levels of the corresponding terpene synthases. For example, the gene encoding the linalool synthase was expressed almost in a cultivar that produces linalool. These results indicated that the accumulation of volatile terpenes in sweet basil is strongly controlled by the expression of each terpene synthase, showing that the terpene synthases are a significant factor for the diversity of terpenes in plant.

Besides the direct products of terpene synthases, further enzymatic modifications by oxidation, reduction, acetylation, or other reactions facilitate the chemical diversity of volatile terpenes (Fig. 37.4). Alcohol dehydrogenase reacts with geraniol to produce citral (a mixture of geranial and neral) in lemon basil and in lemongrass (Iijima et al. 2006; Singh-Sangwan et al. 1993) (Fig. 37.4). In the case of (-)-menthol production in peppermint, (-)-limonene generated from GDP by limonene synthase is continuously catalyzed by seven enzymes involving cytochrome P450 (-)-limonene-3-hydroxylase, (-)-*trans*-isopiperitenol dehydrogenase, (-)-isopiperitenone reductase, (+)-*cis*-isopulegone isomerase, (+)-pulegone reductase, and (-)-menthone reductase (Bohlmann and Keeling 2008).

Other than mono- and sesquiterpenes, apocarotenoids (norisoprenoids), which mainly contain C_{13}-skeletal ketones such as ionones and geranyl acetone, are also important aroma compounds in the fruits and flowers of many plants. Apocarotenoids are produced from carotenoids by carotenoid cleavage dioxygenases (CCDs) (Auldridge et al. 2006) (Fig. 37.5). CCDs use oxygen to cleave the double bounds in a carotenoid, generating aldehydes and ketones. The genes encoding the CCDs related to aroma have been isolated from many fruits, such as tomato and melon fruits, and grape berries (Ibdah et al. 2006; Mathieu et al. 2005; Simkin et al. 2004). Two CCDs have been isolated from tomato fruits, one of which is expressed during fruit ripening as with the CCD of melon fruits (Ibdah et al. 2006; Simkin et al. 2004). In the case of grapes, its gene expression level increased until veraison stage (Mathieu et al. 2005).

The substrate specificities of CCDs are broad, but the structures of the products strongly depend on the substrate carotenoids. When phytoene, lycopene, and β-carotene are enzymatically catalyzed by CCDs, geranylacetone, pseudoionone, and β-ionone are generated, respectively. In contrast, when δ-carotene is the substrate,

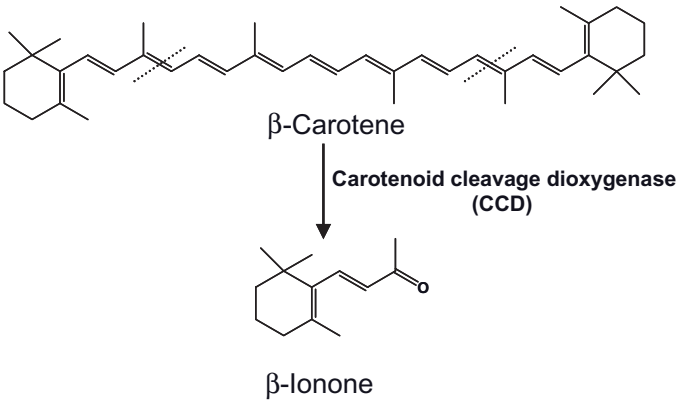

Figure 37.5. The reaction mechanism of CCD in β-carotenoid to produce β-ionone.

both α-ionone and pseudoionone are produced. Other than these compounds, 6-methyl-5-heptene-2-one and citral have also been suggested as products of CCD reaction (Lewinsohn et al. 2005). In the studies of tomato and watermelon fruits using different genetic background lines, it was found that the profiles and intensities of the produced volatile apocarotenes were correlated with carotenoid composition patterns (Lewinsohn et al. 2005). These results indicate that the structures and contents of substrate carotenoids strongly affect the production of the apocarotenoids as aroma.

Phenolics Volatile phenolic compounds, including benzenoid and phenylpropenes, also significantly contribute to special aromas in many vegetables and fruits. While phenolic compounds are known to be generated from phenylalanine as the common precursor, the molecular mechanisms for their biosynthesis have been clarified for a limited number of compounds.

2-Phenylethanol, which shows a floral aroma for various fruits, is produced by the catalysis of three enzymatic steps from L-phenylalanine. The genes encoding two of the three enzymes (i.e., phenylalanine decarboxylase and aldehyde reductase) have recently been isolated from tomato fruits (Tieman et al. 2006, 2007). Phenylalanine decarboxylase directly exerts on phenylalanine to produce 2-phenylethylamine (Tieman et al. 2006). Then, 2-phenylethylamine is presumed to convert to 2-phenylacetaldehyde by an amine oxidase, dehydrogenase, or transaminase. Finally, aldehyde reductase reacts on phenylacetaldehyde to produce 2-phenylethanol (Tieman et al. 2007). In tomato fruits, the overexpression of phenylalanine decarboxylase resulted in 10-fold greater accumulation levels of 2-phenylacetaldehyde, 2-phenylethanol, and 1-nitro-2-phenylethane than those in the wild type (Tieman et al. 2006).

Benzylalcohol and its relatives are also important aroma compounds in many plant foods. They are shown to be produced from phenylalanine by labeling experiments and by a metabolic flux analysis in flowers (Boatright et al. 2004; Orlova et al. 2006) (Fig. 37.6). In petunia flowers, two pathways, β-oxidative (CoA dependent) and non-β-oxidative (CoA independent), were suggested to contribute to the formation of benzenoid compounds (Boatright et al. 2004). In the β-oxidative pathway, *trans*-cinnamic acid produced from phenyl alanine is thioesterified by CoA

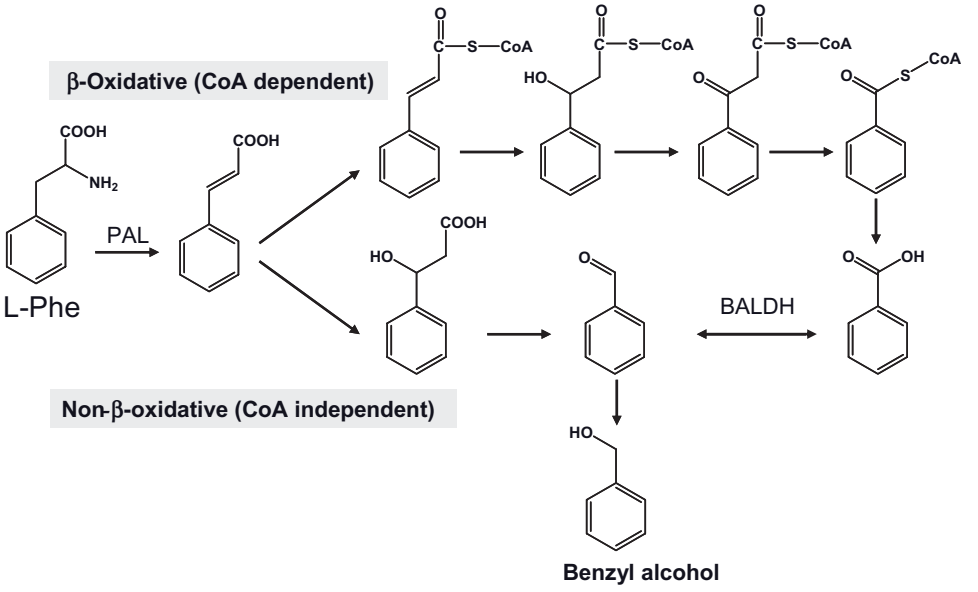

Figure 37.6. Predicted benzenoid aroma compound pathway from L-phenylalanine. L-phe, L-phenylalanine; PAL, phenylalanine lygase; BALDH, benzaldehyde dehydrogenase.

ligase, and benzoyl-CoA is produced after several reaction steps. Furthermore, benzoyl-CoA is hydrolyzed to benzoic acid by thioesterase, and benzaldehyde and bezylalcohol are produced by dehydrogenase. On the other hand, in the non-β-oxidative pathway, benzaldehyde is produced from *trans*-cinnamic acid by nonoxidative conversion, and subsequent dehydrogenase converts it to benzyl alcohol. However, at present, the detailed reaction mechanisms of both pathways are unclear the detailed reaction mechanisms of both pathways are partially clarified at the molecular level (Long et al. 2009).

Salicylate, which is also a kind of benzenoid, obtains its volatility by methylation as methyl salicylate. Methyl salicylate is a common aroma compound in some plant foods, such as tomatoes. The methylation of salicylic acid is catalyzed by *O*-methyltransferase, with S-adenosyl-L-methionine (SAM) as the methyl donor (Negre et al. 2002; Ross et al. 1999). On the other hand, methyl anthranilate, which shows a similar structure to methyl salicylate with just a replacement of the hydroxyl group with the amine group, is the main aroma compound in Concord grape juice, and is used as a flavoring ingredient in candies. However, its methylation in the Concord grapes occurs in a different way from methyl salicylate. Methyl anthranilate involves an AAT from anthranyl-CoA and methanol, anthranyloyl-CoA: methanol acyltransferase (AMAT) (Wang and De Luca 2005). The expression of *AMAT* in Concord grapes has been shown to be upregulated in response to veraison and to be correlated with the increases of anthranilic acid and methanol in ripening grapes (Wang and De Luca 2005).

Phenylpropenes such as eugenol, methyl chavicol, and *t*-anethole are generated by divergence from the lignin biosynthesis pathway (Fig. 37.7). Eugenol is a characteristic aroma compound in many plant foods, with an especially high presence in spices such as cloves and allspice. However, the mechanism of the biosynthesis

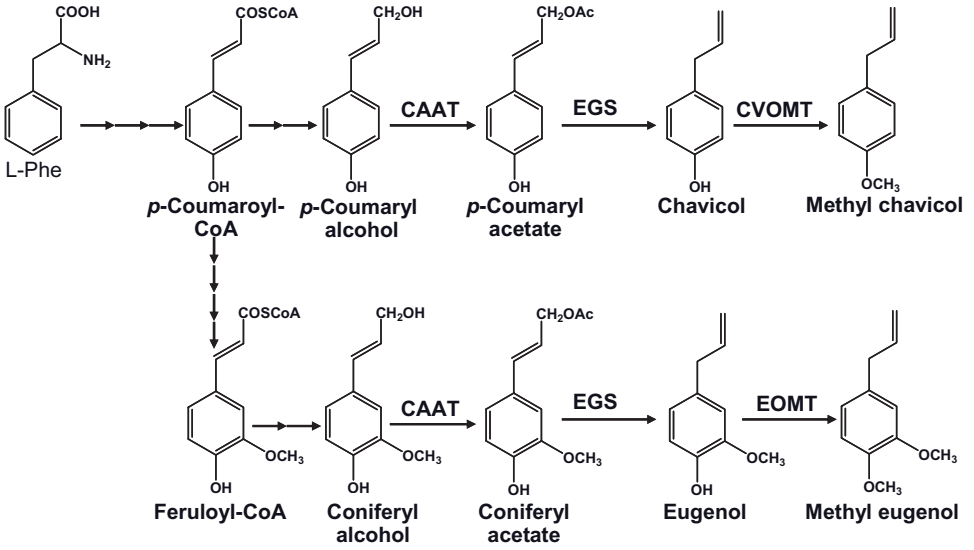

Figure 37.7. Biosynthesis pathway for the production of phenylpropenes from L-phenylalanine in basil glandular trichomes. CAAT, coniferyl alcohol acetyl transferase; EGS, eugenol synthase; CVOMT, chavicol *O*-methyltransferase; EOMT, eugenol *O*-methyltransferase.

was unclear until a few years ago. Koeduka and others (2006) isolated eugenol synthase and isoeugenol synthase from sweet basil leaf glandular trichomes and petunia flowers, respectively. Eugenol/isoeugenol synthases use coniferyl acetate and NADPH as substrates to form eugenol and isoeugenol (Vassao et al. 2006) (Fig. 37.7), for which the reaction mechanism was revealed by a further examination of the crystal structure of eugenol synthase (Louie et al. 2007). Coniferyl acetate, a substrate of eugenol synthase, is synthesized by acetyl transferase from coniferyl alcohol derived from the phenylpropanoid pathway (Fig. 37.7). The cDNA encoding coniferyl AAT has been isolated and characterized in the petunia flower, which produces isoeugenol as a volatile compound (Dexter et al. 2007). Further methylation on eugenol and chavicol catalyzed by *O*-methyltransferase with SAM as the methyl donor (Gang et al. 2002; Lewinsohn et al. 2000) produces methyl eugenol and methyl chavicol as aroma compounds (Fig. 37.7). On the other hand, methyl cinnamate, which is also found in many herbs, was found to be synthesized from cinnamic acid with SAM by the catalysis of carboxyl methyltransferase (Kapteyn et al. 2007).

Sulfur- and Nitrogen-Containing Flavor Compounds While sulfur- and nitrogen-containing flavor compounds are present in a limited number of fruits and vegetables, mainly in the *Allium* species and in Brassicaceae plants, they have intense impacts on our perception of those food flavors. In the *Allium* species like garlic and onion, the main flavor compounds are polysulfides and thiosulfinates, which originate from sulfur-containing amino acids, (+)-*S*-alk(en)yl cysteine sulfoxides (CSOs) (Jones et al. 2004). Among these, *S*-allyl cysteine sulfoxide (ACSO, alliin) is characteristic in garlic, and *trans*-*S*-1-propenyl cysteine sulfoxide (PreCSO, isoalliin) and *S*-propyl cysteine sulfoxide (PCSO, propiin) are the main precursors

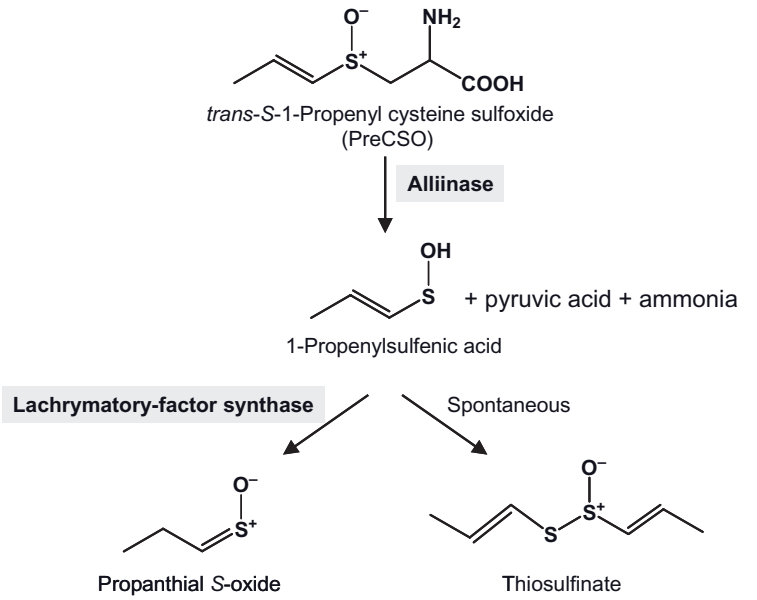

Figure 37.8. Enzymatic reaction of alliinase and lachrymatory-factor synthase from *trans*-S-1-propenyl cysteine sulfoxide (PreCSO) to volatile flavor compounds.

of the onion flavor. On the other hand, *S*-methyl cysteine sulfoxide (MCSO, Methiin) is contained in both *Allium* and Brassicaceae plants, such as cabbage, broccoli, and cauliflower (Jones et al. 2004).

Alliinase cleaves these CSOs to form thiosulfinates with pyruvate and ammonia, and a further second reaction leads them to polysulfides and thiosulfoxides (Fig. 37.8). The alliinase in many *Allium* plants has been investigated for a long time, and the genes responsible for alliinase have been isolated and functionally characterized in onion, garlic, and Chinese chive (Lancaster et al. 2000; Rabinkov et al. 1994; Van Damme et al. 1992). Onion aliinase showed a preferred reaction with PreCSO over other CSOs (Coolong and Randle 2003), indicating that PreCSO contributes to the aroma in onion. Furthermore, onion emits a characteristic volatile, propanthial *S*-oxide, which is involved in the irritating lachrymatory effect (Fig. 37.8). It is produced by lachrymatory-factor synthase following allinase action on PreCSO, but not spontaneous reaction (Imai et al. 2002). The recombinant protein from lachrymatory-factor synthase has shown high substrate specificity on PreCSO, which is the main precursor of onion, in the reaction mixture with alliinase (Imai et al. 2002).

Brassicaceae plants, such as radish, broccoli, mustard, and cauliflower, commonly contain glucosinolates as the precursors of volatile compounds, isothiocyanates, thiocyanates, and nitriles. These volatiles are generated as degradation products by the reaction of myrosinase on glucosinolates after mechanical or herbivory damages to a plant (Bones and Rossiter 2006; Kliebenstein et al. 2005; Rask et al. 2000). Recently, glucosinolates and their degradation products have drawn attention from both chemical ecology and food science (Kliebenstein et al. 2005; Rask et al. 2000; Shroff et al. 2008). Glucosinolates are anti-herbivore and anti-pathogen chemical defenses in plants. Glucosinolates (Mewis et al. 2006) and isothiocyanates are also

known to show anticarcinogenic, antioxidative, and antimicrobial activities (Fahey et al. 1997; 2003). The model plant species *Arabidopsis thaliana* also contains glucosinolates; therefore, extensive research on *A. thaliana* has contributed to an understanding of the mechanisms for the biosynthesis of glucosinolates and their degradations (Wittstock and Halkier 2002).

Glucosinolates are derived from amino acids and are divided into aliphatic, aromatic, and indole glucosinolates depending on the natures of the amino acids in their origins. Methylthio- or methylsulfinyl glucosinolates are synthesized from methionine by several enzymes through chain-elongation steps (Kliebenstein et al. 2005). Recently, the R2R3-Myb transcription factors, which upregulate the genes involved in the enzymes for synthesis of methionine-derived glucosinolates, have been identified (Hirai et al. 2007; Sonderby et al. 2007). In *A. thaliana*, knockout plants with the myb28 gene, a kind of R2R3-Myb transcription factor, have shown drastic decreases in aliphatic glucosinolate contents and downregulated expressions of the genes related to their biosynthesis (Hirai et al. 2007).

Myrosinase basically catalyzes as thioglucosidase on glucosinolates (Fig. 37.9). However, the produced intermediates are unstable, bringing the subsequent rearrangements into multiple stable products, such as isothiocyanates, nitriles, and epithionitriles. In *Brassica napus*, myrosinase has been found in all of the investigated organs, mainly in the myrosin cells, which are specialized cells scattered at low frequency in plants (Andréasson and Jørgensen 2003; Andreasson et al. 2001). Myrosinase genes have been found in *Arabidopsis* and *B. napus* (Rask et al. 2000; Xue et al. 1995). *B. napus* contains at least 20 genes divided into three subfamilies (Rask et al. 2000). The structural preference of the products is determined by the presence of structural specifier proteins, epithiospecifier protein (ESP) and epithiospecifier modifier1 (ESM1) (Fig. 37.9). In *Arabidopsis*, ESP overexpression lines have shown switching from the formation of isothiocyanates to nitriles. Furthermore, ESP gene expression and the structural outcome of glucosinolate activation are

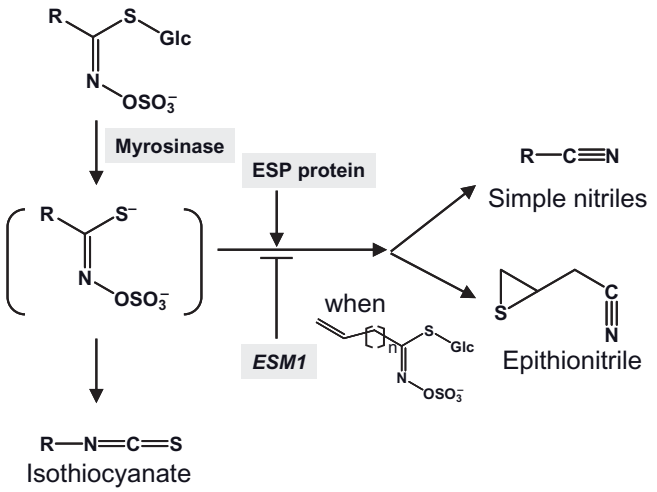

Figure 37.9. Glucosinolate–myrosinase reaction mechanism to produce isothiocyanates, epithionitriles, and simple nitriles.

changeable depending on genotype, age, tissue, and environmental regulation (Wentzell and Kliebenstein 2008). Isothiocyanates are dominantly produced in the flowers and leaves. On the other hand, simple nitriles are more often generated in the roots.

Accumulated? Emitted? Generated after Damage? Aroma Compounds Are Produced and Released from Specialized Organs and under Limited Conditions

Plant volatiles, including aroma compounds, serve many functions related to the ecological and physiological aspects of plants (Pichersky and Gershenzon 2002). Flowers emit volatiles to attract and guide pollinators or to deter unwanted visitors. In vegetative tissues, volatiles are emitted following hervibore and pathogen attacks and are used as toxins for them and as signals for plant defense. In fruits, volatiles are emitted to attract animals, insects, or birds, which eat them and thereby disperse the seeds. Therefore, generally, volatiles are synthesized in specialized organs or parts that are beneficial for each plant.

Lamiaceae plants, such as mints, sages, and basils, have glandular trichomes on the surface of the aerial parts of their leaves, stems, and flowers (Schilmiller et al. 2008). Glandular trichomes constitute gland cells that synthesize volatiles and other secondary metabolites, and a cuticular sac covering each gland cell, into which large amounts of synthesized compounds are secreted and stored. Upon damage to the tissue, the sacs rupture and release their compounds, which in the case of volatiles are easily being evaporated into the atmosphere. While two kinds of glandular trichomes, peltate and capitate glands, exist on the surface of Lamiaceae plants, these metabolites are mainly synthesized and stored in the peltate glands. As for the biosynthesis of volatiles in the glands, the pioneer work on menthol synthesis in mints, resulting in the identification of most of the enzymes involved in menthol production in the glandular fraction (Croteau et al. 2005). Sweet basil produces a characteristic aroma dependent on each cultivar. The peltate glands of basil contain many volatile terpenes and phenylpropanoids (Gang et al. 2001; Iijima et al. 2004b). In lemon basil, citral (geranial and neral), which is the predominant aroma compound, is synthesized from GDP by two enzymatic steps, geraniol synthase and alcohol dehydrogenase in glands (Fig. 37.4). The gene encoding geraniol synthase and its enzymatic activity were exclusively expressed in the glands of basil and have not been detected in other cultivars. In addition, geraniol was further oxidized to the geranial, which undergoes keto-enol tautomerization to form neral in glands (Iijima et al. 2006). On the other hand, some other basil lines contain phenylpropanoids, such as eugenol, methyl eugenol, methyl chavicol, and methyl cinnamate in glands. The enzymes involved in their formation were also revealed to be exclusively expressed in the peltate glandular trichomes (Gang 2005; Kapteyn et al. 2007) (Fig. 37.7).

Citrus fruits accumulate various volatile terpenes (mono- and sesquiterpenes) as essential oils in specialized oil glands in the flavedo (called secretory cavities) and oil bodies in the juice sacs. Expressions of mono- and sesquiterpene synthases are responsible for the production and composition of terpenes and for the differentiation of their structures. In lemon fruits mainly containing (+)-limonene, functional cDNAs encoding two (+)-limonene synthases, (-)-β-pinene synthase and γ-terpinene

synthase, were isolated from the flavedo layer of young green fruits (Lucker et al. 2002). In oranges, a sesquiterpene synthase (Cstps1) responsible for valencene production was identified in the flavedo (Sharon-Asa et al. 2003). The temporal expression of *Cstps1* in orange flavedo during fruit development and ripening showed a consistency with the accumulation level of valencene. Furthermore, *Cstps1* expression and valencene accumulation were upregulated in response to ethylene, indicating that ethylene has an affect on the final ripening stage processes by the production of valencene, although orange is not climacteric (Sharon-Asa et al. 2003).

Climacteric fruits, such as tomatoes, apples, and some varieties of melon, are known to produce and emit many aroma compounds during ripening in response to enhanced ethylene synthesis. Although little is known about systems on how ethylene regulates the biosynthesis of aroma compounds, some research on apple fruit aroma biosynthesis have been performed to discover their relationships (Defilippi et al. 2005; Schaffer et al. 2007; Tsantili et al. 2007). Schaffer and others (2007) generated an apple fruit antisense mutant of l-aminocyclopropane-l-carboxylate oxidase that does not produce internal ethylene. Using this mutant, the effects of ethylene perception on the accumulation of aroma compounds and the expression of the genes potentially related to their biosynthesis (186 genes for aliphatic esters, terpenes, and phenylpropanoids) were systematically demonstrated using microarray analyses. By exogenous ethylene treatment, 30 aroma compounds, mainly aliphatic esters with hexanol, butanol, hexanoic acid, estragole, and α-farnesene, were generated. However, only 17 genes of 186 genes were upregulated during ethylene treatment in the skin of fruits. Seven genes on the aliphatic ester/fatty acid degradation pathway, four genes for sesquiterpene biosynthesis, and six genes for phenylpropanoid biosynthesis were included. In particular, the first and last steps on all of the pathways are regulated by ethylene, indicating that ethylene does not coordinately regulate but selectively regulates the expression of the genes involved in aroma biosynthesis. As well as apple, antisense suppression of the gene involved in ethylene production results in the inhibition of aroma generation in both Charentais melons and tomatoes (Ayub et al. 1996; Oeller et al. 1991). In Charentais melons and tomatoes, ethylene induces the expression of AAT for the production of esters, and alcohol dehydrogenase to produce alcohols, the substrates of AAT (Alexander and Grierson 2002; Flores et al. 2002). Furthermore, tomato LOXs, consisting of a family of at least five isoforms, were independently regulated by ethylene (Griffiths et al. 1999). Among them, Tomlox C, which was expressed in ripening fruits and enhanced by ethylene, was identified as the major LOX involved in flavor volatile production, like hexanal (Chen et al. 2004).

Some aroma compounds are not accumulated beforehand in plants but are generated and emitted rapidly after plants are damaged mechanically or by herbivores. With respect to the interaction of plants with their biotic environment, the molecular mechanism of volatile emission by plant wounding has been demonstrated using the model plant *Arabidopsis*. Two kinds of events are assumed to occur in an aroma generation system. First, substrates or catalyzing enzymes are not synthesized until a plant gets damaged. Second, substrates and catalyzing enzymes are separately localized in a cell, and cell breakage by tissue disruption allows their contact and reaction.

In the first case, the generation of green leaf aroma compounds derived from fatty acid degradation by LOX and HPL is hypothesized. Although free linolenic

acid and 13-hydroperoxide can hardly be detected in an intact *Arabidopsis* leaf, the rapid decrease of galactolipids, the precursors of free fatty acids, and the prominent generation of (*Z*)-3-hexenal have been detected after homogenization (Matsui 2000a). This suggests that tissue disruption triggers the hydrolysis of galactolipids to provide free fatty acids as substrates of the LOX enzyme. D'Auria and others (2007) found that (*Z*)-3-hexenyl acetate was synthesized after (*Z*)-3-hexenal and (*Z*)-3-hexenol production by mechanical wounding on the leaves of *A. thaliana* ecotype Landsberg *erecta*. Taken together, the enzymatic activity and gene expression of acetyl-CoA:(Z)-3-hexen-1-ol acetyltransferase (CHAT), which is responsible for the conversion of (*Z*)-3-hexenol to (*Z*)-3-hexenyl acetate, is increased after wounding. This result suggests that mechanical wounding induces (*Z*)-3-hexenyl acetate production by the activation of the CHAT enzyme. Furthermore, in cucumber, the mechanical wounding of leaves has been reported to raise 9-hydroperoxide lyase activity (Matsui et al. 2006).

For the second case, the reactions of myrosinase on glucosinolates have been well investigated and reviewed. Myrosinase is accumulated in idioblasts called myrosin cells, which are scattered throughout all plant organs. On the other hand, glucosinolates are also known to be accumulated in all of the organs of a plant (Brown et al. 2003) and certain cells, except for myrosin cells (Kelly et al. 1998). Glucosinolates have been reported to be localized in the vacuoles of the aleurone-like cells of *Brassica juncea* (Kelly et al. 1998) and in certain sulfur-containing "S-cells" in the flower stalk of *Arabidopsis* (Koroleva et al. 2000). After the glucosinolate–myrosinase reaction, ESP regulates the formation of more ephithionitriles and simple nitriles than isothiocyanates (Fig. 37.9). Burow and others (2007) found that ESP activity in *Arabidopsis* was developmentally and organ-specifically regulated. In addition, its protein was shown to be present in the epidermal cells of all aerial parts except the anthers and in the cambium cells of the main vascular bundle in leaves. This suggests that myrosinase and ESP are separately localized. However, whether ESP and glucosinolates are co-localized still remains uncertain.

METABOLIC ENGINEERING FOR AROMA COMPOUNDS

The genetic manipulation of the genes involved in the metabolite pathways of plants has been attracting the attention not only of researchers but also of breeders and consumers because many metabolites directly affect the color, flavor, and nutrition of plant foods. The recent progress in metabolic engineering for volatiles in plants, including aroma compounds, has been reviewed in several papers (Aharoni et al. 2005; Dudareva and Negre 2005; Dudareva and Pichersky 2008). *Arabidopsis*, petunia, and tobacco have been the mainly engineered species. As for food plants, tomato, potato, and mint have been used to produce transformants. In particular, tomato is a popular vegetable worldwide and has been preferred as a practical plant model in biological research. Here, therefore, the succeeding examples of metabolic engineering for aroma compounds using tomato are introduced.

In tomato fruits, the effects of the constitutive expression of the genes encoding the enzyme for fatty acid degradation were initially characterized. The overexpression of the yeast Δ-9 desaturase allowed an increase in various aroma compounds derived from fatty acids, such as (*Z*)-3-hexenol, (*Z*)-3-hexenal, and hexenal (Wang

et al. 1996). The overexpression of the tomato alcohol dehydrogenase gene activated ADH activity and increased the ratio of (*Z*)-3-hexenol to (*Z*)-3-hexenal (Speirs et al. 1998). In addition, these tomato fruits were recognized as having a more intense "ripe fruit" flavor (Speirs et al. 1998). On the other hand, transgenic tomato fruits with reduced expression of the tomato LOX C gene showed a marked decrease in the flavor volatiles (*Z*)-3-hexenol, (*Z*)-3-hexenal, and hexanal (Chen et al. 2004).

Genetic modification of the volatile terpene composition in tomato fruits has been achieved by Lewinsohn and others using the terpene synthase genes isolated from heterologous plants (Davidovich-Rikanati et al. 2007; Lewinsohn et al. 2001). When the *S*-linalool synthase gene derived from the *Clarkia breweri* flower was overexpressed in tomato fruits, *S*-linalool was successfully produced with 8-hydroxy linalool (Lewinsohn et al. 2001). On the other hand, the introduction of the *Ocimum basilicum* (sweet basil) geraniol synthase gene under the control of the tomato ripening-specific polygalacturonase promoter synthesized a large amount of geraniol (Davidovich-Rikanati et al. 2007). Interestingly, in this transgenic line, further metabolized monoterpene aroma compounds, citral (geranial and neral), geranyl acetate, citronellol, geranic acid, and neric acid, were also accumulated. When these transgenic fruits were organoleptically evaluated by panelists, the majority of them (80%) recognized that these fruits had a stronger aroma, and 60% of them preferred the transgenic fruits over nontransgenic ones. On the other hand, the amounts of carotenoids, such as lycopene, in the transgenic fruits were half of those in the nontransgenic ones. It is known that both monoterpenoid and carotenoid synthesis occur in the plastid of plant cells and that their substrates, GDP and GGDP, are synthesized on the same pathway. Therefore, an increase in monoterpenoid contents, with a decline in carotenoid contents, suggests that the GGDP pool available for carotenoid synthesis was deprived by monoterpene synthesis thorough GDP because of high levels of geraniol synthase expression in the transgenic lines.

CONCLUSION AND PERSPECTIVE

This chapter introduced the recent progress that has been made on the synthesis and metabolism of aroma compounds by focusing on plant foods. The isolation and functional characterization of the genes using comparative genome information, which is called the functional genomics approach, has facilitated an understanding of the molecular mechanisms for aroma compound biosynthesis. Furthermore, this will enlarge the potential applications to breeding and will improve metabolic engineering. However, at present, regulating the production of a specific aroma compound, increasing or decreasing it, is still somewhat far from a practical approach. In many cases of metabolic engineering, even though the targeted gene expression and its enzymatic activity are effected, the amount of the targeted compound is less than expected. These results suggest that the substrate pool is insufficient in the engineered plant or that the modification of one metabolic pathway may affect other metabolic pathways. Furthermore, each plant cell may have a limited capacity to accumulate a specific compound by a detrimental effect, resulting in the accumulation of further metabolized nontoxic compounds. Therefore, the relationships among the different metabolic pathways in a plant should be carefully considered in metabolite modification. A comprehensive understanding of the interaction of

metabolic pathways can achieve not only an understanding of the detailed metabolic mechanism in a plant but also the means of controlling the production of the targeted compound.

REFERENCES

Aharoni A, Jongsma MA, Bouwmeester HJ. 2005. Volatile science? Metabolic engineering of terpenoids in plants. *Trends Plant Sci* 10(12):594–602.

Aharoni A, Keizer LC, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen AM, De Vos RC, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP. 2000. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *Plant Cell* 12(5):647–62.

Alexander L, Grierson D. 2002. Ethylene biosynthesis and action in tomato: A model for climacteric fruit ripening. *J Exp Bot* 53(377):2039–55.

Andreasson E, Bolt Jorgensen L, Hoglund AS, Rask L, Meijer J. 2001. Different myrosinase and idioblast distribution in *Arabidopsis* and *Brassica napus*. *Plant Physiol* 127(4): 1750–63.

Andréasson E, Jørgensen LB. 2003. Localization of plant myrosinases and glucosinolates. In Romeo JT (ed.), *Integrative Phytochemistry: From Ethnobotany to Molecular Ecology, Recent Advances in Phytochemistry*. Amsterdam: Elsevier, pp. 79–99.

Auldridge ME, McCarty DR, Klee HJ. 2006. Plant carotenoid cleavage oxygenases and their apocarotenoid products. *Curr Opin Plant Biol* 9(3):315–21.

Ayub R, Guis M, Ben Amor M, Gillot L, Roustan JP, Latche A, Bouzayen M, Pech JC. 1996. Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. *Nat Biotechnol* 14(7):862–6.

Beaulieu JC, Grimm CC. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J Agric Food Chem* 49(3): 1345–52.

Beekwilder J, Alvarez-Huerta M, Neef E, Verstappen FW, Bouwmeester HJ, Aharoni A. 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. *Plant Physiol* 135(4):1865–78.

Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N. 2004. Understanding in vivo benzenoid metabolism in petunia petal tissue. *Plant Physiol* 135(4):1993–2011.

Bohlmann J, Keeling CI. 2008. Terpenoid biomaterials. *Plant J* 54(4):656–69.

Bohlmann J, Meyer-Gauen G, Croteau R. 1998. Plant terpenoid synthases: Molecular biology and phylogenetic analysis. *Proc Natl Acad Sci U S A* 95(8):4126–33.

Bones AM, Rossiter JT. 2006. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 67(11):1053–67.

Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* 62(3):471–81.

Burow M, Rice M, Hause B, Gershenzon J, Wittstock U. 2007. Cell- and tissue-specific localization and regulation of the epithiospecifier protein in *Arabidopsis thaliana*. *Plant Mol Biol* 64(1–2):173–85.

Buttery R. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In Acree TE, Teranishi R (eds.), *Flavor Science: Sensible Principles and Techniques*. Washington, DC: American Chemical Society, pp. 259–86.

Chen G, Hackett R, Walker D, Taylor A, Lin Z, Grierson D. 2004. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiol* 136(1):2641–51.

Coolong T, Randle W. 2003. Sulfur and nitrogen availability interact to affect the flavor biosynthesis pathway in onion. *J Am Soc Hortic Sci* 128:776–83.

Croteau RB, Davis EM, Ringer KL, Wildung MR. 2005. (-)-Menthol biosynthesis and molecular genetics. *Naturwissenschaften* 92(12):562–77.

D'Auria JC, Pichersky E, Schaub A, Hansel A, Gershenzon J. 2007. Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (Z)-3-hexen-1-yl acetate in *Arabidopsis thaliana*. *Plant J* 49(2):194–207.

Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E. 2007. Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. *Nat Biotechnol* 25(8):899–901.

Defilippi BG, Dandekar AM, Kader AA. 2005. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *J Agric Food Chem* 53(8):3133–41.

Dexter R, Qualley A, Kish CM, Ma CJ, Koeduka T, Nagegowda DA, Dudareva N, Pichersky E, Clark D. 2007. Characterization of a petunia acetyltransferase involved in the biosynthesis of the floral volatile isoeugenol. *Plant J* 49(2):265–75.

Dudareva N, Negre F. 2005. Practical applications of research into the regulation of plant volatile emission. *Curr Opin Plant Biol* 8(1):113–18.

Dudareva N, Pichersky E. 2008. Metabolic engineering of plant volatiles. *Curr Opin Biotechnol* 19(2):181–9.

Dudareva N, Pichersky E, Gershenzon J. 2004. Biochemistry of plant volatiles. *Plant Physiol* 135(4):1893–902.

El-Sharkawy I, Manriquez D, Flores FB, Regad F, Bouzayen M, Latche A, Pech JC. 2005. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. *Plant Mol Biol* 59(2):345–62.

Fahey JW, Wade KL, Stephenson KK, Chou FE. 2003. Separation and purification of glucosinolates from crude plant homogenates by high-speed counter-current chromatography. *J Chromatogr A* 996(1–2):85–93.

Fahey JW, Zhang Y, Talalay P. 1997. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci U S A* 94(19): 10367–72.

Flores F, El Yahyaoui F, de Billerbeck G, Romojaro F, Latche A, Bouzayen M, Pech JC, Ambid C. 2002. Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais cantaloupe melons. *J Exp Bot* 53(367):201–6.

Fridman E, Wang J, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E. 2005. Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. *Plant Cell* 17(4):1252–67.

Gang DR. 2005. Evolution of flavors and scents. *Annu Rev Plant Biol* 56:301–25.

Gang DR, Lavid N, Zubieta C, Chen F, Beuerle T, Lewinsohn E, Noel JP, Pichersky E. 2002. Characterization of phenylpropene O-methyltransferases from sweet basil: Facile change of substrate specificity and convergent evolution within a plant O-methyltransferase family. *Plant Cell* 14(2):505–19.

Gang DR, Wang J, Dudareva N, Nam KH, Simon JE, Lewinsohn E, Pichersky E. 2001. An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol* 125(2):539–55.

Gershenzon J, Croteau R. 1993. Terpenoid biosynthesis: The basic pathway and formation of monoterpenes, sesquiterpenes, and diterpenes. In Moore T (ed.), *Lipid Metabolism in Plant*. Boca Raton, FL: CRC Press, pp. 339–88.

Gershenzon J, Dudareva N. 2007. The function of terpene natural products in the natural world. *Nat Chem Biol* 3(7):408–14.

Goepfert S, Poirier Y. 2007. Beta-oxidation in fatty acid degradation and beyond. *Curr Opin Plant Biol* 10(3):245–51.

Griffiths A, Barry C, Alpuche-Soils AG, Grierson D. 1999. Ethylene and developmental signals regulate expression of lipoxygenase genes during tomato fruit ripening. *J Exp Bot* 50(335):793–8.

Hirai MY, Sugiyama K, Sawada Y, Tohge T, Obayashi T, Suzuki A, Araki R, Sakurai N, Suzuki H, Aoki K, Goda H, Nishizawa OI, Shibata D, Saito K. 2007. Omics-based identification of *Arabidopsis* Myb transcription factors regulating aliphatic glucosinolate biosynthesis. *Proc Natl Acad Sci U S A* 104(15):6478–83.

Hornung E, Walther M, Kuhn H, Feussner I. 1999. Conversion of cucumber linoleate 13-lipoxygenase to a 9-lipoxygenating species by site-directed mutagenesis. *Proc Natl Acad Sci U S A* 96(7):4192–7.

Howe GA, Lee GI, Itoh A, Li L, DeRocher AE. 2000. Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. *Plant Physiol* 123(2):711–24.

Ibdah M, Azulay Y, Portnoy V, Wasserman B, Bar E, Meir A, Burger Y, Hirschberg J, Schaffer AA, Katzir N, Tadmor Y, Lewinsohn E. 2006. Functional characterization of CmCCD1, a carotenoid cleavage dioxygenase from melon. *Phytochemistry* 67(15):1579–89.

Iijima Y, Davidovich-Rikanati R, Fridman E, Gang DR, Bar E, Lewinsohn E, Pichersky E. 2004a. The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. *Plant Physiol* 136(3):3724–36.

Iijima Y, Gang DR, Fridman E, Lewinsohn E, Pichersky E. 2004b. Characterization of geraniol synthase from the peltate glands of sweet basil. *Plant Physiol* 134(1):370–9.

Iijima Y, Wang G, Fridman E, Pichersky E. 2006. Analysis of the enzymatic formation of citral in the glands of sweet basil. *Arch Biochem Biophys* 448(1–2):141–9.

Imai S, Tsuge N, Tomotake M, Nagatome Y, Sawada H, Nagata T, Kumagai H. 2002. Plant biochemistry: An onion enzyme that makes the eyes water. *Nature* 419(6908):685.

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA. 2004. Biosynthesis of the flavour precursors of onion and garlic. *J Exp Bot* 55(404):1903–18.

Kapteyn J, Qualley AV, Xie Z, Fridman E, Dudareva N, Gang DR. 2007. Evolution of cinnamate/p-coumarate carboxyl methyltransferases and their role in the biosynthesis of methylcinnamate. *Plant Cell* 19(10):3212–29.

Kelly PJ, Bones A, Rossiter JT. 1998. Sub-cellular immunolocalization of the glucosinolate sinigrin in seedlings of *Brassica juncea*. *Planta* 206(3):370–7.

Kliebenstein DJ, Kroymann J, Mitchell-Olds T. 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. *Curr Opin Plant Biol* 8(3):264–71.

Kobayashi A, Tsuda Y, Hirata N, Kubota K, Kitamura K. 1995. Aroma constituents of soybean [*Glycine max* (L.) Merril] milk lacking lipoxygenase isoenzymes. *J Agric Food Chem* 43(9):2449–52.

Koeduka T, Fridman E, Gang DR, Vassao DG, Jackson BL, Kish CM, Orlova I, Spassova SM, Lewis NG, Noel JP, Baiga TJ, Dudareva N, Pichersky E. 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc Natl Acad Sci U S A* 103(26):10128–33.

Koroleva OA, Davies A, Deeken R, Thorpe MR, Tomos AD, Hedrich R. 2000. Identification of a new glucosinolate-rich cell type in *Arabidopsis* flower stalk. *Plant Physiol* 124(2): 599–608.

Lancaster JE, Shaw ML, Joyce MD, McCallum JA, McManus MT. 2000. A novel alliinase from onion roots. Biochemical characterization and cDNA cloning. *Plant Physiol* 122(4): 1269–79.

Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam KH, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E. 2001. Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 127(3):1256–65.

Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Meir A, Zamir D, Tadmor Y. 2005. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. *J Agric Food Chem* 53(8):3142–8.

Lewinsohn E, Ziv-Raz II, Dudai N, Tadmor Y, Lastochkin E, Larkov O, Chaimovitsh D, Ravid U, Putievsky E, Pichersky E, Shoham Y. 2000. Biosynthesis of estragole and methyleugenol in sweet basil (*Ocimum basilicum* L). Developmental and chemotypic association of allylphenol O-methyltransferase activities. *Plant Sci* 160(1):27–35.

Li D, Xu Y, Xu G, Gu L, Li D, Shu H. 2006. Molecular cloning and expression of a gene encoding alcohol acyltransferase (MdAAT2) from apple (cv. Golden Delicious). *Phytochemistry* 67(7):658–67.

Long MC, Nagegowda DA, Kaminaga Y, Ho KK, Kish CM, Schnepp J, Sherman D, Weiner H, Rhodes D, Dudareva N. 2009. Involvement of snapdragon benzaldehyde dehydrogenase in benzoic acid biosynthesis. *Plant J* 59(2):256–65.

Louie GV, Baiga TJ, Bowman ME, Koeduka T, Taylor JH, Spassova SM, Pichersky E, Noel JP. 2007. Structure and reaction mechanism of basil eugenol synthase. *PLoS One* 2(10):e993.

Lucker J, El Tamer MK, Schwab W, Verstappen FW, van der Plas LH, Bouwmeester HJ, Verhoeven HA. 2002. Monoterpene biosynthesis in lemon (*Citrus limon*). cDNA isolation and functional analysis of four monoterpene synthases. *Eur J Biochem* 269(13): 3160–71.

Manriquez D, El-Sharkawy I, Flores FB, El-Yahyaoui F, Regad F, Bouzayen M, Latche A, Pech JC. 2006. Two highly divergent alcohol dehydrogenases of melon exhibit fruit ripening-specific expression and distinct biochemical characteristics. *Plant Mol Biol* 61(4–5):675–85.

Mathieu S, Terrier N, Procureur J, Bigey F, Gunata Z. 2005. A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: Functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation. *J Exp Bot* 56(420): 2721–31.

Matsui K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Curr Opin Plant Biol* 9(3):274–80.

Matsui K, Kurishita S, Hisamitsu A, Kajiwara T. 2000a. A lipid-hydrolysing activity involved in hexenal formation. *Biochem Soc Trans* 28(6):857–60.

Matsui K, Miyahara C, Wilkinson J, Hiatt B, Knauf V, Kajiwara T. 2000b. Fatty acid hydroperoxide lyase in tomato fruits: Cloning and properties of a recombinant enzyme expressed in *Escherichia coli*. *Biosci Biotechnol Biochem* 64(6):1189–96.

Matsui K, Ujita C, Fujimoto S, Wilkinson J, Hiatt B, Knauf V, Kajiwara T, Feussner I. 2000c. Fatty acid 9- and 13-hydroperoxide lyases from cucumber. *FEBS Lett* 481(2):183–8.

Matsui K, Minami A, Hornung E, Shibata H, Kishimoto K, Ahnert V, Kindl H, Kajiwara T, Feussner I. 2006. Biosynthesis of fatty acid derived aldehydes is induced upon mechanical wounding and its products show fungicidal activities in cucumber. *Phytochemistry* 67(7):649–57.

McGarvey DJ, Croteau R. 1995. Terpenoid metabolism. *Plant Cell* 7(7):1015–26.

Mewis I, Tokuhisa JG, Schultz JC, Appel HM, Ulrichs C, Gershenzon J. 2006. Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry* 67(22):2450–62.

Negre F, Kolosova N, Knoll J, Kish CM, Dudareva N. 2002. Novel S-adenosyl-L-methionine : salicylic acid carboxyl methyltransferase, an enzyme responsible for biosynthesis of methyl salicylate and methyl benzoate, is not involved in floral scent production in snapdragon flowers. *Arch Biochem Biophys* 406(2):261–70.

Oeller PW, Lu MW, Taylor LP, Pike DA, Theologis A. 1991. Reversible inhibition of tomato fruit senescence by antisense RNA. *Science* 254(5030):437–9.

Orlova I, Marshall-Colon A, Schnepp J, Wood B, Varbanova M, Fridman E, Blakeslee JJ, Peer WA, Murphy AS, Rhodes D, Pichersky E, Dudareva N. 2006. Reduction of benzenoid synthesis in petunia flowers reveals multiple pathways to benzoic acid and enhancement in auxin transport. *Plant Cell* 18(12):3458–75.

Pichersky E, Gershenzon J. 2002. The formation and function of plant volatiles: Perfumes for pollinator attraction and defense. *Curr Opin Plant Biol* 5(3):237–43.

Pichersky E, Niyogi K. 2006. Physiology and metabolism: The second coming of plant biochemistry and physiology. *Curr Opin Plant Biol* 9(3):217–9.

Pichersky E, Noel JP, Dudareva N. 2006. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. *Science* 311(5762):808–11.

Rabinkov A, Zhu XZ, Grafi G, Galili G, Mirelman D. 1994. Alliin lyase (alliinase) from garlic (*Allium sativum*). Biochemical characterization and cDNA cloning. *Appl Biochem Biotechnol* 48(3):149–71.

Rask L, Andreasson E, Ekbom B, Eriksson S, Pontoppidan B, Meijer J. 2000. Myrosinase: Gene family evolution and herbivore defense in Brassicaceae. *Plant Mol Biol* 42(1): 93–113.

Ross JR, Nam KH, D'Auria JC, Pichersky E. 1999. S-Adenosyl-L-methionine : salicylic acid carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense, represents a new class of plant methyltransferases. *Arch Biochem Biophys* 367(1): 9–16.

Schaffer RJ, Friel EN, Souleyre EJ, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma JH, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD. 2007. A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiol* 144(4):1899–912.

Schilmiller AL, Last RL, Pichersky E. 2008. Harnessing plant trichome biochemistry for the production of useful compounds. *Plant J* 54(4):702–11.

Schwab W, Davidovich-Rikanati R, Lewinsohn E. 2008. Biosynthesis of plant-derived flavor compounds. *Plant J* 54(4):712–32.

Sharon-Asa L, Shalit M, Frydman A, Bar E, Holland D, Or E, Lavi U, Lewinsohn E, Eyal Y. 2003. Citrus fruit flavor and aroma biosynthesis: Isolation, functional characterization, and developmental regulation of Cstps1, a key gene in the production of the sesquiterpene aroma compound valencene. *Plant J* 36(5):664–74.

Shiojiri K, Kishimoto K, Ozawa R, Kugimiya S, Urashimo S, Arimura G, Horiuchi J, Nishioka T, Matsui K, Takabayashi J. 2006. Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *Proc Natl Acad Sci U S A* 103(45):16672–6.

Shroff R, Vergara F, Muck A, Svatos A, Gershenzon J. 2008. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *Proc Natl Acad Sci U S A* 105(16):6196–201.

Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ. 2004. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles beta-ionone, pseudoionone, and geranylacetone. *Plant J* 40(6):882–92.

Singh-Sangwan N, Sangwan RS, Luthra R, Thakur RS. 1993. Geraniol dehydrogenase: A determinant of essential oil quality in lemongrass. *Planta Med* 59(2):168–70.

Sonderby IE, Hansen BG, Bjarnholt N, Ticconi C, Halkier BA, Kliebenstein DJ. 2007. A systems biology approach identifies a R2R3 MYB gene subfamily with distinct and overlapping functions in regulation of aliphatic glucosinolates. *PLoS One* 2(12):e1322.

Speirs J, Lee E, Holt K, Yong-Duk K, Steele Scott N, Loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiol* 117(3):1047–58.

Suzuki Y, Ise K, Li C, Honda I, Iwai Y, Matsukura U. 1999. Volatile components in stored rice [*Oryza sativa* (L.)] of varieties with and without lipoxygenase-3 in seeds. *J Agric Food Chem* 47(3):1119–24.

Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr Opin Plant Biol* 9(3):297–304.

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ. 2006. Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proc Natl Acad Sci U S A* 103(21):8287–92.

Tieman DM, Loucas HM, Kim JY, Clark DG, Klee HJ. 2007. Tomato phenylacetaldehyde reductases catalyze the last step in the synthesis of the aroma volatile 2-phenylethanol. *Phytochemistry* 68(21):2660–9.

Tikunov Y, Lommen A, de Vos CH, Verhoeven HA, Bino RJ, Hall RD, Bovy AG. 2005. A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiol* 139(3):1125–37.

Torres-Penaranda AV, Reitmeier CA. 2001. Sensory descriptive analysis of soymilk. *J Food Sci* 66(2):352–6.

Tsantili E, Gapper NE, Arquiza JM, Whitaker BD, Watkins CB. 2007. Ethylene and alpha-farnesene metabolism in green and red skin of three apple cultivars in response to 1-methylcyclopropene (1-MCP) treatment. *J Agric Food Chem* 55(13):5267–76.

Van Damme EJ, Smeets K, Torrekens S, Van Leuven F, Peumans WJ. 1992. Isolation and characterization of alliinase cDNA clones from garlic (*Allium sativum* L.) and related species. *Eur J Biochem* 209(2):751–7.

Vara-Ubol S, Chambers E, Chambers DH. 2004. Sensory characteristics of chemical compounds potentially associated with beany aroma in foods. *J Sens Stud* 19(1):15–26.

Vassao DG, Gang DR, Koeduka T, Jackson B, Pichersky E, Davin LB, Lewis NG. 2006. Chavicol formation in sweet basil (*Ocimum basilicum*): Cleavage of an esterified C9 hydroxyl group with NAD(P)H-dependent reduction. *Org Biomol Chem* 4(14): 2733–44.

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE. 1996. Changes of fatty acids and fatty acid-derived flavor compounds by expressing the yeast delta-9 desaturase gene in tomato. *J Agric Food Chem* 44(10):3399–402.

Wang J, De Luca V. 2005. The biosynthesis and regulation of biosynthesis of Concord grape fruit esters, including 'foxy' methylanthranilate. *Plant J* 44(4):606–19.

Wentzell AM, Kliebenstein DJ. 2008. Genotype, age, tissue, and environment regulate the structural outcome of glucosinolate activation. *Plant Physiol* 147(1):415–28.

Wittstock U, Halkier BA. 2002. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci* 7(6):263–70.

Xue J, Jorgensen M, Pihlgren U, Rask L. 1995. The myrosinase gene family in *Arabidopsis thaliana*: Gene organization, expression and evolution. *Plant Mol Biol* 27(5):911–22.

Yahyaoui FE, Wongs-Aree C, Latche A, Hackett R, Grierson D, Pech JC. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. *Eur J Biochem* 269(9):2359–66.

PART VII

VEGETABLE COMMODITIES

CHAPTER 38

Avocado (*Persea Americana* Mill.) Oil

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INTRODUCTION

The first comprehensive list of volatile molecules present in food matrices comprised a few hundred compounds. At the beginning of the 1970s, less than 1500 flavor chemicals had been identified in food products. It is well-known that nature itself produces most of the world's food flavors, many of which were investigated following the advent of gas chromatography (GC) and mass spectrometry (MS), which marked a real turning point for flavor research. In the early stages of research in this field, attention was devoted to the development of methods in order to acquire deeper knowledge on the profiles of food volatiles; however, this analytical task was made troublesome due to the complexity of many real-world samples. Over the last decade, flavor research has benefited from improvements in instrumental analytical chemistry, and, nowadays, the number of known flavors has increased more than fourfold, reaching over 7000 compounds (D'Acampora Zellner et al. 2008).

In the case of oil, quality may be defined in a number of ways, but flavor sensory perception by consumers is the ultimate determinant. Evaluation of the sensory quality of vegetable oils involves perception of both favorable and unfavorable sensory attributes, with evaluation of sensory defects being used to classify oils into various grades (Angerosa 2000).

Commercially important fruit oil is extracted from the fresh or dried mesocarp of avocado. Avocado oil has been identified for its potential use in cosmetics and skin care products (Freitas et al. 2000; Werman and Neeman 1988). The main fatty acids present in avocado oil pulp are oleic (54–76%), linoleic (11–16%), palmitoleic (5–12%), and linolenic (about 1%). In avocado crops, the proportion of oleic acid increases and that of palmitic acid decreases in comparison to the other fatty acids by retarding the harvest (Freitas et al. 1992; Haiyan et al. 2007; Ozdemir and Topuz 2004).

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Avocado oil quality may be classified from commercial, nutritional, or organoleptic characteristics. The nutritional value arises from high levels of oleic acid and minor components (unsaponifiable matter), whereas aroma is strongly influenced by volatile compounds. Fatty acid contents in avocado and in olive oils are comparable. However, the unique and delicate flavor of olive oil is a determinant factor for its higher human consumption as dressing oil (Kalua et al. 2007; Ridolfi et al. 2002). Avocado oil studies are prominent in scientific literature, but the reported data are more concerned with its health properties. Of all the edible oils, only olive oils have had its flavor attributes studied in detail. The most important external factors influencing olive oil oxidation are temperature, light, and oxygen concentration. At high temperatures, there is an increase in the rate of oxidation but a reduction in the solubility of oxygen. The concentration of alkoxy radicals increases relative to the initially formed peroxy radicals, and polymeric compounds are formed from alkoxy and alkyl radicals. At low or moderate temperatures, the rate of oxidation is slow. Hydroperoxides are the major compounds formed, and their concentration increases until the advanced stages of oxidation when they decompose into minor volatile compounds, in particular carbonyl compounds that may modify olive oil aroma (Velasco and Dobarganes 2002).

FORMATION AND COMPOSITION OF VOLATILE FRACTION

The profile of volatile compounds present in avocado oil determines aroma and quality. Hence, it is critical to understand the formation and characteristics of those compounds that promote certain favorable aroma attributes. Many compounds, mainly terpenoids, aldehydes, alcohols, esters, and hydrocarbons, were found in the volatile fraction of avocado oil.

Volatile compounds in fruit oils are mainly produced by oxidation of polyunsaturated free fatty acids (linolenic and linoleic acids). Endogenous plant enzymes (lipoxygenase) are mostly responsible for the positive aroma perceptions, whereas chemical oxidation and exogenous enzymes, usually from microbial activity, are associated with off-flavor, referred to as oxidative rancidity. The lipoxygenase pathway is initiated by enzyme release when fruit tissues are disrupted. The reaction pathway involves a series of enzymes that oxidize (lipoxygenase) and cleave (hydroperoxide lyase) polyunsaturated fatty acids to produce aldehydes. These are subsequently reduced to alcohols (by alcohol dehydrogenase) and are esterified to produce esters (by alcohol acyltransferase) (Kalua et al. 2007; Ozdemir and Topuz 2004; Sanchez and Salas 2000).

Hydroperoxides of unsaturated fatty acids formed by autoxidation are very unstable and break down into a wide variety of volatile flavor compounds as well as nonvolatile products. It is widely accepted that hydroperoxide decomposition involves homolytic cleavage of the –OOH group, giving rise to an alkoxy radical and a hydroxyl radical. The alkoxy radical undergoes β-scission on the C–C bond, with the formation of an aldehyde and an alkyl or vinyl radical (Ho and Shahidi 2005).

The aromas of aldehydes are generally described as green, painty, metallic, beany, and rancid, and they are often responsible for the undesirable flavors in fats and oils. Hexanal has long been used as an index of oxidative deterioration in foods. Hexanal and 2,4-decadienal are the primary oxidation products of linoleate. The

autoxidation of linoleate generates 9- and 13-hydroperoxides of linoleate. Cleavage of 13-hydroperoxide will lead to hexanal, and breakdown of 9-hydroperoxide will lead to 2,4-decadienal. The retro-aldol reaction of 2,4-decadienal, mediated by moisture, produces 2-octenal, hexanal, and acetaldehyde. 2,4-Decadienal is known to be one of the most important flavor contributors to deep fat-fried foods (Morales et al. 1994).

ANALYTICAL IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS (VOCs)

The most common methods used for the evaluation of volatile compounds involve techniques with an enrichment step, since, because of the very low concentration of the most part of them, the sensitivity of methodologies like direct injection or static headspace (HS) is very poor so that often does not allow their detection, being their concentrations under gas chromatographic detectability thresholds (Angerosa et al. 2004).

It is usually necessary to combine different methods to obtain a complete extraction of all the volatile compounds, resulting in extracts truly representative of the sample aroma. The more commonly applied methods to aroma extraction from vegetable oils are simultaneous distillation–extraction (SDE), dynamic headspace (DHS), and solid phase microextraction (SPME).

The term HS is applied for various gas extraction techniques where volatile sample constituents are transferred into a gas. The sample, placed in a closed container, may be in contact and in equilibrium with the extracting gas (static or equilibrium HS), or the volatile compounds may be stripped off in a continuous flow of an inert gas through or above the liquid phase (DHS).

SDE has traditionally been applied in the analysis of plant materials and has appeared to afford the most favorable uptake for mono- and sesquiterpenes, as well as their oxygenated analogues. SDE via solvent extraction of the distillate combines the advantages of liquid–liquid and steam distillation extraction and has been used to isolate volatile organic compounds with very high recovery rates (Marriott et al. 2001).

DHS is commonly known as purge and trap. Following the extraction, the volatiles are focused on a sorbent cartridge or a cryo-trap. In any case, in the optimization of this technique, the following points should be noted: (1) the temperature must be selected within a narrow range, since at temperatures less than 20°C, most part of the volatile compounds cannot be stripped in an effective way; (2) the upper limit is established by possible oxidative degradation of the oil matrix; (3) the extraction parameters (sample size, flux, and stripping time) are established by the extracting conditions from the matrix selected so that the greater number of compounds with a concentration higher than gas chromatographic detectable thresholds is stripped; (4) the number, the quality, and the quantity of volatile compounds depend on the adsorbent material; and (5) the desorption had not produced artifacts (Angerosa et al. 2004).

Introduced in the early 1990s, SPME has since been used among others for the aroma analysis of various food products. Lately, the SPME technique has been applied for the analysis of flavors from vegetable oil-containing products

(Jelen et al. 2000) and for avocado pulp and oil (López et al. 2004; Moreno et al. 2003). SPME is a powerful tool in HS analysis due to its nondestructive character, to the velocity of response, and to the access to the low retention zone of the chromatogram since it is solvent free. These techniques are extremely handy from the point of being easy-to-use, economics, and green analytical chemistry through minimizing solvent usage. Nevertheless, as any method, SPME has limitations including the selectivity of each fiber and the competition for linkage sites. Due to the mechanism based on the physicochemical equilibrium of vapor and liquid phases, SPME generates particular partition constants depending on the structures of the compounds. Even if the number of the compounds that can be sampled with this method is only slightly less in comparison with DHS, with SPME, it is possible to analyze more than 130 chemical compounds, most of them belonging to the following chemical classes: aldehydes, alcohols, esters, hydrocarbons, and ketones (Angerosa et al. 2004).

According to Vichi and others (2003) SPME demonstrated higher extraction efficiency for characteristic compounds of virgin olive oil aroma, such as lipid oxidation-derived compounds, particularly alcohols. However, the highest extraction of total terpenoid compounds was obtained with SDE. So, a specific extraction technique could be taken into consideration according to the class of volatile compounds to be determined in vegetable oils.

GC is undoubtedly the method of choice for the separation of a wide range of VOCs in both aqueous and gaseous samples (Huybrechts et al. 2003). Its separation power is extremely useful in combination with MS detection to identify numerous analytes in complex mixtures. For the quantification of VOCs, flame ionization detection (FID) and MS detection are the most common techniques, with the possibility to use both detectors in parallel. In the latter case, MS and FID have the complementary roles of identification and quantification, respectively (Demeestere et al. 2008). The absolute response of an MS detector is less stable in function of time than that of an FID. This is generally known, but how large this instability is and to what extent it affects accurate quantification are poorly documented. A straightforward analytical approach is to use an internal standard that can compensate detector instabilities as a function of time. However, despite the fact that the principle of internal standard addition is well-known, its use for the quantitative analysis of particularly gaseous VOCs by thermal desorption (TD)–GC–MS is scarce. A major bottleneck in VOC analysis is the matrix of the calibration mixture. Indeed, next to the determination of VOCs in liquid samples, the quantification of airborne VOCs by TD–GC–MS is another field of research gaining wide interest. In these cases, calibration is ideally performed with accurate gaseous standard mixtures. Unfortunately, accurate gas standards are expensive and are not easy to generate, particularly in the case of reactive, polar, or less volatile analytes and at low concentrations. For this reason, routine thermal desorption calibration is often carried out by introducing liquid standard solutions to the sampling end of sorbent tubes in a stream of carrier gas.

In the DHS–TD method, the analytes are released by thermal desorption and are transferred to the GC column. If sorbent sampling is used, an additional cryogenic refocusing zone is necessary prior to injection in order to obtain sharp chromatographic peaks. The recent application of sorbent microtraps eliminates the need for cryogenic cooling at the head of the column. Flash heating provides narrow

chromatographic peaks as well as high resolution (Wang and Chen 2001). Purge and trap provides reliable data but is time-consuming and labor intensive, particularly when many samples are involved. In addition, it requires complex instrumentation. Hence, purge and trap is a priori not a technique with online and real-time monitoring capability. Another major drawback of the DHS method is the high amount of water vapor generated at the purge stage and the chromatographic problems associated with it. Excess water vapor causes peak distortion and plugging of the cryo-trap. Water removal has been carried out using hygroscopic membranes (Nafion), cryotrapping, adsorbent trapping, desiccants, or a dry purge stage (Kolb 1999). The development and commercialization of microtraps, being positioned between the sample sorbent and the GC injector, have strengthened the traditional TD–GC–MS. Microtraps can be held at sub-ambient temperatures and are operated fully automatically. Thus, the microtrap technology keeps the low limits of detection advantage of TD while improving the injection through its cost effectiveness and easiness to operate (Demeestere et al. 2008).

TD–GC–MS nowadays most often makes use of split/splitless injection. Therefore, the mass of analyte entering the detector is different from the mass of analyte present on the sorbent tube. Variations of the split ratio affect the mass of analyte entering the detector relative to that loaded on the sorbent material. This particular issue, that is, the difference in mass between what is on the sorbent tube and what is "seen" by the detector, and the effect of the matrix phase (gas or liquid) on this mass ratio, is poorly described in literature. Therefore, the common practice of using liquid calibration mixtures for gaseous samples may be questionable because it supposes that the matrix phase does not affect the split ratio (Demeestere et al. 2008). A bottleneck of VOC quantification by TD–GC–MS deals with complex multianalyte matrices. Samples taken in food, flavor, and fragrances typically contain up to hundreds of VOCs. Apart from the challenge to identify them all correctly, the analyst is confronted with the difficulty having a calibration mixture that contains all of the observed analytes. It is often unpractical and highly expensive or even impossible to obtain standards for all compounds identified (Brankov et al. 1999). In these cases, it is not possible to calculate the response factors of all analytes by conventional quantitative GC–MS methods, and quantification becomes subject to estimation errors. According to Demeestere and others (2008), there is no well-documented approach on how to deal with the quantification of VOCs by TD–GC–MS.

The research for new flavor compounds is an ever-growing field, being continuously influenced by consumer demands. For this reason, the investigation and identification of odor-active compounds, especially key odor notes, in food samples, as also the determination of their relevance and release from the matrix, are important for the characterization of a food (Mamede and Pastore 2006). In this respect, gas chromatography–olfactometry (GC-O) is considered a useful analytical and sensorial tool, with a vast number of investigations carried out on food flavor. The introduction of the GC-O technique was a breakthrough in analytical aroma research and marked the beginning of the discrimination of a multitude of volatiles in odor-active and non-odor-active matrixes. On the basis of the results attained, new flavor creations emerged. Furthermore, many extraction techniques have been developed, in combination, to enhance the quality of the flavor results, even though none of the commonly used extraction methods alone is able to give a complete

reproduction of a flavor's profile. The application of diverse extraction procedures, on an identical matrix, appears to be the best choice, enabling to achieve a more extensive screening. Likewise, this is true for the GC-O methods where the exploitation of different methods may give complementary information on a given matrix. According Ikeda and others (2006), the combined SDE or SPME with GC-O allows fast and careful methods to identify odorants from vegetable oils.

VOLATILE COMPOUNDS IN AVOCADO PULP OIL

According to López and others (2004), the volatile compounds on avocado fresh pulp are surprisingly limited. An experimental design was used by these authors to investigate the effect of microwave time, pH, and avocado leaves on avocado flavor using SPME–GC–MS. In unprocessed avocado puree, six different volatile compounds were found: ethanol, 3-methylbutanol, acetic acid, 3-hydroxy-2-butanone, hexanol, and pentanol. In contrast, the compounds found in processed avocado were aldehydes, alcohols, and ketones, with 2-heptenal [*E*] being the most abundant of these compounds followed by octanal, 1-octen-3-one, and 2-octenal [*E*]. In avocado leaves, the main volatile compounds were identified as estragol, terpenoids, and 2-hexenal. In particular, terpenoids derived from lipid degradation showed an interesting pattern depending on the variety and extracting method. According to Morales and others (1995), 2-hexenal is the most abundant volatile compound in olive oils from different cultivars, accounting for 50–70% of the total volatiles in the HS of such oils, and it contributes positively to the typical and appreciated "green note."

Moreno and others (2003) evaluated the effect of extraction processes on the volatile profile of avocado pulp oil: microwave–squeezing oil, hexane-based oil from avocado pulp dehydrated in a microwave oven, hexane-based oil from avocado pulp dehydrated at 70°C in a vacuum oven, and acetone-based oil from avocado fresh pulp (Table 38.1). As can be observed in Table 38.1, there are four volatile compounds when the oil is extracted by microwave–squeezing. The largest number of volatiles appears when the oil is solvent extracted. This may be due to the effect of both thermal extraction with solvents and a greater decomposition effect on the fatty acids. The samples exposed to microwaves contained terpenoids and aldehydes, such as hexanal (linoleic acid derivative), octanal, and nonanal (oleic acid derivatives). The oil extracted with hexane contained propionic acid, decanol, 2,4-decadienal, and aromatic hydrocarbons such as tridecane and undecane, as well as terpenoids. The largest number of aromatic hydrocarbons was detected in this sample. Meanwhile, the oil extracted with acetone contained mainly terpenoids, aldehydes, and short-chain fatty acids. Some of the terpenoids found were α-bergamotene, α-humulene, α-copaene, α-cubebene, α-farnesane, β-caryophyllene, and β-bisabolene. The authors have concluded that the volatile profile of avocado oil was closely dependent on the extraction procedure and that a greater deterioration of the oils was produced with solvents rather than with microwaves.

The experimental study carried out by Freitas and others (2009) shows the terpenes are the dominant HS compounds in avocado pulp oils measured by SPME–GC–MS. Some of the terpenoids found were α-cubebene, copaene, α-bergamotene,

TABLE 38.1. Volatile Compounds Identified in Avocado Oil

	Extraction Methods			
Compounds	Microwave + Cold-Pressed	Microwave + Hexane	Hexane	Acetone
Hexanal	X	X		
Benzene, 1,4-dimethyl			X	
Benzene, 1,2-dimethyl			X	X
Heptanal		X		
1,2,4-Trimethylbenzene			X	
n-Decane			X	
n-Octanal		X		
Benzene, 4-ethyl-1,2-dimethyl			X	
n-Undecane			X	
Nonanal	X	X		
Benzoic acid 2-hydroxymethylester			X	
n-Dodecane			X	
Decyl aldehyde		X		
Undecane, 2,6-dimethyl			X	
2-Proponoic acid, 2-ethylhexyl ester			X	
trans,*trans*-2,4-Decadienal				X
Tridecane			X	
2,4-Decadyenal		X		
trans,*trans*-2,4-Decadienal (isomer)				X
α-Cubebeno		X		X
2-Docen-1-al		X		
Propanoic acid, 2-methyl-3-hydroxy-2,4,-trimethylpenthyl ester			X	
α-Copaene		X		X
Tetradecane			X	
β-Caryophyllene	X	X		X
trans-α-Bergamotene		X		
α-Bergamotene				X
α-Humulene		X		X
Cyclotetradecane		X		
Germacrene D				X
n-Pentadecane			X	
(E,E)-α-farnesene				X
β-Bisabolene				X
Caryophyllene		X		X
Propanoic acid, 2-methyl1-(1,1-dimethyl)-2-methyl-1,3-propamedyl ester			X	

Extracted from Moreno and others (2003); compounds were identified by SPME–GC–MS.

α-bisabolene, β-bisabolene, β-caryophyllene, and α-caryophyllene. The numbers of peaks were changed between 25 (cold-pressed oil) and 71 (ethanol-based oils). Using the same method, Moreno and others (2003) have identified 4, 12, and 36 volatile compounds, respectively, in the cold-pressed oil, acetone-based oil, and hexane-based oil, and Haiyan and others (2007) have identified 9 and 11 peaks,

respectively, in the volatile compounds present in commercial avocado oils (cold-pressed and refined). So, the ethanol has promoted an increase in the amount of identified volatiles, probably due to thermodynamic effects associated with ethanol polarity. The other noticeable effect of ethanol-based extraction was the appreciable number of hydrocarbons, particularly sesquiterpenes, in avocado oil. Sinyinda and Gramshaw (1998), using GC-MS, has identified a similar terpene profile in the avocado pulp.

The differences between cold-pressed and ethanol-based oils have been found in the nature and proportions of the compounds generated. As can be observed in Table 38.1, the 2,4-decadienal presented a multiple peak in the ethanol-based oil. In addition 2-octenal, responsible by off-flavor, was observed in the ethanol-based oil only. In the literature, 2,4-decadienal and 2-octenal have been reported as two of the numerous aldehydes formed by the free radical oxidation of oils containing linoleic acid (Guillen and Ruiz 2004). Additionally, 2-pentenal and 2-heptenal, the main rancidity indicators in vegetable oils, were not observed in cold-pressed and ethanol-based avocado oils.

Although the avocado oil was affected by ethanol-based extraction, its degradation produced lower proportions of harmful aldehydes and higher proportions of terpenoids than those found in hexane-based oil by Moreno and others (2003). An interesting fact is noteworthy: the absence of nonanal (oleic acid derivative) in ethanol-based oil. According to Morales and others (1994), the early measurement of nonanal (which was not detected at all or only at trace levels, in extra-virgin olive oil samples) could be an appropriate method to detect the beginning of the thermoxidation. The authors have applied a thermoxidation process to develop new insights on the evolution of the volatile compounds responsible for virgin olive oil flavor. The ratio of hexanal/nonanal has been used to differentiate between oxidized and good-quality virgin olive oil samples. Sensory evaluation of the samples and peroxide value agreed on the evolution of the oxidation.

FINAL CONSIDERATIONS

Using the SPME–GC–MS method, Moreno and others (2003) have identified 4, 12, and 36 volatile compounds, respectively, in the cold-pressed, acetone-based, and hexane-based oils, and Haiyan and others (2005) have identified 9 and 11 peaks, respectively, in the volatile compounds present in commercial avocado oils (cold-pressed and refined). Haiyan and others (2005) have identified seven aldehydes (2-butenal, pentanal, pentenal, pentenal isomer, hexanal, heptanal, and nonanal) as major compounds in the volatile profile of commercial avocado refined oil. According to the authors, those compounds have indicated a lipid oxidation and a lower sensorial quality in commercial avocado oils. Hexanal has been reported to be a volatile compound present in avocado pulp, together with octanal and nonanal. The latter compound also indicates that lipid oxidation had taken place. However, the amount of hexanal does not distinguish oxidized oils as this compound originates from both the enzymatic and chemical oxidation pathways (Kalua et al. 2007; Sinyinda and Gramshaw 1998).

Some undesirable volatiles appearing in avocado oil are probably due to the oxidation of polyunsaturated fatty acids during avocado pulp drying with convective

air. According to Moreno and others (2003), this effect can be enhanced by drying the avocado pulp in a microwave oven. Refined oils had a lower total peak area than the corresponding crude or cold-pressed oil. So, the effect of oil refining was to cause a general reduction in the amount of all volatiles.

According to Freitas and others (2009), ethanol seems to be a better alternative than hexane in reducing the oxidation of oleic acid during vegetable oil extraction. Nevertheless, further investigations are needed to evaluate, simultaneously, the agronomic and technological aspects of the volatile compounds found in avocado oil.

REFERENCES

Angerosa F. 2000. Sensory quality of olive oils. In Harwood J, Aparicio R (eds.), *Handbook of Olive Oil: Analysis and Properties*. Gaithersburg, MD: Aspen Publications, pp. 393–458.

Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro GF. 2004. Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality. *J Chromatogr A* 1054(1–2):17–31.

Brankov E, Rao ST, Porter PS. 1999. Identifying pollution source regions using multiply censored data. *Environ Sci Technol* 33(13):2273–7.

D'Acampora Zellner B, Dugo P, Dugo G, Mondello L. 2008. Gas chromatography–olfactometry in food flavor analysis. *J Chromatogr A* 1186(1–2):123–43.

Demeestere K, Dewulf J, De Roo K, De Wispelaere P, Langenhove HV. 2008. Quality control in quantification of volatile organic compounds analysed by thermal desorption–gas chromatography–mass spectrometry. *J Chromatogr A* 1186(1–2):348–57.

Freitas SP, Arruda NP, Gobbi CN, Resende CM. 2009. Volatile compounds in cold-pressed and ethanol-based avocado oil (*Persea americana* Mill.). In *Proceedings of the 8th SLACA- Simpósio Latino Americano de Ciência de Alimentos*. São Paulo: UNICAMP, p. 1.

Freitas SP, Jablonka FH, Lago RCA, Hartman L. 1992. Extraction aqueuse enzimatique de l'huile d'avocat a partir de la pulpe fraîche. *Rev Fr Corps Gras* 41(11–12):365–71.

Freitas SP, Lago RCA, Carneiro FP, Torquilho DF. 2000. Natural antibiotics—A by product in aqueous enzymatic extraction of avocado oil from fresh pulp. In *AOCS Annual Meeting & Expo, 2000, San Diego—California. Inform*, Vol. 11. Champaign, IL: AOCS Press, pp. S126–S126.

Guillen MD, Ruiz A. 2004. Study by means of 1H nuclear magnetic resonance of the oxidation process undergone by edible oils of different natures submitted to microwave action. *Eur J Lipid Sci Technol* 106(10):680–7.

Haiyan Z, Bedgood DR Jr., Bishop AG, Prenzler PD, Robards K. 2007. Endogenous biophenol, fatty acid and volatile profiles of selected oils. *Food Chem* 100(4):1544–51.

Ho C-T, Shahidi F. 2005. Flavor components of fats and oils. In Shahidi F (ed.), *Bailey's Industrial Oil and Fat Products*, 6th Ed. Hoboken, NJ: Wiley, pp. 387–411.

Huybrechts T, Dewulf J, Langenhove HV. 2003. State-of-the-art of gas chromatography-based methods for analysis of anthropogenic volatile organic compounds in estuarine waters, illustrated with the river Scheldt as an example. *J Chromatogr A* 1000(1–2):283–7.

Ikeda G, Tomizawa A, Imayoshi Y, Iwabuchi H, Hinata T, Sagara Y. 2006. Flavor design of sesame-flavored dressing using gas chromatography/olfactometry and food kansei model. *Food Sci Technol Res* 12(4):261–9.

Jelen HH, Obuchowska M, Zawirska-Wojtasiak RE. 2000. Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J Agric Food Chem* 48(6):2360–7.

Kalua CM, Allen MS, Bedgood DR, Bishop AG, Prenzler PD, Robards K. 2007. Olive oil volatile compounds, flavor development and quality: A critical review. *Food Chem* 100(1): 273–86.

Kolb B. 1999. Headspace sampling with capillary columns. *J Chromatogr A* 842(1–2): 163–205.

López MG, Guzmán GR, Dorantes AL. 2004. Solid-phase microextraction and gas chromatography—mass spectrometry of volatile compounds from avocado puree after microwave processing. *J Chromatogr A* 1036(1):87–90.

Mamede MEO, Pastore GM. 2006. Study of methods for the extraction of volatile compounds from fermented grape must. *Food Chem* 96(4):586–90.

Marriott PJ, Shellie R, Cornwell C. 2001. Gas chromatographic technologies for the analysis of essential oils. *J Chromatogr A* 936(1–2):1–22.

Morales MT, Alonso MV, Rios JJ, Aparicio, R. 1995. Virgin olive oil aroma: Relationship between volatile compounds and sensory attributes by chemometrics. *J Agric Food Chem* 43(11):2925–31.

Morales MT, Aparicio R, Rios JJ. 1994. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J Chromatogr A* 668(2):455–62.

Moreno AO, Dorantes L, Galíndez J, Guzmán RI. 2003. Effect of different extraction methods on fatty acids, volatile compounds, and physical and chemical properties of avocado (*Persea americana* Mill.) oil. *J Agric Food Chem* 51(8):2216–21.

Ozdemir F, Topuz A. 2004. Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post-harvesting ripening period. *Food Chem* 86(1):79–83.

Ridolfi M, Terenziani S, Patumi M, Fontanazza G. 2002. Characterization of the lipoxygenases in some olive cultivars and determination of their role in volatile compounds formation. *J Agric Food Chem* 50(4):835–9.

Sanchez J, Salas JJ. 2000. Biogenesis of olive oil aroma. In J Harwood and R Aparicio (eds.), *Handbook of Olive Oil: Analysis and Properties*. Gaithersburg, MD: Aspen Publications.

Sinyinda S, Gramshaw JW. 1998. Volatiles of avocado fruit. *Food Chem* 62(4):483–7.

Velasco J, Dobarganes C. 2002. Oxidative stability of virgin olive oil. *Eur J Lipid Sci Technol* 104(9–10):661–76.

Vichi S, Castellote AI, Pizzale L, Conte LS, Buxaderas S, López-Tamames E. 2003. Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. *J Chromatogr A*, 983(1):19–33.

Wang J-L, Chen W-L. 2001. Construction and validation of automated purge-and-trap–gas chromatography for the determination of volatile organic compounds. *J Chromatogr A* 927(11–12):143–54.

Werman MJ, Neeman I. 1988. Avocado oil production and chemical characteristics. *J Am Oil Chem Soc* 64(2):229–32.

CHAPTER 39

Cabbage Flavor

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INTRODUCTION

Fresh cabbage flavor is more complex than expected. At its best, it can be sweet, fruity, and rich; at its worst, it can be bitter, musty, and flat. Historically, cabbage flavor has been underappreciated, and the primary measures of cabbage quality have been physical characteristics such as size, shape, and density. Recently, flavor has received increased attention as an important measure of cabbage quality. This new focus on cabbage flavor is motivated primarily by a dual desire to secure and extend shares of increasingly competitive markets and to promote the consumption of fresh cabbage, which is a good source of vitamin C and secondary metabolites (i.e., glucosinolates) with human health potential.

Investigations of cabbage flavor have been intermittent, though often very focused, over the past several decades. By the end of the 1970s, glucosinolates, sulfides, sugars, and some alcohols and aldehydes were identified as key determinants of cabbage flavor, with glucosinolates considered the most influential (Buttery et al. 1976; Freeman and Mossadeghi 1973; MacLeod 1976). This early work focused primarily on determining the quantity of known flavor compounds in fresh and blanched cabbage combined with the objective to determine the influence of horticultural practices on flavor. In the early 1980s, several key deficiencies in the literature were identified with regard to our knowledge of cabbage flavor (Fenwick et al. 1983). Up to that point, there had been limited effort to employ sensory panels to link cabbage physical and chemical characteristics with perceived flavor, and there was a general lack of understanding of just what constituted "good" cabbage flavor from the consumers' perspective. Also, chemical concentrations were reported in relative amounts, making it difficult to compare findings among studies.

Subsequent work addressing these gaps in our understanding has confirmed the assumed importance of glucosinolates and their breakdown products directly to bitterness and pungency, and indirectly to perceived sweetness. More importantly, we currently have a better sense of the complexity of cabbage flavor and recognize

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the critical importance of textural and aromatic attributes to consumer acceptance of fresh cabbage. Recent advances in our understanding on how growing conditions affect flavor and flavor components have improved our ability to produce cabbage with desirable flavor characteristics. Despite this progress, significant research gaps regarding cabbage flavor still exist, particularly in determining absolute amounts of flavor components other than glucosinolates and sugars in fresh cabbage, as well as quantifying their relationship to fruity/vegetal flavors and overall consumer desirability.

DESCRIPTORS AND COMPONENTS OF CABBAGE FLAVOR

A list of cabbage flavor descriptors is presented in Table 39.1. Primary importance has traditionally been placed on those attributes that contribute to the strength of cabbage flavor, particularly pungency. Other strength-related descriptors include bitterness and those grouped as off-flavors. The flavor chemistry associated with these descriptors is dominated by glucosinolates, a group of sulfur-containing, amino acid-derived compounds ubiquitous to the *Brassicacea*. Intact glucosinolates can

TABLE 39.1. Reported Descriptors of Cabbage Flavor

Descriptor	Determinant or Measure	Reference
Gustatory		
Bitter	Glucosinolates[a]	Buttery and others (1976), MacLeod (1976), Radovich and others (2003), Van Doorn and others (1998)
Sweet	Sugars, total soluble solids, glucosinolates	MacLeod (1976), Radovich and others (2003), Yano and others (1990)
Olfactory/lachrymatory		
Alliaceous	Glucosinolates, aliphatic aldehydes, sulfides	Buttery and others (1976), MacLeod (1976)
Fruity	pH, aromatic aldehydes	Buttery and others (1976), Martens (1985)
Green/grassy	Aliphatic alcohols and aldehydes	Buttery and others (1976), MacLeod (1976), Radovich and others (2003)
Musty	Glucosinolates, sulfides	Chin and others (1996), Radovich and others (2003)
Pungent/hot/sharp	Glucosinolates	Freeman and Mossadeghi (1973), Radovich and others (2004a)
Sulfurous	Sulfides, glucosinolates	Buttery and others (1976), MacLeod and Nussbaum (1977)
Texture/mouthfeel		
Crisp	% Dry matter	MacLeod (1976)
Firm	Puncture resistance	Yano and others (1990)
Juicy	% Dry matter, dripping rate[b]	MacLeod (1976), Yano and others (1990)

[a]Includes hydrolysis products.
[b]Calculated as liquid (g) collected from 15-g finely shredded cabbage under 15 kg of pressure for 3 min (Yano et al. 1990).

have organoleptic activity; 2-propenyl glucosinolate is perceived as bitter, for example. However, most glucosinolates are enzymatically hydrolyzed upon tissue disruption (e.g., chewing) to produce aglucones that are generally much more reactive than their parent compounds. The details of this reaction are described elsewhere in several excellent reviews (Fahey et al. 2001; Fenwick et al. 1983; Rosa et al. 1997) and will not be addressed here. However, it is important to note that the conformation and corresponding organoleptic activity of glucosinolate products are dependent on hydrolysis conditions such as pH and the presence of cofactors. These are discussed here as appropriate. Because of their importance to crop ecology and human health as well as flavor, more information is available on glucosinolate concentrations than on other flavor components in cabbage. The primary challenge to the reviewer is reconciling the various units used to report glucosinolate concentrations in the literature. In discussing glucosinolate concentrations here, units have been standardized to millimoles per kilogram using the appropriate molecular weights. Glucosinolate concentrations in the literature are reported as both fresh and dry weights. In standardizing units, no attempt was made to adjust for moisture content since dry matter concentration in cabbage can vary significantly (see below). Concentrations are noted here as either on a fresh or dry weight basis as reported in the original work.

While flavor strength certainly impacts acceptance of fresh cabbage, the relatively weak link between the strength of cabbage flavor and consumer desirability has led to the speculation that aldehydes and alcohols underlying perceptions of fruity and vegetal flavors have been significantly overlooked in investigations of cabbage flavor (Martens 1985; Radovich 2003; Yano et al. 1990). In addition, textural components such as juiciness, firmness, and crispness have been identified as important variables that can contribute significantly to perception and consumer acceptance of cabbage flavor (Martens 1985; Yano et al. 1990).

Pungency

Pungency, reminiscent of mild horseradish or black mustard, is a common characteristic of fresh cabbage. Pungency in cabbage is sometimes alternately described as "hot" or "sharp" flavors (Chin et al. 1996). Pungency has been previously assumed to contribute positively to the overall consumer acceptance of cabbage flavor, and pungency scores by taste panels have been shown to be at least weakly correlated with corresponding desirability scores (MacLeod 1976; Radovich et al. 2003). In addition, the lack of pungency in cabbage is associated with "flat" flavor (Rosa et al. 1997). However, despite the positive contribution of pungency to the overall perception of flavor, even low levels of pungency may be unacceptable in some markets. Specifically, coleslaw manufacturers in North America have invested considerable efforts toward minimizing hotness in cabbage in order to maximize consistency in the flavor of their dressed product. These efforts have focused primarily on identifying low glucosinolate varieties and on managing cabbage production fields to minimize glucosinolate levels in heads grown for the fresh market (Ball et al. 1999; Radovich et al. 2004a).

Allyl isothiocyanate (AITC), a hydrolysis product of allyl glucosinolate (sinigrin), is the primary chemical influencing pungency in cabbage. Using a 3-point intensity scale and 20 panelists, Yano and others (1987) reported an increase in the perceived

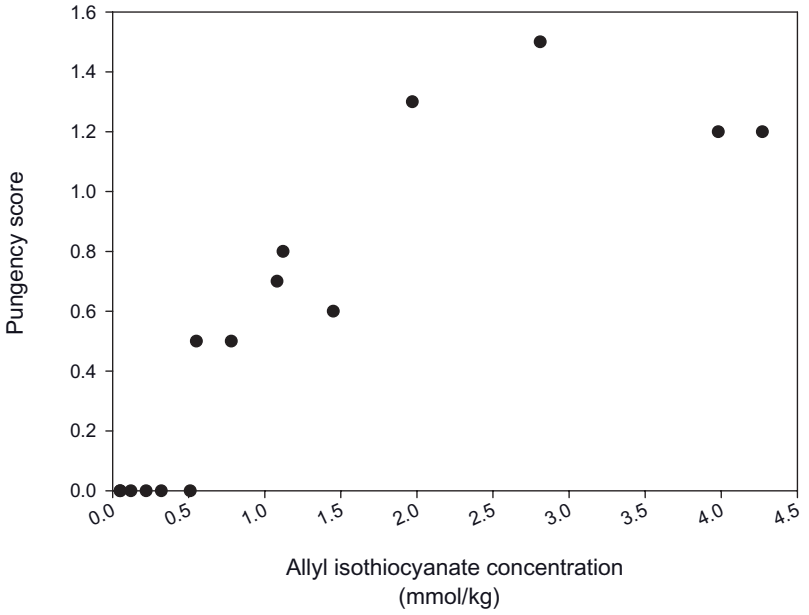

Figure 39.1. Relationship between allyl isothiocyante (AITC) concentrations and consumer perception of pungency in raw cabbage. Pungency scores represent mean values from 20 panelists. *Source*: Yano and others (1987).

pungency of cabbage with increasing AITC concentrations in the range of 0.5–2.0 mmol/kg, on a fresh weight basis (Fig. 39.1). Pungency was not perceived when cabbage tissue AITC concentrations were below 0.5 mmol/kg, and panelists could not detect differences among cabbage cultivars when AITC concentrations exceeded 2.0 mmol/kg. The parent glucosinolate of AITC, sinigrin, usually represents a considerable proportion (~30%) of the total amount of glucosinolates in fresh cabbage, and Ball and others (1999) reported a strong ($r^2 = 0.95$), positive correlation between total glucosinolate and sinigrin concentrations among 12 cabbage cultivars. Therefore, total glucosinolate levels measured relatively easily using the colorimetric method may be a rapid assay for pungency in fresh cabbage. In fact, a positive relationship has been reported between total glucosinolates and perceived pungency among cabbage varieties when total glucosinolate concentrations were <18 mmol/kg on a dry weight basis (Radovich et al. 2004b). The inability of panelists to perceive pungency differences via tasting at high AITC or glucosinolate concentrations may be attributed in part to sensory fatigue.

Other glucosinolates have been suggested to contribute significantly to horseradish, watercress, radish, and acrid notes in cabbage, particularly in the headspace above cooked cabbage. These include 2-phenylethyl (gluconasturtiin), 3-methylthiopropyl (glucoibervirin), and 4-methylthiobutyl (glucoerucin) glucosinolates (Buttery et al. 1976; Fenwick et al. 1983). Although found in very low concentrations and not always detected (Radovich et al. 2005; Rosa et al. 1997; VanEtten et al. 1976), the low odor thresholds of these compounds relative to AITC suggest that they should not be ignored as potentially important in the perception of pungency (Buttery et al. 1976).

Bitterness

Bitterness is negatively associated with consumer preference, although it generally is of less importance in raw cabbage than other flavor characteristics such as sweetness, pungency, texture, and aromatic components (MacLeod 1976; Radovich et al. 2003; Van Doorn et al. 1998). The two primary bitter components in cabbage are the intact glucosinolate sinigrin and 5-vinyl-oxazolidine-2-thione (goitrin), a hydrolysis product of 2-hydroxy-3-butenyl glucosinolate (progoitrin). Although both compounds influence the perception of bitterness, goitrin is probably most important in fresh cabbage because sinigrin would be largely hydrolyzed to the pungent compound AITC upon chewing. However, cooking would denature the enzyme responsible for cleaving the glucose moiety from the parent glucosinolate, and sinigrin would therefore be expected to play a larger role in the perception of bitterness in cooked cabbage. Although no work has been done to quantify the relationship between goitrin or sinigrin concentrations and perception of bitterness in cabbage, the compounds have a strong, positive, and linear relationship with bitterness in brussels sprouts. Van Doorn and others (1998) found trained panelist perception of bitterness in cooked brussels sprouts to increase with combined sinigrin and progoitrin concentrations in uncooked sprouts within the range of 1.2–5.1 mmol/kg on a fresh weight basis. This range exceeds the combined sinigrin and progoitrin concentrations of ≤1.2 mmol/kg generally reported for cabbage (Radovich et al. 2005; Rosa et al. 1998; VanEtten et al. 1976).

Off-Flavors

Descriptors generally grouped together as off-flavors in cabbage include "sulfurous," "earthy," and "musty" (Martens 1985; Radovich et al. 2003; Yano et al. 1990). Although sulfides and glucosinolates undoubtedly contribute greatly to these notes (Fenwick et al. 1983), very little work has been done to quantify the relationship between the content of these compounds and the perceived off-flavor in cabbage. 1-Cyano-2,3-epithiopropane, produced when sinigrin is hydrolyzed in the presence of an epispecifier protein, may contribute musty, sulfurous notes in cabbage (Chin et al. 1996). 2-Propenyl nitrile, another potential hydrolysis product of sinigrin, can also contribute to objectionable aromas (MacLeod 1976; Srisangnam et al. 1980).

Sweetness

Sugar (primarily glucose, fructose, and sucrose) can comprise as much as 20–40% of the total dry matter content in the edible portion of cabbage heads (Radovich et al. 2005; Rosa et al. 2001). Sweetness is considered a positive attribute of fresh cabbage flavor, and the relationship between sweetness scores and sensory quality in cabbage can be stronger than pungency and bitterness (Martens 1985; Padilla et al. 2007; Radovich et al. 2003). However, no work has been done to quantify the relationship between sugar levels and the perception of sweetness. Also, the perception of sweetness is at least partially dependent on cabbage flavor strength, and high sugar concentrations may be masked by high glucosinolate concentrations.

Fruity and Vegetal Flavors

Although bitterness, pungency, and sweetness are dominant components of fresh cabbage flavor, consumer preference has only been weakly correlated with intensity scores or concentrations of the chemicals that influence these traits. AITC and sugar levels, expected to influence sweetness and pungency, were poorly correlated with flavor desirability scores in Japan (Yano et al. 1990). In the United States, the descriptors "sweet," "hot," and "bitter" explained only 13% of the variability in taste scores, suggesting the potential for a strong aromatic component to fresh cabbage flavor (Radovich et al. 2003). "Fruity" was identified as the most important descriptor in explaining variability in fresh cabbage sensory quality among samples from different years and locations, exceeding "sweet," "bitter/sharp," "total strength," "sulfurous," and "earthy" in importance (Martens 1985).

Important contributors to fruity and grassy/green flavors likely include 2-hexenal (*trans*), 3,1-hexenol (*cis*), 2,4-heptadienal (*trans*, *trans*), 2,4-decadienal (*trans*, *trans*), benzaldehyde, and phenylacetaldehyde (Buttery et al. 1976; MacLeod 1976). While sulfides contribute sulfurous aromas that may be considered undesirable, they are thought to contribute positively to the "typical cabbage" flavor that may include alliaceous (i.e., onion and garlic) notes. Dominant sulfides in cabbage include dimethyl sulfide, butylmethyl sulfide, dipropyl sulfide, dimethyl disulfide, methylbutyl disulfide, methylpropyl disulfide, dimethyl trisulfide, and 2,4,5-trithiahexane (Buttery et al. 1976; MacLeod and Nussbaum 1977; Shankaranarayana et al. 1974). Unfortunately, there has been little work to characterize the absolute amounts of relevant alcohols, aldehydes, or sulfides in cabbage, and no efforts to relate these compounds with fruity or vegetal descriptors have been reported.

Texture

The influence of texture on the perception of cabbage flavor has been largely underestimated until recently. It is important because it may explain flavor differences in cabbage not explained by chemical components and because it may be the dominant factor determining consumer preference in some markets. Employing a trained panel and comprehensive statistical analysis, Martens (1985) found the descriptive terms juiciness, chewing resistance, and crispness to be more important than sweetness, bitterness, or flavor strength in explaining variability in fresh cabbage quality among samples from different years and locations. Consumer preference of fresh shredded cabbage is positively related to juiciness scores (Yano et al. 1990). Dry matter content (%DM) is related to textural components; chewing resistance (a negative attribute) increases, while juiciness and crispness (positive attributes) decrease with increased %DM (Martens 1985; Yano et al. 1990). %DM in fresh market cabbage generally ranges from 6% to 12% depending on variety, climate, and irrigation regime (Kleinhenz and Wszelaki 2003; Radovich et al. 2005). Threshold levels of %DM for the perception of textural differences have not been conclusively determined, although evidence suggests that DM values of 6–8% may correspond with ideal textural quality (Radovich et al. 2004a, 2005). Yano (1990) reported a strong correlation between the perception of juiciness and dripping rate, calculated as liquid (gram) collected from 15-g finely shredded cabbage under 15 kg of pressure for 3 min. Texture desirability was positively and linearly correlated with dripping

rate ranging from 0.5 to 1.5 g/15 g. These authors found dripping rate to be more strongly associated with panelist perception of texture than puncture resistance of mesophyll.

FACTORS AFFECTING CABBAGE FLAVOR

Genotype

There is a large genetic influence on cabbage flavor that is reflected in differences in sensory characteristics among cabbage genotypes (Padilla et al. 2007; Radovich et al. 2003; Yano et al. 1990). This is due to the genetic variability in concentrations of compounds associated with flavor in cabbage. Variability in concentrations of glucosinolates, DM, and sugars have all been well documented (Kleinhenz and Wzselaki 2003; Radovich et al. 2004b; Rosa et al. 1996; VanEtten et al. 1976; Wszelaki and Kleinhenz 2003), and there is undoubtedly genetic variability to be found in the other less studied flavor components of cabbage as well. Although genotype selection is often restricted to a relatively small pool of cultivars that are well adapted to the growing conditions of the target production area, there may still be opportunity for selecting cultivars with acceptable agronomic traits that also have relatively high flavor quality. This is probably most practically done by incorporating informal tasting by a few, experienced evaluators into existing cultivar trials. If the number of genotypes to be screened for flavor is large, chemical and physical determinants of flavor such as glucosinolate concentrations and %DM may have potential as screening tools for specific characteristics such as pungency or texture (see above).

Growing Environment

As with other crops, the flavor of cabbage is strongly influenced by a growing environment. Because the list of biotic and abiotic environmental factors that may influence cabbage flavor is rather long, it is perhaps most useful to frame the discussion in terms of plant stress. Conditions that induce stress (e.g., high air temperatures, soil moisture deficit, close plant spacing) generally have the greatest impact on cabbage flavor, particularly when stress occurs during head development.

Planting Date Reports that planting date impacts the levels of glucosinolates and sugars in fresh cabbage have long supported the hypothesis that manipulating planting date can influence cabbage flavor, and this has recently been confirmed with direct measures of planting date effects on flavor (Padilla et al. 2007). Cabbage planted in the summer and harvested in the fall tends to be lower in glucosinolates, higher in sugars, and is perceived to be sweeter and has better flavor than spring-planted cabbage harvested in the summer (Bible et al. 1980; Padilla et al. 2007; Radovich et al. 2004b; Rosa et al. 1997, 2001). Supraoptimal air temperatures experienced during head development of summer-harvested cabbage is likely a primary contributing factor to this phenomenon. Altering planting date to optimize flavor is not a practical option for growers, nor is reducing head temperatures in the field generally feasible. However, glucosinolate concentrations and pungency in fresh

cabbage may be minimized via irrigation during periods of high air temperatures, as first suggested by Bible and others (1980). These authors reported that plant water availability affects the response of cabbage glucosinolate levels to planting date and that no planting date effect was observed in plots receiving consistent irrigation.

Irrigation Cabbage is generally drought tolerant and is usually only minimally irrigated after seedling establishment. However, mitigating periods of drought stress with supplemental irrigation can influence cabbage flavor. Cabbage grown under water stress conditions exhibited a fourfold increase in AITC concentrations over cabbage grown with regular irrigation, and this corresponded with differences in flavor as measured through triangle testing (Freeman and Mossadeghi 1973). Mitigation of low moisture stress via drip irrigation during head development has recently been shown to increase fructose and glucose concentrations and to reduce glucosinolate concentrations in cabbage heads (Radovich et al. 2005). Panelists were also able to detect flavor differences between irrigated and unirrigated cabbages in triangle tests (Radovich et al. 2004a). It is suggested that replacing at least 50% of the crop evapotranspiration (Et_c) lost throughout head development may be adequate to mitigate excess pungency and to maximize the perception of sweetness in fresh cabbage (Radovich et al. 2005).

Other Factors Several other factors in the production field can influence flavor and flavor components in cabbage, particularly glucosinolate concentrations. Most notably, glucosinolate concentrations generally decrease with increasing tissue maturity. This results in greater flavor strength in younger leaves near the core of cabbage heads (MacLeod 1976). Also, as heads expand and mature in the field, the proportion of older leaves to younger leaves increases and the total level of glucosinolates in heads generally declines (Rosa et al. 1997). Suojala and others (1999) report that delaying harvest for 3–4 weeks increased sweetness and reduced off-flavors and bitterness in cabbage harvested in the fall.

Plant nutrition and spacing also influence the flavor chemistry of *Brassica* vegetables, although no work has been done to measure the effect on flavor directly. Rosa and others (1998) provide a comprehensive review of the effect of plant nutrition on glucosinolate concentrations. The role of sulfur nutrition is particularly important, given the dependency of cabbage flavor on sulfur-containing compounds. Many soils, particularly heavy soils, contain adequate sulfur so that glucosinolate production is not limited. However, excessive nitrogen may reduce glucosinolate concentrations under low to moderate levels of soil sulfur. Close plant spacing can increase glucosinolate concentrations in cabbage, probably as a result of increased intraspecific competition (MacLeod and Nussbaum 1977).

Storage

Cabbage heads are generally stored at 0–1°C under high humidity (>85%), and there may be some decrease in sensory quality when heads are stored under these conditions for several months. In particular, atypical flavors and mustiness may develop, and some decrease in textural quality has also been reported (Hansen 1979;

Mihov 2001; Suojala et al. 1999). The development of off-flavors appears to be exacerbated in the presence of high atmospheric CO_2 concentrations (>4%), and ventilation in the storage unit may help preserve sensory quality in storage (Hansen 1979). There is variability among genotypes in the stability of sensory quality during storage, with some cultivars reportedly maintaining quality during periods of relatively long storage (Mihov 2001; Suojala et al. 1999).

Cooking

Cooking influences cabbage flavor, primarily by reducing the potential for enzymatic degradation of glucosinolates. This is particularly important in the case of sinigrin, which, rather than hydrolyzing to AITC, either persists as the bitter parent compound or converts nonenzymatically to 2-propenyl nitrile to produce objectionable odors (MacLeod 1976; Srisangnam et al. 1980). Blanching cabbage for less than 4 min results in the preservation of some flavors characteristic of fresh cabbage, while increasing cooking time to 10 min greatly reduces cabbage flavor.

REFERENCES

Ball S, Skog L, Smith RB, Murr D, McKeown A. 1999. Reducing pungency in commercial coleslaw by controlling glucosinolate concentrations. Project Report 648, Department of Plant Agriculture, University of Guelph, Ontario.

Bible B, Ju H, Chong C. 1980. Influence of cultivar, season, irrigation and date of planting on thiocyanate ion of cabbages. *J Amer Soc Hort Sci* 105:88–91.

Buttery RG, Guadangi DG, Ling LC, Seifert RM, Lipton W. 1976. Additional volatile components of cabbage, broccoli and cauliflower. *J Agric Food Chem* 24:829–32.

Chin HW, Zeng Q, Lindsay RC. 1996. Occurrence and flavor properties of sinigrin hydrolysis products in fresh cabbage. *J Food Sci* 6:101–4.

Fahey JW, Zalcmann AT, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56:5–51.

Fenwick GR, Heaney RK, Mullin WJ. 1983. Glucosinolates and their breakdown products in food and food plants. *CRC Crit Rev Food Sci Nutr* 18:123–201.

Freeman GG, Mossadeghi N. 1973. Studies on the relationship between water regime and flavour strength in watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek), cabbage (*Brassica oleracea capitata*) and onion (*Allium cepa*). *J Hortic Sci* 48:365–78.

Hansen H. 1979. Organoleptic evaluation of white cabbage stored in commercial storage rooms at different levels of CO_2. *Acta Hortic* 93:29–34.

Kleinhenz MD, Wszelaki A. 2003. Yield and relationships among head traits in cabbage (*Brassica oleracea* L., Capitata group) as influenced by planting date and cultivar. I. Fresh market. *HortScience* 38:1349–54.

MacLeod AJ. 1976. Volatile flavor components of the Cruciferae. In Vaughan JG, MacLeod AJ, Jones BMG (eds.), *The Biology and Chemistry of the Cruciferae*. London: Academic Press, pp. 307–30.

MacLeod AJ, Nussbaum ML. 1977. The effect of different horticultural practices on the chemical flavor composition of some cabbage cultivars. *Phytochemistry* 16:861–5.

Martens M. 1985. Sensory and chemical quality criteria for white cabbage studied by multivariate analysis. *Lebensm Wiss Technol* 18:100–4.

Mihov K. 2001. Changes in quality of white cabbage (*Brassica oleracea* var. *capitata* L.) under storage. *Bulg J Agri Sci* 7:427–34.

Padilla G, Cartea ME, Soengas P, Ordas A. 2007. Characterization of fall and spring plantings of Galician cabbage germplasm for agronomic, nutritional, and sensory traits. *Euphytica* 154:63–74.

Radovich TJK, Kleinhenz MD, Delwiche JF, Liggett RE. 2004a. Triangle tests indicate that irrigation timing affects fresh cabbage sensory quality. *Food Qual Prefer* 15:471–6.

Radovich TJK, Kleinhenz MD, Streeter JG, Miller AR, Scheerens JC. 2004b. Planting date affects total glucosinolate concentrations in six commercial cultivars of cabbage (*Brassica olereacea* L., Capitata group). *HortScience* 40:106–10.

Radovich TJK, Kleinhenz MD, Sanchez-Vela A, Scheerens JC, Schult B. 2003. Fresh cabbage sensory quality: Components and the impact of production factors. *Acta Hortic* 628:787–95.

Radovich TJK, Kleinhenz MD, Streeter JG. 2005. Irrigation timing relative to head development influences yield components, sugar levels, and glucosinolate concentrations in cabbage. *J Am Soc Hortic Sci* 130:943–9.

Rosa E, David M, Gomes MH. 2001. Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. *J Sci Food Agric* 81:1145–9.

Rosa EAS, Heaney RK, Fenwick GR, Portas CAM. 1997. Glucosinolates in crop plants. *Hortic Rev* 19:99–215.

Rosa EAS, Heaney RK, Portas CAM, Fenwick GR. 1996. Changes in glucosinolate concentrations in *Brassica* crops (*B. oleracea* and *B. napus*) throughout growing seasons. *J Sci Food Agric* 71:237–44.

Shankaranarayana ML, Raghavan B, Abraham KO, Natarajan CP. 1974. Volatile sulphur compounds in food flavors. *CRC Crit Rev Food Technol* 4:395.

Srisangnam C, Salunke DK, Reddy NR, Dull GG. 1980. Quality of cabbage II. Physical, chemical and biochemical modification in processing treatments to improve flavor of blanched cabbage (*Brassica oleracea* L.). *J Food Qual* 3:233.

Suojala T, Pessala R, Heinio RL. 1999. Effect of harvest time on the sensory quality of white cabbage. Agri-Food Quality II: Quality Management of Fruits and Vegetables. *J Sci Food Agric* 80(10):238–41.

Van Doorn HE, van der Kruk GC, van Holst GJ, Raaijmakers-Ruijs NCME, Postma E, Groeneweg B, Jongen WHF. 1998. The glucosinolates sinigrin and progoitrin are important determinants for taste preference and bitterness of brussels sprouts. *J Sci Food Agric* 78:30–8.

VanEtten CH, Daxenbichler ME, Williams PH, Kwolek WF. 1976. Glucosinolates and derived products in cruciferous vegetables. Analysis of the edible part from twenty-two varieties of cabbage. *J Agric Food Chem* 24:452–5.

Wszelaki A, Kleinhenz MD. 2003. Yield and relationships among head traits in cabbage (*Brassica oleracea* L., Capitata group) as influenced by planting date and cultivar. II. Processing. *HortScience* 38:1355–9.

Yano M, Itoh H, Saijo R. 1987. Allyl isothiocyanate and its contribution to pungency in cabbage. *J Food Sci Technol (Japan)* 34:608–11.

Yano M, Saijo R, Sugawara W, Ohta H. 1990. Influence of physical and chemical properties on consumer preference of shredded cabbage. *Nippon Shokuhin Kogyo Gakkaishi* 37:478–83.

CHAPTER 40

Carrot Flavor

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INTRODUCTION

Carrots are among the top 10 most important vegetable crops in terms of area devoted to its production and tonnage of crop production (Simon and Goldman 2007). According to the United Nations' Food and Agriculture Organization (FAO) as cited by Lucier and Biing (2007), three countries produced nearly half of the world's carrots during 2003–2005. The United States was the third leading producer of all carrots, just behind Russia, with each producing about 7% of the world output. Both distantly followed China, which produced 34% of the world's carrots. FAO data indicate that worldwide carrot production averaged 24 million metric tons during 2003–2005—up 51% from 1993–1995. Much of the world increase over the past decade was due to a nearly threefold increase in China's output.

Although carrots are believed to be originally from subtropical countries, nowadays, carrots are grown and consumed by people worldwide. Carrots are one of the most popular vegetables in the United States and fresh-market carrot consumption has been increasing over the past few decades (Lucier and Biing 2007). According to the United States Department of Agriculture (USDA), carrots are the fifth most consumed vegetable in the country; the average American consumed 11.63 lb of carrots in 2005 (Lucier and Biing 2007), increased from 9.5 lb in 2003 (Corselius 2007). This indicates that carrots are one of the dietary staples in the United States. Nevertheless, carrot is also now a dietary staple in many Asian countries like China and Indonesia. The uniqueness of its flavor and its health benefits perhaps are factors related to its worldwide acceptance.

Carrots are truly one of nature's wonder foods. Not only are they universally relished for their delicious flavor and satisfying crunch but they also provide a wide range of health benefits (Peters 2006). Carrots are valuable for their taste, good digestibility, and high contents of provitamin A, other carotenoids, and fibers. Both epidemiological and nutritional studies have pointed out its positive impact on human health (Kjellenberg 2007; Sulaeman et al. 2002a). Increased health awareness

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has increased the consumption of fruits and vegetables, including carrots, which are one of the favorite choices.

Many varieties have been developed to fulfill the consumer demand for certain characteristics of carrot products according to their intended use. For example, for juice purposes, consumers want a nice and appealing color, sweet taste, and good aroma. In Indonesia, imported carrot from Australia and China has become more popular and the import volume is continuously increasing despite increased domestic production, due to those desirable attributes. It is said that a good carrot is a flavorful carrot, and variety selection, more than anything else, is the key to tasty carrots.

According to the World Carrot Museum (http://www.carrotmuseum.com/), there are two main types of cultivated carrots: (1) Eastern/Asiatic carrots, which are often called anthocyanin carrots because of their purple roots, although some have yellow roots, and (2) Western or carotene carrots, which have orange, red, or white roots. According to Peters (2006), carrot varieties grown in the United States can be grouped into four major categories according to their shape and length:

1. *Nantes.* These roots are medium long (5–8 in.), slender (~1.25 in.), and cylindrical with rounded tips. Good examples include Scarlet Nantes and Nantes Coreless.
2. *Chantenay.* These roots are short to medium long (3–6 in.) with broad shoulders (~2 in.) and taper to a blunt tip. Good examples include Red Core Chantenay and Kurota Chantenay.
3. *Imperator.* These roots are long (8–14 in.) with slender shoulders (~1.5 in.) that taper slightly to a pointed tip. A good example is the Japanese Imperial Long.
4. *Danvers.* These roots are medium long (6–10 in.) with broad shoulders (~2 in.) that taper to a distinctly pointed tip. Good examples are Scarlet Keeper and St. Valery.

Perhaps a more important criterion when choosing a carrot variety is the intended use (Peters 2006). The primary uses of carrot would be for (1) fresh market, (2) cooking, (3) juicing, and (4) storage. Fresh-market carrots are crunchy and sweet with an exceptional carrot flavor when consumed raw. The Nantes and Chantenay types are the best for this use. Most varieties are suitable for cooking, which makes even somewhat bitter carrots palatable. Danvers types are best for cooking and their broad-shouldered roots are perfect cut into disks in a stew. The best juicing carrots are high in water content and lower in sugar than other types. High-sugar carrots tend to be too sweet as a juice. A great juicing variety is the Danvers-type Scarlet Keeper. Mature, topped carrots can be kept in storage without rotting for 4–5 months. Danvers and Chantenay types are the best for this purpose, with Scarlet Keeper and Red Core Chantenay being particularly good. Most of the commercial carrots are Imperator types because they have high fiber and hold up very well in the field, although their flavor is generally not as sweet as the other types (Peters 2006).

It is believed that the secret of carrot flavor comes from many components contained in the carrot roots, which differ among varieties. This chapter will discuss the uniqueness of carrot flavor, the compounds responsible for the flavor, as well as the effects of handling and processing on the carrot flavor.

THE UNIQUENESS OF CARROT FLAVOR

Sensory Attributes of Carrots

The flavor of carrot contributes to the flavor of many food products. Examples include soups, vegetable salads, pizzas, chips, cakes, and breads. Depending on the variety, in general, carrots have a pleasant flavor. No wonder many recipes and diets include carrot as one of their ingredients. Its unique and pleasant flavor makes the carrot a first vegetable introduced to babies to build their acceptance of various foods. Gerrish and Mennella (2001) reported that carrot puree enhances food acceptance in formula-fed infants.

Carrot flavor is unique and rich. Speaking about carrot flavor, we may have several words to express what we feel and taste. Some of them are piney, woody, terpy, succulent, grassy, brassy, earthy, tender, juicy, soapy, bitter, and sweet. Perhaps, due to the complexity of carrot flavor, the number and terminology of attributes judged during sensory evaluation was different among the researchers.

Baardseth and others (1995) measured 13 attributes to evaluate the sensory qualities of raw carrot: whiteness, color hue (yellow/red), color strength, intensity of taste, sweet taste, fruity taste, acid taste, bitter taste, "earthy taste," juiciness, crispness, firmness, and aftertaste. Meanwhile, Alasalvar and others (2001) used only nine attributes, that is, cut carrot foliage, sweetness, oiliness, fruitiness, bitterness, soapiness, petrol, woodiness, and aftertaste. Rosenfeld and others (2002) evaluated more attributes (17) of raw carrot, that is, intensity of odor, terpene odor, green odor, whiteness, hue, color strength, intensity of taste, terpene flavor, green flavor, sweet taste, acidic taste, bitter taste, earthy flavor, firmness, crispness, juiciness, and aftertaste. Szymczak and others (2007) also used 13 different descriptors to describe the sensory quality of raw carrot from a different genotype: odor of raw carrot, sweet odor, off-odor, color of the outer part of the roots, color of the inner part of the roots, flesh firmness, flesh juiciness, flesh crunchiness, flavor of raw carrot, sweet taste, sour taste, bitter taste, and off-flavor. Berger and others (2008) used nine different descriptors to measure the sensory properties of raw carrot: aromatic odor, carrot-like odor, butter-like odor, aromatic taste, carrot-like taste, sweet taste, tender texture, fibrous texture, and juicy texture.

The above-mentioned attributes are all for raw carrots. For cooked and processed carrots, more attributes are evaluated depending on the type of processing. For example, Baardseth and others (1995) measured 22 attributes for assessing the sensory quality of carrot chips: the intensity of odor, sweet odor, acid odor, sickly sweet odor, burnt odor, whiteness, color hue, color strength, uniform color, same size and shape, hat formation, blister formation, intensity of taste, sweet taste, sickly sweet taste, bitter taste, salty taste, burnt taste, firmness, crispness, fatness, and aftertaste.

Evaluating the Sensory Attributes of Carrots

To evaluate the sensory attributes of carrot, a sensory analysis should be performed in a sensory laboratory equipped according to International Organization for Standardization (ISO) standard. A trained panel, consisting at least 12 persons, should be selected and trained according to ISO standard. Several techniques may

TABLE 40.1. Quantitative Quality Descriptors Utilized for Carrot Cultivars

Descriptor	Anchoring Points	Definition
Odor of raw carrot	None—very intensive	Raw carrot odor characteristic
Sweet odor	None—very intensive	Pleasant, sweet aroma
Off-odor	None—very intensive	Unusual odor for carrot
Color of outer part of carrot	Bright—dark color	Visual evaluation of brightness of flesh carrot
Color of inner part of carrot	Bright—dark color	Visual evaluation of brightness of flesh carrot
Flesh firmness	Firm—soft	Degree of force to chew carrot
Flesh juiciness	Not juicy—very juicy	Amount of liquid released when chewing sample
Flesh crunchiness	Not crunchy—very crunchy	Mouth feel of carrot crunchiness
Flavor of raw carrot	None—very intensive	Characteristic fresh raw carrot flavor
Sweet taste	None—very intensive	Basic sweet taste
Sour taste	None—very intensive	Basic sour taste
Bitter taste	None—very intensive	Basic bitter taste
Off-flavor	None—very intensive	Unusual flavor for carrot
Overall quality impression	Low—high quality	Score for general sensory quality

Source: Adapted from Szymczak and others (2007).

be used for sensory evaluation; however, for most attributes previously mentioned, a quantitative descriptive analysis (QDA) is preferred. In this procedure, the panelist should understand the definition of each attribute and how to measure it. An example of descriptors, definition, and anchoring points for those descriptors is shown in Table 40.1.

In spite of many terms used to evaluate the sensory properties of carrots, there are at least three to four attributes related to flavor commonly observed: sweetness, bitterness, and odor, and texture, which will be discussed more below.

Factors Affecting Carrot Flavor

Carrot flavor is quite complicated and is affected by many compounds contained in the carrot roots. Carrots contain hundreds of terpenoids, which are volatile flavor compounds, and nonvolatile compounds such as sugar, amino acids, and phenolic substances. Many studies as reviewed by Howard and others (1995) indicated that raw carrot flavor is affected by free sugar content, volatile constituents, bitter compounds, and free amino acids. Harsh turpentine flavor is associated with elevated levels of terpenoids and reduced sugar content, while sweetness and overall preference are related to high sugar content and reduced volatile components.

Different cultivars have different and unique flavors, and this is related with its chemical composition. For example, Nantes-type carrots contain few terpenoids but lots of sugar and are famous for being sweet, juicy, and tender (Poncavage 1998). At the other extreme of carrot flavor, weighing in on the heavy side, are Imperator-type carrots; they are more strongly flavored because they contain more terpenoids.

Nantes-type carrots run the risk of being watery and bland, in spite of their sweetness, while Imperators can be harsh and bitter. Combining the sugar of a Nantes with just a bit of the stronger flavors we will get a rich, sweet, creamy carrot with a classic taste (Poncavage 1998).

Carrot flavor is believed to be influenced by genetics, and therefore, the selection of variety is the most important factor to consider when expecting carrots that fit the intended use. However, other factors such as location, climate (temperature and light), soil type, cultivation, and processing also contribute to flavor development.

A study by Baardseth and others (1995) noted differences in sensory properties in carrot cultivated from the north to south of Norway due to generic variations. The Meridia variety was whiter, fruitier, acid, juicy, and crisp, and had a less bitter and earthy aftertaste than Fontana, Newburg, and Nandrin varieties. The variety Newburg, on the other hand, had more hue (most red), color strength, intensive taste, and sweetness than the others. Carrots cultivated at different sites, also showed differences in sensory properties.

Furthermore, Alasalvar and others (2001) studied the sensory profile of raw carrots of four different colored carrots. They found significant differences for some of the flavor attributes, including sweetness (Fig. 40.1). The purple carrot got the highest score, and orange and white genotypes had higher levels of terpenes than the other genotypes. They found no significant differences for bitterness and aftertaste. "Bolero," the most popular commercial cultivar on the Danish

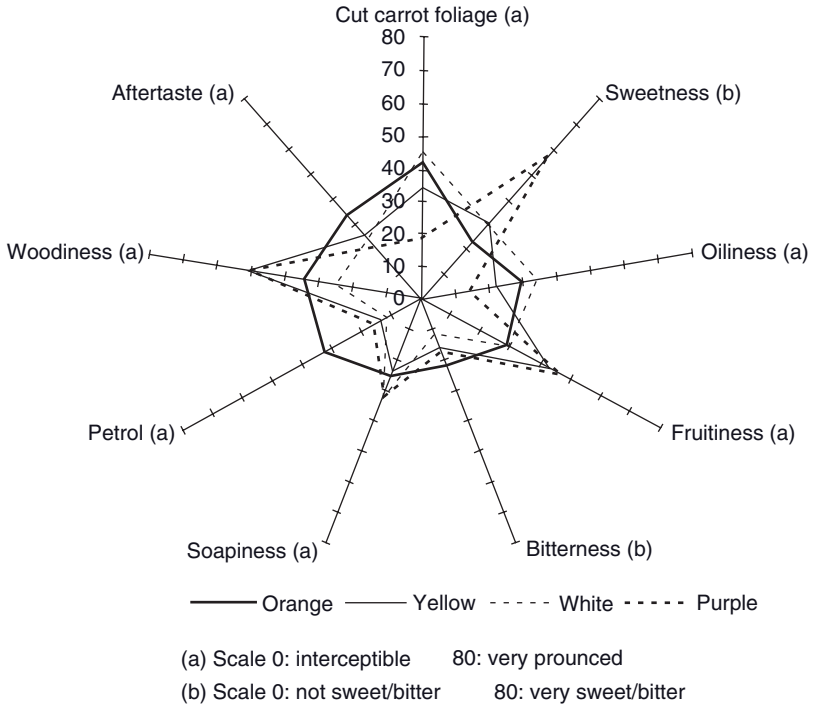

Figure 40.1. Flavor profile analysis of colored carrot varieties (Alasalvar et al. 2001). Used with permission of the American Chemical Society.

market, was described as having the highest sweetness, hay aroma, and the lowest carrot flavor.

Similar findings were observed by Varming and others (2004) on six carrot cultivars grown in Denmark and by Szymczak and others (2007) for carrot cultivars of a different genotype similar with what Alasalvar and others (2001) utilized. It was found that the carrot cultivar had an effect on most sensory and flavor component variables. Carrot cultivars of different genotypes differ in respect to sensory quality. Sensory attributes, which most significantly influenced overall sensory quality impression, were off-odor intensity, flesh firmness, raw carrot flavor intensity, bitter taste, and off-flavor intensity. Regarding the consumer preferences, orange-colored carrot cultivars were preferred by consumers to other colored ones. Liking of carrot flavor is strongly and positively correlated with juiciness and crunchiness of storage roots (Szymczak et al. 2007).

The influence of temperature and plant density on sensory quality and volatile terpenoids was demonstrated by Rosenfeld and others (2002). By partial least squares regression (PLSR), two-thirds of the flavor compound variables were found to correlate significantly with one or more of the nine sensory attributes, and all of the sensory attributes were significantly correlated with one or more of the consumer liking test variables of bitterness, sweetness, and liking.

The sensory properties including the flavor profile of raw carrot may be used for predicting the carrot product quality such as carrot chips. By describing the raw material either by chemical and color parameters or by sensory parameters, the producers of carrot chips can choose the raw carrots that meet the requirement of the desired quality of carrot chips (Baardseth et al. 1995).

CHEMICAL COMPOSITION OF CARROT AND ITS RELATIONSHIP WITH CARROT FLAVOR

Major Substances

Raw and fresh carrots contain mainly moisture (88%), carbohydrate (9.6%), protein (1%), ash (1%), and other minor components (less than 1%) (Table 40.2). The ash content is usually between 5% and 10% in dry matter. The lipid content of carrot root is very low (less than 0.5%). Main minor components of carrot roots are carotenoids, mainly α- and β-carotenes, and a small amount of lutein/zeaxanthin. In addition, carrots contain several vitamins, phenolic substances, and volatile compounds that are believed to influence the carrot flavor. The chemical composition of the carrot root varies over time, between cultivars and as a result of cultivation measures (Alasalvar et al. 2001; Holley et al. 2000; Kjellenberg 2007; Simon 1982).

Carbohydrates Carrots contain carbohydrates mainly in the form of free sugars, and only a small amount is in form of starch. Carrots also contain dietary fiber. About half of the dry matter content in carrot is soluble sugar. Soluble sugars are the main form of storage compounds in carrots. They account for 34–70% of the dry weight (Soujala 2000). It is reported that at harvest, the sugar content mainly consists of the disaccharide sucrose and the two monosaccharides, glucose and

TABLE 40.2. Proximate Composition of Raw Fresh Carrot

Nutrient	Units	Value per 100 g	Number of Data Points	Standard Error
Water	g	88.29	33	0.429
Energy	kcal	41.0	0	0
Energy	kJ	173.0	0	0
Protein	g	0.93	19	0.008
Total lipid (fat)	g	0.24	26	0.018
Ash	g	0.97	19	0.014
Total carbohydrate	g	9.58	0	0
Total dietary fiber	g	2.8	4	0
Total sugar	g	4.74	0	0
Sucrose	g	3.59	11	0.28
Glucose (dextrose)	g	0.59	11	0.141
Fructose	g	0.55	11	0.097
Starch	g	1.43	2	0

Source: USDA (2007).

fructose. Fresh carrots contain 8.17% free sugar, consisting of 3.39% sucrose, 1.89% β-glucose, 1.45% α-glucose, 1.05% fructose, and 0.39% unknown (Alabran and Mabrouk 1973). Alabran and Mabrouk (1973) also reported other "sugarlike" compounds (sugar phosphate), that is, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, nucleoside phosphate, and mono-, di-, and triphosphates, which account about 0.80% in fresh carrots. Similar findings were reported by Baardseth and others (1995) and by Howard and Dewi (1995).

Nitrogenous Compounds Fresh carrots contain about 1.31% free nitrogenous compounds (Alabran and Mabrouk 1973). Protein content in raw carrots is 0.93% (USDA 2007). Aspartic acid, α-alanine, serine, and glutamic acid in the free form are abundant in fresh carrots and account for about 68% of the total free nitrogenous compounds (Alabran and Mabrouk 1973). Other important amino acids that exist in quite significant amount are valine and threonine. Together with amino sugars, like glucosamine, the free amino acids account for 90% of the total free nitrogenous compounds (Alabran and Mabrouk 1973). These amino acids may play an important role in flavor development during processing.

Lipids The total lipid content in carrots is approximately 0.24%. The composition of the carrot oil is complex. It is mainly genetically determined. The amounts of different oils vary, however, depending on growing conditions (Heatherbell and Wrolstad 1971).

Organic Acids The total amount of organic acids is about 0.2% in fresh weight (Åkesson 2003) The most common organic acids in carrots are pyruvatic, oxalic, acetic, isocitric, and malic acid (Phan et al. 1973).

Minerals Fresh carrot is a good source of some minerals. Potassium is quite abundant in fresh carrot. It accounts (in mg/100 g fresh material) for 320 followed

by sodium, 69; phosphorus, 35; calcium, 33; and magnesium, 12. Other important minerals, but in small quantity, are fluoride (3.2), iron (0.30), zinc (0.24), manganese (0.143), selenium (0.1), and copper (0.045) (USDA 2007).

Minor Components

There is a large amount of minor components that are secondary metabolites in carrots. The ones mentioned here are more or less connected to the sensory properties, especially taste and flavor (Kjellenberg 2007).

Carotenoids Carrots are the most important source of dietary carotenoids in Western countries including the United States (Block 1994; Törrönen et al. 1996). Carrots contain the highest amount of β-carotene of the common fruits and vegetables (Desobry et al. 1998). Raw fresh carrots contain (μg/100 g fresh material) β-carotene 8285, α-carotene 3477, and lutein/zeaxanthin 256 (USDA 2007). β-Carotene constitutes 60–80% of the carotenoids in carrots followed by α-carotene (10–40%), lutein (1–5%), and the other minor carotenoids (0.1–1.0%) (Chen et al. 1995). Among the provitamin A carotenoids, β-carotene showed the highest vitamin A activity on a molar basis (Biesalski 1997; Van Vliet et al. 1996). Other carotenoids are estimated to have only half of β-carotene's potency (Castenmiller and West 1998). Processing may convert some of these carotenoids into cis isomers (Lessin et al. 1997) and epoxy-carotenoids (Ball 1998) that may have lower vitamin A activities (Ball 1998; Johnson et al. 1996). This is confirmed, for example, by Sulaeman and others (2001) as shown in Table 40.3.

Higher levels of carotenoids are normally found in the phloem than in the xylem (Koch and Goldman 2005). Other carotenoids that may be contained in carrot varieties are γ- and ξ-carotenes, lycopene, and β-zeacarotene. However, the most predominant in orange and yellow carrots are α- and β-carotenes (Simon and Wolff 1987). Lycopene is found in red carrots (Surles et al. 2004). Xanthophylls, such as lutein, are common in yellow carrots. In purple carrots, beside the carotenoids, there are other pigments, anthocyanins, belonging to the flavonoids (Surles et al. 2004). An example of the concentrations of carotenoids found in different types of carrots is found in Table 40.4.

TABLE 40.3. Carotenoid Content (μg/100 g w/w and μg/100 g d/w) and Vitamin A Activity (μg RAE/100 g w/w) of Fresh Carrot Slices

	w/w	d/w
Lutein	394	3,270
α-Carotene	3,160	26,224
β-Carotene	8,626	71,585
Total carotenoids	12,180	101,079
Vitamin A activity[a]	851	7,058

Source: Adapted from Sulaeman and others (2001).

[a]Retinol activity equivalent $(\mathrm{RAE}) = \frac{\mu g\ \alpha\text{-carotene}}{24} + \frac{\mu g\ \beta\text{-carotene}}{12}$.

TABLE 40.4. Concentrations of Carotenoids in Different Types of Raw Carrots (μg/100 g carrot, fresh weight) (mean ± SD)

Carrot Type	α-carotene	β-carotene	Lycopene	Lutein	Total
Orange	2,200 ± 800	12,800 ± 3,300	nd	260 ± 80	15,200 ± 4,100
Purple	4,100 ± 1,200	12,300 ± 5,100	nd	1,100 ± 730	17,500 ± 7,000
Red	110	3,400 ± 890	6,100 ± 600	320 ± 260	9,800 ± 1,400
Yellow	50	180 ± 170	nd	510 ± 270	710 ± 380
White	nd	6 ± 3	nd	9 ± 2	14 ± 1

Source: Adapted from Surles and others (2004).

nd, not detected.

TABLE 40.5. Vitamin Composition in Raw Fresh Carrot

Vitamin	Units	Value per 100 g	Number of Data Points	Standard Error
Total Vitamin C	mg	5.9	21	1.13
Thiamin	mg	0.066	21	0.011
Riboflavin	mg	0.058	19	0.013
Niacin	mg	0.983	19	0.215
Pantothenic acid	mg	0.273	9	0.145
Vitamin B_6	mg	0.138	19	0.03
Total folate	mcg	19.0	19	5.175
Folate, DFE	mcg	19.0	0	0
Total choline	mg	8.8	0	0
Betaine	mg	0.4	1	0
Vitamin A (IU)	IU	16,706.0	0	0
Vitamin A potency (RAE)	mcg	835.0	0	0
Vitamin E (alpha-tocopherol)	mg	0.66	11	0.269
Tocopherol, beta	mg	0.01	11	0.005
Tocopherol, gamma	mg	0.00	11	0
Tocopherol, delta	mg	0.00	11	0
Vitamin K (phylloquinone)	mcg	13.2	4	0

Source: USDA (2007).

DFE, dietary folate equivalent.

Vitamins Table 40.5 shows the vitamin composition of fresh carrot. Besides provitamin A carotenoids, other major vitamins contained in carrot roots are vitamin C, choline, folate, and niacin. Alasalvar and others (2001) reported the amount of vitamin C is between 3 and 5 mg/100 g fresh weight in orange varieties and about 1–2 mg/100 g fresh weight in white and yellow varieties. Carrots also contain vitamin K, vitamin E, thiamin, pantothenic acid, vitamin B_6, riboflavin, and betaine.

Volatiles and Essential Oils Kjellenberg (2007) reviewed many studies about the volatile compounds in carrots and their importance regarding the taste and flavor of carrot. Between 30 and 40 volatile substances are commonly detected in carrots. Shamaila and others (1996) reported that a total of 37 volatile compounds

were isolated and identified from fresh carrot. About 98% of the total volatile compound mass in carrot is reported as mono- and sesquiterpenes (Kjeldsen et al. 2001), and the remains are alcohols, styrene, and alkane (Alasalvar et al. 2001). Usually between 17 and 20 different simple terpenes contribute to the typical carrot flavor (Simon 2002).

Alasalvar and others (2001) examined four different colored carrots, orange, purple with orange core, yellow, and white for their content of phenolics, antioxidant vitamins, and sugars as well as for their volatiles and sensory responses. A total of 35 volatiles were identified in all carrots, 27 positively. The 33 volatile components found in orange carrots were propanol, α-thujene, α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene, *cis*-ocimene, *trans*-ocimene, γ-terpinene, terpinolene, 2,5-dimethylstyrene, undecane, camphor, borneol, terpinen-4-ol, linalyl acetate, β-citronellol, bornyl acetate, α-santalene, longifolene, β-caryophyllene, α-selinene, *trans*-α-bergamotene, α-humulene, *cis*-β-farnesene, γ-elemene, α-zingiberene, valencene, β-bisabolene, and γ-bisabolene. White carrot contained the highest content of volatiles followed by orange, purple, and yellow (Table 40.6).

TABLE 40.6. Mean Concentrations of Volatile Compounds in Different Raw Carrot Varieties (ppm)

Compound	Orange	Purple	Yellow	White
α-Thujene	0.001	tr	0.013	0.046
α-Pinene	0.180	0.017	0.018	1.242
Camphene	0.005	0.002	tr	0.065
Sabinene	0.023	0.001	0.537	1.624
β-Pinene	0.089	0.043	0.103	0.597
Myrcene	0.351	0.494	0.196	0.791
α-Phellandrene	0.170	0.066	0.081	0.326
α-Terpinene	0.010	0.002	0.020	0.116
p-Cymene	0.057	0.017	0.004	0.209
Limonene	0.236	0.066	0.049	0.638
cis-Ocimene	0.103	0.013	nd	0.041
trans-Ocimene	0.016	0.001	tr	0.004
γ-Terpinene	0.569	0.056	0.044	1.210
Terpinolene	3.465	0.810	0.569	7.290
2,5-Dimethylstyrene	0.079	0.012	0.007	0.205
Undecane	0.023	0.004	0.001	0.005
Bornyl acetate	tr	nd	tr	0.098
β-Cartophyllene	0.749	1.025	0.332	1.251
trans-α-Bergamotene	0.023	0.010	0.011	0.022
α-Humulene	0.035	0.052	0.014	0.061
cis-β-Farnesene	0.006	0.008	0.012	0.001
β-Bisabolene	0.054	0.007	0.019	0.064
γ-Bisabolene	0.424	0.245	0.338	0.344
Monoterpenes	5.275	1.588	1.634	14.199
Sesquiterpenes	1.291	1.347	0.726	1.743
Total volatiles	6.668	2.951	2.368	16.250

Source: Adapted from Alasalvar and others (2001).

tr, trace (<0.001 ppm); nd, not detected.

The most frequent essential oils in carrots are the monoterpenes sabinene, β-myrcene, α-terpinolene, and β-caryophyllene together with some sesquiterpenes.

Although genetics plays a role in determining the number of volatile compounds, the actual amounts of the different volatile compounds are, however, dependent on the environment (Rosenfeld et al. 2002). In addition to carrot line or cultivar, factors such as maturity, storage, processing, and climate also influence concentrations of carrot volatiles (Heatherbell and Wrolstad 1971; Rosenfeld et al. 2002; Simon 1982). The effect of climate (temperature) and plant density on the volatile terpenoids of carrots was investigated by Rosenfeld and others (2002). It was found that higher temperatures (18 and 21°C) led to a higher content of terpenoid volatiles in carrot (Table 40.7). These researchers also found that there were no significant differences in the concentrations of bornyl acetate, β-farnesene, and α-humulene, but not the other terpenes, when the plant density was "dense" as compared with "normal."

Phenolic Substances Phenols are biosynthesized along the polyketide (acetyl-coenzyme A) or the shikimic pathway (Kjellenberg 2007). Phenolic compounds in fruits and vegetables are of great interest in two respects (Alasalvar et al. 2001). First, they contribute to the sensory qualities of fruits and vegetables: color, astringency, bitterness, and aroma. Second, some phenolics possess pharmacological properties and are used for therapeutic purposes. Their contribution to the resistance of fruits to parasite attack appears to be well established by earlier research, although their physiological function and modes of action are still being investigated. Therefore, the metabolism of phenolics may be used as a good indicator to evaluate the quality and storability of carrots.

TABLE 40.7. Effects of Temperature on Terpenes (peak area; mVmin × 10^8)

Terpene	9 °C	12 °C	15 °C	18 °C	21 °C	Significance Level
α-Pinene	2.48	2.47	2.52	2.32	2.67	NS
Camphene	0.10	0.12	0.14	0.17	0.19	NS
β-Pinene	1.08^{a}	1.16^{a}	1.50^{ab}	1.56^{ab}	2.44^{b}	$p < 0.01$
β-Myrcene	1.29^{a}	1.52^{a}	2.34^{a}	3.35^{ab}	4.79^{b}	$p < 0.001$
α-Terpinene	0.13	0.20	0.14	0.26	0.15	NS
Linonene	1.76	2.03	1.61	2.04	1.73	NS
β-Phellandrene	0.28	0.33	0.27	0.30	0.31	NS
Ocimene	0.43^{a}	0.63^{a}	0.49^{ab}	1.00^{bc}	1.02^{c}	$p < 0.01$
γ-Terpinene	3.40	3.64	3.18	3.77	4.53	NS
α-Terpinolene	12.3^{a}	12.8^{a}	10.1^{ab}	10.4^{ab}	6.7^{b}	$p < 0.05$
Bornyl acetate	2.03^{a}	2.81^{ab}	3.36^{bc}	3.58^{bc}	4.19^{c}	$p < 0.01$
trans-Caryophyllene	5.33^{a}	4.98^{a}	6.81^{ab}	7.61^{ab}	9.03^{b}	$p < 0.05$
β-Farnesene	0.82^{a}	0.99^{ab}	1.25^{b}	1.90^{c}	2.65^{d}	$p < 0.001$
α-Humulene	0.80^{a}	0.76^{a}	1.12^{ab}	1.44^{bc}	1.92^{c}	$p < 0.001$
Farnesene	3.50	4.05	4.14	4.12	4.18	NS

Source: Adapted from Rosenfeld and others (2002).

Mean values with the same letter are not significantly different ($p \geq 0.05$) by Tukey's multiple-comparison test. NS, not significant.

The most common phenolic substances in carrot are hydroxycinnamic acid derivates (Kjellenberg 2007). Alasalvar and others (2001) identified and analyzed the most common phenolic compound in carrots of different color as listed in Table 40.8. In total, 11, 16, 10, and 9 phenolic compounds were determined for the first time in orange, purple, yellow, and white carrots, respectively. Of these, chlorogenic acid was the most predominant phenolic compound in all carrot varieties. Purple carrots contained the highest total phenolics and the lowest is the white ones. Orange carrots contained total phenolics about 16.21 mg/100 g. Gębczyński (2006) reported that fresh carrot contained total polyphenols of about 20.9 mg/100 g.

Kjellenberg (2007) reviewed many studies regarding the bitter taste of carrots, and he recognized that some specific phenolic substances are often mentioned and investigated. One of them is the phytoalexin 6-methoxymellein (6-MM) (3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin), which is a secondary metabolite that inhibits the growth of many microorganisms.

Polyacetylenes Although various reports pointed to 6-MM as a key player imparting the bitter taste in carrots, activity-guided fractionation experiments recently gave evidence that not this isocoumarin but bisacetylenic oxylipins contribute mainly to the off-taste. Among these, (Z)-heptadeca-1,9-dien-4,6-diyn-3-ol, (Z)-3-acetoxy-heptadeca-1,9-dien-4,6-diyn-8-ol, and (Z)-heptadeca-1,9-dien-4,6-diyn-3,8-diol (falcarindiol) have been successfully identified (Czepa and Hofmann

TABLE 40.8. Mean Concentrations (mg/100 g fresh weight) of Phenolic Compounds in Different Raw Carrot Varieties

Compound	Orange	Purple	Yellow	White
3′-Caffeoylquinic acid	0.28[a]	0.88[b]	0.09[c]	0.09[c]
cis-3′-Caffeoylquinic acid	nd	1.94	nd	nd
5′-Caffeoylquinic acid	8.50[a]	54.08[b]	4.41[c]	4.47[c]
Caffeic acid	nd	2.42	nd	nd
3′-p-Coumaroylquinic acid	0.54[a]	0.91[b]	0.20[c]	0.31[d]
3′-Feruloquinic acid	0.21[abc]	7.30[d]	0.19[c]	0.26[ab]
3′,4′-Dicaffeoylquinic acid	2.08[a]	2.78[b]	1.30[c]	1.06[d]
5′-Feruloyquinic acid	0.11[a]	0.96[b]	0.51[c]	0.39[d]
cis-5′-Caffeoylquinic acid	nd	0.49	nd	nd
5′-p-Coumaroylquinic acid	0.13[a]	0.74[b]	0.11[a]	nd
4′-Feruloyquinic acid	0.40	nd	nd	nd
3′,5′-Dicaffeoylquinic acid	3.80[a]	0.44[b]	0.75[c]	1.74[d]
3′,4′-Diferuloylquinic acid	0.07[a]	0.53[b]	0.12[a]	0.31[c]
3′,5′-Diferuloylquinic acid	0.09[a]	1.17[b]	0.04[a]	0.06[a]
Total phenolics	16.21[a]	74.64[b]	7.72[c]	8.69[c]

Source: Adapted from Alasalvar and others (2001).

Means followed by the same letter, within a row, are not significantly different ($p > 0.05$). nd, not detected.

2004). Falcarindiol, FaDOH, has recently been detected as a bitter-tasting constituent in carrot beside 6-MM.

Compounds Responsible for Carrot Flavors

Carrots have a complex flavor (Alasalvar et al. 2001). From many studies, it is clear that there is no single compound that accounts for a distinctively carrot-like flavor. The chemical substances responsible for carrot flavor are quite complex. There are many factors that influence carrot flavor, including nonvolatile chemical constituents such as free sugars, phosphates, nitrogenous compounds, bitter compounds, phenolics, and organic acids. Alabran and Mabrouk (1973) suggested that the nonvolatile chemical constituents (sugars and amino acids) are primarily responsible for the taste of fresh carrot and that the contribution of volatile components is small compared with that of nonvolatile compounds. Meanwhile, Simon and others (1980) emphasized the importance of both sugars and volatile terpenes in determining raw carrot flavor. However, most researchers suggest that the characteristic flavor of carrots is mainly due to the volatile constituents, which are mostly made up of terpenes and sesquiterpenes.

The taste or flavor of carrots is a unique composition between sweet, fruity, and a more harsh or bitter flavor. Many factors affect the balance between the different flavors in carrots and thus contribute to the final taste (Kjellenberg 2007). Sweet taste is more common in the center and lower tip part of the carrot. The phloem is mostly sweeter and also more bitter than the xylem. Bitter taste is more often detected in the upper and outer part of the carrot.

Aroma/Odor of Carrot The overall "flavor sensation" of carrots may be divided into sensations due to "taste" and to "aroma" (Alabran and Mabrouk 1973). The nonvolatile components of carrots (sugars and amino acids) are the taste-bearing compounds, and the volatile components are responsible for aroma. It is agreed that the characteristic aroma of raw carrots and processed carrots is due mostly to their volatile components. However, due to the delicate flavor of carrots, Alabran and Mabrouk (1973) had the opinion that the contribution of essential oils may be small in comparison with that of the nonvolatile, taste-bearing components.

Sweet Taste The amount of sugar in carrots has a clear correlation with the perception of sweetness. The amount of sugar can also contribute in masking the bitter taste in carrots. One possible reason for the increases in bitter taste during storage is decreasing sugar content. The sugar in carrots consists mainly of sucrose, glucose, and fructose. Alabran and Mabrouk (1973) reported four free sugars and six sugar phosphates were identified in fresh carrots. Sucrose accounts for 44% of the total free sugar content, which amounts to 8.14% of fresh carrots. Fructose content approaches one-fourth of the total reducing sugars in fresh carrots.

Alasalvar and others (2001) observed the differences ($p < 0.05$) in relative sweetness, the contents of vitamin C and α- and β-carotenes, and certain flavor characteristics among the colored carrot varieties examined as shown in Table 40.9. With respect to sweetness, the purple carrot was significantly sweeter than other three varieties ($p < 0.05$). This may be due to the high sucrose level found in the purple

TABLE 40.9. Mean Sugars, Vitamin C, and α- and β-Carotenes Content in Different Raw Carrot Varieties

	Sugars (g/100g)							
Color	Fructose	Glucose	Sucrose	Total Sugars	Relative Sweetness	Vitamin C (mg/100 g)	α-Carotene (mg/100 g)	β-Carotene (mg/100 g)
Orange	1.34[a]	1.44[a]	2.69[a]	5.47[a]	6.07[a]	5.33[a]	3.99[a]	6.94[a]
Purple	0.58[b]	0.69[b]	4.11[b]	5.38[a]	5.62[b]	nm	8.73[b]	16.13[b]
Yellow	1.31[a]	1.77[a]	1.96[cd]	5.04[a]	5.54[ab]	1.98[b]	tr	tr
White	1.47[a]	1.59[a]	2.33[ad]	5.39[a]	6.05[ab]	1.25[c]	nd	nd

Source: Adapted from Alasalvar and others (2001).

Means followed by the same letter, within a column, are not significantly different ($p > 0.05$); nm, not measured (due to pink color). Relative sweetness was calculated relative to sucrose (fructose, 1.73; glucose, 0.74; and sucrose, 1.00). tr, trace; nd, not detected.

variety. However, this observation does not agree with the total sugar contents and consideration of their relative sweetness, indicating that other components may be influencing the sweetness response. It has been found by Howard and others (1995) and by Simon and others (1980) that high levels of terpenoids in orange carrot mask the overall sweetness response. Rosenfeld and others (2002) also found similar results, concluding that a high content of certain terpenes in the carrot root seems to lead to a high score for bitterness, which again might suppress the perception of sweet taste.

According to Kjellenberg (2007) during the seedling phase, no soluble sugar is stored; in the second phase, only reducing sugar is stored; and in the third phase, starting some 50 days after sowing, mainly sucrose is stored in the carrot root. The reduction in sugar during storage mainly concerns sucrose. The total amount of sugars did not differ so much between different parts of the carrot.

Bitter Taste Bitter taste along with harsh flavor is often the reason for consumer rejection of carrot products, such as carrot puree in the infant diet, and is therefore a major problem for vegetable processors. It is also a major problem with regard to strained carrots (Talcott and Howard 1999).

No particular compound has been found that explains all phenomena connected to the harsh and bitter flavors of carrots. The appearances of such flavors are probably due to a multiplicity of compounds (Kjellenberg 2007). Several researchers have identified that one compound responsible for the bitter taste in carrot is 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, more commonly known as isocoumarin or 6-MM. 6-MM is a secondary metabolite that inhibits the growth of many microorganisms (Talcott and Howard 1999).

Talcott and Howard (1999) found relationships between isocoumarin and sweetness, bitterness, sour flavor, and preference. There is a negative correlation between isocoumarin and sweetness as well as preference and a positive correlation between isocoumarin and bitterness and sour flavor.

From the Talcott and Howard (1999) study, it was found that the 6-MM concentrations >94 mg/kg (recognition threshold) will negatively impact most flavor attributes of strained carrots. However, perception of bitterness may be at a much lower concentration due to the effects of additional phytoalexins produced simultaneously with 6-MM as demonstrated by the TX and GA genotypes.

Though the bitter taste in stored carrot had previously been related to the content of 6-MM, in fresh carrots, the level of 6-MM was below the threshold of bitterness (Rosenfeld et al. 2002). Therefore, there should be other compounds contributing to the bitter taste. Sesquiterpenes isolated from canned carrots were found responsible for some of the bitter taste (Shallenberger et al. 1960). Simon and others (1980) indicated that harsh flavor, which is described as terpene flavor (green flavor and earthy flavor) was most likely caused by terpenes. This was confirmed by Holley and others (2000), who found that carrot lines with the highest °Brix/terpinolene ratios had a lower degree of bitterness, indicating a relationship between sweet and bitter taste in carrots.

Working with carrots grown in controlled climate chambers at 9, 12, 15, 18, and 21°C and at two plant densities, Rosenfeld and others (2002) confirmed previous studies (Holley et al. 2000; Simon et al. 1980) and concluded that the factor most responsible for the bitter taste in raw carrot is terpenes, which suppress the

perception of sweet taste in carrots. They showed a high positive correlation between terpenes (α-terpinene, β-myrcene, trans-caryophyllene, farnesene, α-humulene) and sensory variables (terpene flavor, green flavor, earthy flavor, bitter taste, aftertaste): 83% of the variation in terpenes was able to predict 82% of the sensory variables by means of PLSR. They also found that terpinolene decreased with increasing growth temperature and probably plays only a minor role in masking the sweet taste in carrots.

Terpenes are connected both to the typical carrot taste as to harsh flavors (Kjellenberg 2007). There are a large number of terpenes in carrots mainly in the carrot oil. They are more common in the upper part and in the phloem. The concentration of terpenes increases during growth. Higher temperatures during growing season also increase the amount of terpenes. Terpenes can mask for sweet taste but can also be less detectable by increasing sugar concentration.

Experiments confirmed terpinolene to be the most abundant terpene. However, terpinolene was far more abundant in carrots grown at 9°C than in carrots grown at 21°C. Since carrots grown at low temperature were less bitter, the contribution from terpinolene to bitter taste must have been less than its quantity indicated. Buttery and others (1968) found terpinolene to account for 38% of the total amount of essential oils in carrots, and the odor threshold of terpinolene was calculated to be 200 ppb. In comparison, aldehydes such as 2-nonenal, 2-decanal, and octanal with odor thresholds of 0.008–0.7 ppb contributed much more to the total flavor than terpinolene. There is reason to believe that terpenes other than terpinolene contribute more to the bitter taste. Such terpenes might show increasing values with increasing growth temperature, which were mainly γ-terpinene, α-pinene, ocimene, camphene, caryophyllene, β-pinene, β-myrcene, α-humulene, and β-farnesene.

That terpene determines the flavor of carrot was demonstrated by Rosenfeld and others (2002). The correlation between terpene and the flavor component of carrot is presented in Table 40.10.

Phenolic substances, as 6-MM, are synthesized along the polyketide or shikimic pathway as a reaction of stress and increased respiration in the carrot. Together with other compounds, they can contribute to the bitter taste in carrot. Polyacetylenes, such as falcarindiol, are formed from oleic acid probably as a part of the defense against pathogens. Falcarindiol is, however, always present in carrots, more commonly in the upper and outer part and in the phloem. There is a correlation between the amount of falcarindiol and the bitter taste in carrots. The sweet and bitter taste

TABLE 40.10. Correlation Matrices for Terpenes and Sensory Variables

	Bitter	Green	Earthy	Perpene	Aftertaste
α-Terpinolene	–0.83	–0.80	–0.89	–0.89	–0.85
β-Pinene	0.82	0.78	0.80	0.84	0.79
β-Myrcene	0.87	0.85	0.80	0.89	0.88
Caryophyllene	0.87	0.95	0.73	0.86	0.86
β-Farnesene	0.83	0.90	0.65	0.82	0.79
α-Humulene	0.80	0.91	0.63	0.80	0.76
Total	0.60	0.71	0.39	0.55	0.58

Source: Adapted from Rosenfeld and others (2002).

in carrots is dependent both on genetic and environmental factors. The choice of cultivars and cultivation methods can therefore highly affect the taste of carrots before they reach the consumer.

Holley and others (2000) reported that terpinolene and caryophyllene were found in the highest concentration among different carrot lines. The concentrations of terpinolene with caryophyllene accounted for >70% of the total volatile terpenoids, in agreement with previous studies (Heatherbell and Wrolstad 1971). Terpinolene has been associated with "cooked carrot" aroma and flavors in processed carrots (Howard et al. 1995) and has also been described as being "perhmy" (Heatherbell and Wrolstad 1971). Simon (1982) reported that terpinolene influenced harsh flavor, sweetness, as well as preference for raw carrots. Caryophyllene has been described as possessing a terpene odor with elements of "cloves" and "turpentine" (Budavari 1989).

Using descriptive analysis, Gills and others (1999) found significant differences in the perception of sweet taste and of color, and in levels of °Brix and percentage of sugar among all cultivars, but perceived intensity of sweetness was not related to the levels of °Brix or percentage of sugar. No significant differences were found among cultivars in harsh carroty, green, astringent, and earthy flavors, and in the perception of sour taste. Intensity ratings for perceived hardness were nonsignificant in either study.

Gills and others (1999) reported that differences in sensory profiles existed among all cultivars, but no trend was evident in the relation of sweetness to harsh flavor. This finding was in agreement with that of Holley and others (2000). While the refractive index appears to correlate positively with sucrose, the use of "°Brix" as an indication of "total sugars" has been questioned, given the negative correlation with glucose and fructose (Simon et al. 1980). Furthermore, refractive index does not necessarily correlate well with sweetness, suggesting that other molecules such as volatile terpenoids also impact perceived sweetness (Simon 1985). In agreement, Howard and others (1995) reported a better correlation between total sugar concentration and sweet flavor scores than "°Brix" readings.

FLAVOR FROM CARROT SEEDS

Not only the root is used as a source of flavor but also the seeds. Many efforts have been carried out to isolate the essential oil from the seeds of carrots. Carrot seeds are considered carminative, stimulant, and very useful in cases of flatulence, windy colic, hiccups, dysentery, and chronic coughs. Raw carrots are sometimes given to children for expelling worms, and the boiled roots, mashed to a pulp, are sometimes used as a cataplasm for application to ulcers. Carrot seeds are excellent in treating obstructions of the viscera and in jaundice (for which they were formerly considered a specific).

Carrot seed, which contains about 0.5–1.6% (v/w) essential oil, is widely used in the formulation of certain alcoholic liquors and fragrances as an aromatic ingredient (Özcan and Chalchat 2007). Its essential oil is also used for medicinal purposes such as diuretic or stomachic (Mazzoni et al. 1999). A problem with the use of carrot seed is the high intensity of the harsh carrot-like flavor; research on carrot breeding is needed to solve this problem. Nevertheless, unacceptable carrot flavor can be

perceived by consumers; this appears to be due to suboptimal postharvest storage conditions and harsh handling during distribution. Bitter and harsh as well as flat, insipid flavors in carrots have been described in response to ethylene exposure, mechanical stress, or storage in low-oxygen atmospheres (Simon 1985).

These oils are usually mixtures of hydrocarbons of various groups generally containing terpenes and oxidized aromatic derivatives. The seed is a traditional "morning after" contraceptive, and there is some evidence to uphold this belief.

An essential oil obtained from the seed has also been used cosmetically in anti-wrinkle creams. A concentrated extract of the seeds and root makes a very good insecticide.

Özcan and Chalchat (2007) investigated the essential oil and edible oil compositions of carrot seeds from Konya by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and reported that the oil yields of essential and edible oils from carrot seeds were 0.83% and 7.84%, respectively. The major constituents of seed essential oil were carotol (66.78%), daucene (8.74%), (Z,Z)-α-farnesene (5.86%), germacrene D (2.34%), *trans*-α-bergamotene (2.41%), and β-selinene (2.20%), whereas carotol (30.55%), daucol (12.60%), and copaenol (0.62%) were the important components of edible carrot seed oil. However, the dominant component of both oils was carotol.

EFFECT OF HANDLING AND PROCESSING ON CARROT FLAVOR COMPONENT

The flavor components of carrot may be changed as the effect of storage and processing. The quality of vegetables deteriorates gradually during storage in response to endogenous factors and environmental conditions. Processes such as transpiration, respiration, activation of growth, and attacks by pathogens lead not only to quantitative losses but also to quality losses, which can destroy the marketability of the product or remain invisible (Soujala 2000).

Soujala (2000) reported that delaying the harvest improves storability up to the end of September or the beginning of October under northern conditions, whether the risk for storage diseases is high or not. After this point in time, storability remains at the same level. It is shown that the timing of harvest is an essential factor affecting the yield, quality, and storability of carrots. Varming and others (2004) reported that storage of carrots was characterized by an increase of a range of aroma components, but the changes in flavor compounds were not correspondingly observed by the sensory analysis.

Blanching may decrease the volatiles responsible for carrot flavor. Shamaila and others (1996) reported that most volatiles, in particular terpenoids (sabinene, β-pinene, β-myrcene, limonene, trans-caryophyllene, α-humulene, β-bisabolene, and α-farnesene) decreased by at least 50% within 60 s of blanching. Furthermore, Shamaila and others (1996) reported that ratings on quality attributes of color, texture, raw carrot aroma, sweetness, flavor, and overall impression decreased with blanching time, while cooked carrot aroma increased. There were correlations ($p < 0.05$) between blanching times, flavor volatiles, and sensory attributes.

Minimal processing and edible coating of mini peeled carrot was reported not to affect fresh carrot flavor or aroma, sweetness, bitterness, harshness, or taste

preference (Howard and Dewi 1996). It is reported that total terpenoids declined 72% after 17 days storage, with the major loss occurring within 3 days after minimal processing. α- and β-carotenes declined 18% and 14% within 3 days after minimal processing with no further loss. Peel tissue contained less β-carotene than phloem tissue, and its removal was not responsible for the loss of β-carotene. The edible coating did not affect terpenoid or carotene content. A previous study (Howard and Dewi 1995) found that coating treatments had a minor effect on sugar content. Carrots treated with the lowest rate had a higher total sugar content than noncoated carrots or those treated at the highest rate. Howard and Dewi (1995) found that sensory scores for fresh carrot flavor, fresh carrot aroma, and overall acceptability were higher for coated carrots. An application rate of 0.23–0.49 L/min appeared adequate for protection against surface discoloration and for retention of flavor.

The development of a white material on the surface of freshly peeled carrots can be inhibited by a 20- to 45-s dip in a 60°C, pH 1.0 solution. This treatment insures better retention of the original carrot color and flavor compared to untreated carrots and can also be easily incorporated into a production system. A level above pH 1.0 will also offer protection, but only for shorter time (Bolin 1992).

Sulaeman and others (2002b) reported that frying carrot slices resulted in a distinctive and attractive flavor that even people who reported not liking carrots may find acceptable. Alabran and Mabrouk (1973) reported that flavor may be influenced by enzymatic browning reactions; enzymatic browning reactions sometimes occur during food processing. In addition, the particular flavor of cooked carrots varies with the cooking temperature and method of cooking, that is, dry heat or moist heat. The taste and aroma of the browning reaction by-products varies depending on the reacting amino acids. The aromas resulting from the heating of glucose in fresh carrots along with aspartic acid, α-alanine, glutamic acid, valine, threonine, serine, and arginine are reminiscent of rock candy, sweet caramel, caramel, rye bread, chocolate, maple syrup, and buttery notes, respectively. Different aromas are produced by the same reaction mixtures when the temperature is raised to 180 °C.

After lengthy storage, carrots may accumulate phenols and often develop off-flavor and undesirable color (Sarkar and Phan 1979). This process is influenced by the ethylene content. When applied at moderate levels, ethylene caused an increase in phenols in the tissue, especially isochlorogenic acid, and in the roots. Longer exposure at a moderate level and short exposure at high levels induced formation of new compounds: isocoumarin, eugenin, and two others still unidentified. Further studies indicated that these compounds are probably synthesized via the acetate pathway. Low levels of ethylene produced by carrots may induce slowly the production of isocoumarin during storage and may render them bitter. Effects of the other phenols on taste and other qualities should be studied.

Ethylene-induced formation of isocoumarin was characterized in relation to ethylene-enhanced respiration in whole or cut carrots as reported by Lafuente and others (1996). Ethylene concentrations (0.1–5.0 ppm) and temperatures (1–15°C) that increased respiration also favored a more rapid formation of isocoumarin (8-hydroxy-3-methyl-6-methoxy-3,4-dihydro-isocoumarin). Exposing mature carrots to 0.5 ppm C_2H_4 for 14 days at 1 or 5°C resulted in isocoumarin contents of

20 and 40 mg/100 g peel, respectively. These levels were easily detected as a bitter flavor in the intact carrot roots. Immature carrots formed higher levels of isocoumarin than mature carrots; 180 mg/100 g peels were detected in young carrots stored 14 days at 5°C in air containing 0.5 ppm C_2H_4. Freshly harvested carrots exposed to 5 ppm C_2H_4 accumulated fourfold higher isocoumarin levels than those formed by carrots stored 30 days at 5°C before exposure to C_2H_4. An atmosphere of 100% O_2 potentiated the effect of C_2H_4 on isocoumarin formation, resulting in a fivefold increase over that found in carrots treated with C_2H_4 in air. A storage atmosphere of 0.5 ppm C_2H_4 in 1% O_2 resulted in isocoumarin levels about one-half those attained in 0.5 ppm C_2H_4 in air. Sliced, cut, or dropped carrots exposed to C_2H_4 showed greater isocoumarin accumulation rates than intact uninjured carrots. Peeled baby carrots, however, had little capacity to form isocoumarin. In general, the more rapid the respiratory rose in response to C_2H_4, the more rapidly isocoumarin accumulated. The greater the respiratory response to ethylene, the higher the level of isocoumarin formed.

Working with commercially processed strained carrots obtained from three different processing locations over a 1-year span, Talcott and others (2001) reported that concentrations of 6-MM, soluble phenolics, and organic acids in relation to high moisture content were critical factors for strained carrot taste. Strained carrot color could not be attributed to processing location or chemical composition and was likely due to raw product variation between cultivars. Variation between each processing location was greater than variation within each location, and overall differences between lots were attributed to 6-MM and soluble phenolic acid concentrations. Raw carrots should be screened for indications of stress-induced chemical constituents. Research is needed to better understand the factors that contribute to the taste of commercially processed carrots.

Working with strained processed carrots, Howard and others (1995) found that fresh carrot flavor, aroma, and aftertaste were associated with high total sugar to terpinolene ratios. Cooked flavor and aftertaste attributes were associated with elevated levels of terpinolene.

Future research is still needed to explore the effect of handling and processing on the flavor and on consumer acceptance and to find the best technique to develop the desirable flavors in the product and minimize the undesirable ones.

REFERENCES

Alabran DM, Mabrouk AF. 1973. Carrot flavour, sugars and free nitrogenous compounds in fresh carrots. *J Agric Food Chem* 21:205–8.

Alasalvar C, Grigor JM, Zhang D, Quantick PC, Shahidi F. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *J Agric Food Chem* 49(3):1410–16.

Åkesson U. 2003. Sensorisk kvalitet I morötter. Inst F Trädgärdsvetenskap. Alnarp, Sweden: Swedish University of Agricultural Sciences.

Baardseth P, Rosenfeld HJ, Sundt TW, Skrede G, Lea P, Slinde E. 1995. Evaluation of carrot varieties for production of deep-fried carrot chips: II. Sensory aspects. *Food Res Int* 28(6):513–19.

Ball GFM. 1998. *Bioavailability and Analysis of Vitamins in Foods*. New York: Chapman & Hall.

Berger M, Küchler T, Maaßen A, Stockfisch MB, Steinhart H. 2008. Correlations of carotene with sensory attributes in carrots under different storage conditions. *Food Chem* 106:235–40.

Biesalski HK. 1997. Bioavailability of vitamin A. *Eur J Clin Nutr* 51(Suppl. 1):S71–5.

Block G. 1994. Nutrient sources of provitamin A carotenoids in the American diet. *Am J Epidemiol* 139(3):290–3.

Bolin HR. 1992. Retardation of surface lignification on fresh peeled carrots. *J Food Process Preserv* 16(2):99–104.

Budavari S. 1989. *The Merck Index*, 11th edition. Rahway, NJ: Merck and Co.

Buttery RH, Seifert RM, Guadagni DG, Black DR, Ling LC. 1968. Characterization of some volatile constituents of carrots. *J Agric Food Chem* 16:1009–15.

Castenmiller JJM, West CE. 1998. Bioavailability and bioconversion of carotenoids. *Annu Rev Nutr* 18:19–38.

Chen BH, Peng HY, Chen HE. 1995. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J Agric Food Chem* 43(7):1912–18.

Corselius K. 2007. Carrots to the core. The Rodale Institute®. http://www.newfarm.org/ (accessed August 23, 2007).

Czepa A, Hofmann T. 2004. Quantitative studies and sensory analysis on the influence of cultivar, spatial tissue distribution, and industrial processing on the bitter off-taste of carrots (*Daucus carota* L.) and carrot products. *J Agric Food Chem* 52:4508–14.

Desobry SA, Netto FM, Labuza TP. 1998. Preservation of beta-carotene from carrots. *Crit Rev Food Sci Nutr* 38(5):381–96.

Gębczyński P. 2006. Content of selected antioxidative compounds carrot and in frozen product prepared for consumption. *EJPAU* 9(3):#3.

Gerrish CJ, Mennella JA. 2001. Flavor variety enhances food acceptance in formula-fed infants. *Am J Clin Nutr* 73(6):1080–5.

Gills LA, Resurreccion AVA, Hurst WC, Reynolds AE, Phatak SC. 1999. Sensory profiles of carrot (*Daucus carota* L.) cultivars grown in Georgia. *HortScience* 34(4):625.

Heatherbell DA, Wrolstad RE. 1971. Carrot volatiles. II. Influence of variety, maturity and storage. *J Food Sci* 36:225–7.

Holley SL, Edwards CG, Thorngate JH, Fellman, Matiinson DS, Sorensen EJ, Dougherty RH. 2000. Chemical characterization of different lines of *Daucus carota* L. roots. *J Food Qual* 23:487–502.

Howard LR, Braswell D, Heymann H, Lee Y, Pike LM, Aselage J. 1995. Sensory attributes and instrumental analysis relationships for strained processed carrot flavor. *J Food Sci* 60(1):145–6.

Howard LR, Dewi T. 1995. Sensory, mincrobiological and chemical quality of mini-peeled carrots as affected by edible coating treatment. *J Food Sci* 60(1):142–4.

Howard LR, Dewi T. 1996. Minimal processing and edible coating effects on composition and sensory quality of mini-peeled carrots. *J Food Sci* 61(3):643–5.

Johnson EJ, Krinsky NI, Russell RM. 1996. Serum response of all-trans and 9-cis isomers of beta-carotene in humans. *J Am Coll Nutr* 15(6):620–4.

Kjeldsen F, Christensen LP, Edelenbos M. 2001. Quantitative analysis of aroma compounds in carrot (*Daucus carota* L.) cultivars by capillary gas chromatography using large-volume injection technique. *J Agric Food Chem* 49:4342–8.

Kjellenberg L. 2007. *Sweet and Bitter Taste in Organic Carrot*. Alnarp, Sweden: Swedish University of Agricultural Science.

Koch TC, Goldman IL. 2005. Relationship of carotenoids and tocopherols in a sample of carrot root-color accessions and germplasm carrying Rp and rp alleles. *J Agric Food Chem* 53:325–31.

Lafuente MT, Lopez-Galvez G, Cantwell M, Yang SF. 1996. Factors influencing ethylene-induced isocoumarin formation and increased respiration in carrots. *J Am Soc Hortic Sci* 121(3):537–42.

Lessin WJ, Catigani GL, Schwartz SJ. 1997. Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. *J Agric Food Chem* 45(10): 3728–32.

Lucier G, Biing HL. 2007. Factors Affecting Carrot Consumption in the United States. http://www.ers.usda.gov/publications/vgs/2007/03Mar/VGS31901/VGS31901.pdf (accessed March 17, 2008).

Mazzoni V, Tomi F, Casanova J. 1999. A daucane-type sesquiterpene from *Daucus carota* seed oil. *Flav Frag J* 14:268–72.

Özcan MM, Chalchat JC. 2007. Chemical composition of carrot seeds (*Daucus carota* L.) cultivated in Turkey: characterization of the seed oil and essential oil. *Grasas Y Aceites* 58(4):359–65.

Peters S. 2006. Carrots: Enjoyed by Kids of All Ages. The Cutting Edges, eNewsletter 59: 1. http://www.seedsofchange.com/enewsletter/issue_59/carrots.asp (accessed August 23, 2007).

Phan CT, Hsu H, Sarkar SK. 1973. Physical and chemical changes occurring in the carrot root during storage. *Can J Plant Sci* 53:635–41.

Poncavage J. 1998. Carrot flavor secrets. *Org Gardening* 45(6):50–4.

Rosenfeld H, Aaby K, Lea P. 2002. Influence of temperature and plant density on sensory quality and volatile terpenoids of carrot (*Daucus carota* L.) root. *J Sci Food Agric* 82:1384–90.

Sarkar SK, Phan CT. 1979. Naturally-occurring and ethylene-induced phenolic compounds in the carrot root. *J Food Prot* 526–34.

Shallenberger RS, Atkins JD, Moyer JC. 1960. The isolation and partial characterization of a volatile essential oil from bitter canned carrots. *Food Res* 25:419–23.

Shamaila M, Durance T, Girard B. 1996. Water blanching effects on headspace volatiles and sensory attributes of carrots. *J Food Sci* 61(6):1191-5.

Simon PW. 1982. Genetic variation for volatile terpenoids in roots of carrot, *Daucucs carota*. Backcrosses and F2 generations. *Phytochemistry* 21:875–9.

Simon PW. 1985. Carrot flavor: Effects of genotype, growing conditions, storage, and processing. In Patte HE (ed.), *Evaluation of Quality of Fruits and Vegetables*. Westport, CT: AVI Publishing, pp. 315–28.

Simon PW, Goldman IL. 2007. Carrot. In Singh RJ (ed.), *Genetic Resources, Chromosome Engineering, and Crop Improvement Series*, Vol. 3. Boca Raton, FL: CRC Press, pp. 497–517.

Simon PW, Wolff XY. 1987. Carotenes in typical and dark orange carrots. *J Agric Food Chem* 35:1017–22.

Simon PW, Peterson CE, Lindsay RC. 1980. Correlation between sensory and objective parameters of carrot flavor. *J Agric Food Chem* 28:559–62.

Soujala T. 2000. Pre and postharvest development of carrot yield and quality. Academic dissertation, University of Helsinki, p. 47.

Sulaeman A, Keeler L, Giraud DW, Taylor SL, Wehling RL, Driskell JA. 2001. Carotenoid content and physicochemical and sensory characteristics of carrot chips deep-fried in different oils at several temperatures. *J Food Sci* 9:1257–64.

Sulaeman A, Giraud DW, Naslund MM, Driskell JA. 2002a. Mongolian gerbils can utilize provitamin-A carotenoids in deep-fried carrot chips. *J Nutr* 132:211–17.

Sulaeman A, Tan KB, Taylor SL, Driskell JA. 2002b. Sensory acceptability of deep-fried carrot chip product among American and Southeast Asian Consumers: A pilot study. *J Food Qual* 25:453–67.

Surles R, Weng N, Simon PW, Tanumihardjo SA. 2004. Carotenoid profiles and consumer sensory evaluation of specialty carrots (*Daucus carota* L.) of various colors. *J Agric Food Chem* 52:3417–54.

Szymczak P, Gajewski M, Radzanowska J, Dabrowska A. 2007. Sensory quality and consumer liking of carrot cultivars of different genotype. *Veg Crops Res Bull* 67:163–76.

Talcott ST, Howard LR. 1999. Chemical and sensory quality of processed carrot puree as influenced by stress-induced phenolic compounds. *J Agric Food Chem* 47:1362–6.

Talcott ST, Howard LR, Brenes CH. 2001. Factors contributing to taste and quality of commercially processed strained carrots. *Food Res Int* 34:31–8.

Törrönen R, Lehmusaho M, Häkkinen S, Hänninen O, Mykkänen H. 1996. Serum β-carotene response to supplementation with raw carrots, carrots juice or purified β-carotene in healthy non-smoking women. *Nutr Res* 16(4):565–75.

USDA. 2007. National Nutrient Database for Standard Reference, Release 20 (2007). http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl (accessed March 20, 2008).

Van Vliet T, van Schaik F, Schreurs WHP, van den Berg H. 1996. In vitro measurement of β-carotene cleavage activity: Methodological considerations and the effect of other carotenoids on β-carotene cleavage. *Int J Vitam Nutr Res* 66(1):77–85.

Varming C, Jensen K, Moller S, Brockhoff PB, Christiansen T, Edelenbos M, Bjorn GK, Poll L. 2004. Eating quality of raw carrots-correlations between flavour compounds, sensory profiling analysis and consumer liking test. *Food Qual Prefer* 15(6):531–40.

CHAPTER 41

Chili Flavor

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INTRODUCTION

Chilis are one of the most ancient constituents of spices and condiments. The discovery of chilis was credited to Christopher Columbus. It is an irony that Columbus, who wanted to reach India by sea route, found the American continent and discovered the presence of chilis rather than the other exotic spices associated with India. The ancient texts of India do mention the presence of chilis. However, the American continent was attributed with the origin of wild varieties of chilis as such. The usage of the word "chilli," usually spelt as "chili" in the United States, separates the commodity from black or white peppers, which are also pungent spice species and are highly popular in the exotic world of spices.

Chilis in a fresh state are rich sources of vitamin C, and isolation of vitamin C from paprika attracted worldwide attention during the exploratory period of vitamin C metabolism and the establishment of ascorbic acid as one of the most vital nutrients associated with a number of metabolic reactions in human beings. Chilis also possess interesting medicinal properties and are usually known as potential stimulants and are carminative in nature. The ancient texts describe the curative role of chilis in rheumatism and in skin diseases. Modern research did herald chilis as potential sources of antioxidants and other functional ingredients of nutraceutical importance. Ferrari and Aillaud (1971) gave a detailed bibliography concerning the various principal ingredients of chilis. Capsanthin is the most important pigment of capsicum and the pungent principle is capsaicin, which is present in the placenta and is said to retain its pungency even at a concentration as low as 1 ppm.

BIOLOGY AND CLASSIFICATION

The genus *Capsicum* belongs to the family Solanaceae, which has about 90 genera and approximately 2000 species of different habits. Heiser (1969) described around

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

TABLE 41.1. Key to the Cultivated Species of Capsicum

Index Code	Description	Botanical Nomenclature
A	Corolla lobes purple, seeds black	*C. pubescens*
AA	Corolla lobes white or greenish white, rarely purple, seeds light in color	
B	Corolla white with yellow or tan markings on throat, anthers yellow	*C. baccatum*
BB	Corolla without yellow markings on throat, anthers light blue to purple	
C	Corolla usually clear or dingy white, pedicels usually solitary at a node	*C. annuum*
CC	Corolla usually greenish white, pedicels usually more than one at a node	
D	Pedicels usually two per node, erect at anthesis, without distinct constriction with the calyx	*C. frutescens*
DD	Pedicels usually three to five per node, usually curved, with distinct circular constriction with the calyx	*C. chinense*

20 wild species of *capsicum*, most of which are of South American origin and two of the genera, *Capsicum annuum* var. glabriusculum and *Capsicum frutescens*, extending through Middle America to the Southern United States. As such, the species coming under chili peppers are extremely variable particularly in the characters of the fruit, and the variation is parallel. Linnaus recognized two species, that is, *C. annuum* and *C. frutescens*, in the *Species Plantarum*, published in the year 1753. During the next 100 years, a great number of additional species were described based largely on the characters of the fruit, so that when the genus was revised, 100 binomials were described (Irish 1898). The widely accepted classification was given by Heiser and Smith (1953) in which five cultivated species were reported following the key given in Table 41.1.

Small-fruited forms of the species occur widely across Southern United States through Mexico and Central America to Northern America. Heiser and Pickersgill (1975) revised *C. annuum* var. minimum (Miller) Heiser to *C. annuum* var. glabriusculum (Dunal) Heiser and Pickersgill. These fruits are extremely pungent and are not cultivated, and the origin is largely restricted to wild growth. *C. annuum* var. annuum is an economically important chili variety, and it is basically a large-fruited variety grown mostly in Central America. The fruit is a multi-seeded berry, pendulous or erect, and is usually borne singly at the nodes.

C. frutescens L. as a cultivated plant, the species in much less variable with a more restricted distribution than *C. annuum*. The variety Tabasco is the only cultivar grown in the United States. It is a short-lived perennial shrub. The corolla is greenish white, waxy, or shiny. The fruits are usually small and narrow, having green and yellow coloration when immature, turning red at maturity.

Capsicum baccatum L. is South American in origin and the widely cultivated variety is var. pendulum. Countries such as Argentina, Bolivia, Brazil, Chile, Ecuador, and Peru are known to cultivate this variety extensively (Pickersgill 1969).

Capsicum chinense closely resembles *C. frutescens* but can be distinguished by the pedicels, which are usually shorter, thicker, and curved. It is commonly cultivated in South America and in the West Indies. The fruits are long and elongated and are red pinkish in color when mature.

Capsicum pubescens is a distinct species and can be distinguished from other cultivated species by pubescens, by its blue or purple flowers. It is a high-altitude variety and is grown in countries such as Bolivia and Peru.

CULTIVATION AND CLIMATE

Chilis are grown in the tropics from sea levels to 2000 m or more. Generally, an optimum temperature of 24°C and at least 3 months of warm weather is required for good yields. The small fruits especially those of *C. frutescens* are much more tolerant to hot weather. An annual rainfall of 60–125 cm is required to grow chilis, and in India, the crops are grown throughout the year, excluding the period of January to March to avoid frosting. Well-drained heavy soil is preferred, loamy in nature, and the optimum soil pH is 6.0–6.5.

The crop may be sown directly in the field as in the case of Southern United States, or a nursery is raised on seed beds and then transplanted into the field. The usual spacing that is followed in between the rows is 60 cm, and about 17,500 plants could be accommodated per hectare. The usual spacing followed in India is 60 × 15 cm. The hot chili peppers are late maturing (100–115 days) compared to sweet pepper (58–82 days). The average yield of dry red chilis in a rain-fed crop in India is 280 kg/ha; however, the yields are largely variable depending on the fertility of the soil, agro-climatic conditions, and genetic lines.

HISTORY

Historically, the discovery of the American continent by Christopher Columbus is associated with the unraveling of peppers. It was popularly known by the famous quote "Columbus never reached the spices of far East, he did find one that has come to rival them." There is evidence that chilis were eaten by Indians perhaps as early as 7000 BC. Heiser (1969) says that the Indians were actually growing the plants between 5200 and 3400 BC. Chili comes from the dialects of Mexico and Central America. It is presumed that capsicum must have been taken by Columbus to Europe and has found its way to the Southeast Asian countries. *C. annuum* var. *annuum* and *C. frutescens* were spread to most of the warmer regions of the world, and the later species became naturalized in many tropical countries. India is the largest producer of chilis in the world, accounting for about 12–14 lakh tons of production annually, followed by China, with a production of about 4 lakh tons, Mexico with a production of 3 lakh tons, and Pakistan with 3 lakh tons. The extent of pungency depends on the cultivars, and some of the hottest chilis are grown in India. Varieties such as Bhut Jolokia and Naga Jolokia are very popular, and in terms of international pungency units known as Scoville heat units (SHUs), they are rated above 1,000,000 units. The extent of pungency in these cultivars is yet to be commercially exploited, as most of the Indian varieties that are commercially used have SHUs in the range of 10,000–20,000 units only.

DISEASES

Chili crops are susceptible to a number of diseases and the pathogens could be fungi, bacteria, or viruses. The damping of disease usually occurs in the nurseries due to infestation by *Rhizoctonia solani* (Kuhn) and *Pithium* sp. The seed may rot or the seedlings may be killed before emerging from the soil. The soft stems of young seedlings may also be attached after emergence, causing shriveling of the stems. Generally, captan is sprayed on the seeds or on the seed bed. Bacterial spot caused by *Xanthomonas vesicatoria* (Dows) can cause serious injury to capsicums, both on the leaves and on the fruits. The disease causes spots of yellowish-green color on the leaves and the severely spotted leaves turn yellow and brown. The bacteria are also soil borne and therefore crop rotation is of vital importance in avoiding the disease. Blight disease or root rot caused by *Phytophthora capsici* had also been described, and the symptoms include the appearance of dark, water-soaked patches on the fruit, which become coated with the growth of the fungus. *Fusarium annum* causes fusarial wilt characterized by drooping of lower leaves. The disease could attain highly damaging proportions for crops raised on poorly drained land and on temperatures above 27°C. Fruit rots caused by *Colletotrichum capsici* were also found to cause damage to the same extent as that of anthracnose caused by *Gloeosporium piperatum*. The curly top virus and also the tobacco mosaic virus cause immense damage in terms of the yield and quality of the crop. In addition to pathological diseases, a number of physiological disorders were also described causing blossom end rot and sunscald.

CHILI FLAVOR

Chili flavors were characterized as a mixture of pyrazines, that is, 3-isopropyl-2-methoxy pyrazine, 3-butyl-2-methoxy pyrazine, and 3-isobutyl 2-methoxy pyrazine (Murray and Whitfield 1975). The pungency contributing principles are the capsaicinoids, which are vanillylamides of various acids, out of which capsaicin as vanillyl amide of isodecanylic acid is the most important. Some of the most important capsicum-based flavors are as follows:

Source: *C. annuum* L.

Plant part: the ripe fruits

The inner pericarp and seeds are removed, entirely or partly, when a less sharp taste is desired.

Components: A sharp-tasting capsaicinoid (0.3–0.5%), in addition to nordihydro- and homodihydrocapsaicins, was found (Purseglove et al. 1981).

The main aroma constituent was described as 2-methoxy-isobutylpyrazine (van Ruth and Roozen 1994). A steroid saponin mixture called capsicidin with capsioid was the main constituent, which functions antibacterially against yeast and fungi. The carotenoids, partly esterified with fatty acids, mainly capsanthin, α-carotene, and violaxanthin, contribute toward the color. Apart from the organoleptic determination of pungency (SHUs), the capsaicinoids are determined quantitatively with high-performance liquid chromatography (HPLC) (Kurian and Starks 2002). The

quantitative determination of pellargonic acid and vanillylamide, which are available synthetically and are therefore prone to adulteration, is of special importance. Red pepper and the corresponding oleoresins are used for coloring purposes. Methods for their quantitative determination are extensively researched to develop appropriate photometric methods after isolation by thin-layer chromatography (TLC) with simultaneous detection of synthetic dyes.

CHILIS (PEPPERONI)

C. frutescens is a major species being used for the extraction of pepperoni flavor. Due to their higher capsaicinoid content, the fruits are considerably sharper in their pungency, and the basic constituents are the same as those of red pepper. Haymon and Aurand (1971) detected various esters of butyric, valerianic, and caprionic acids; however, no typical aroma constituents were detected.

The component in capsicum, which stimulated the valued pungency, was crystallized and named capsaicin by Thresh in 1946. With the advancement in TLC, Kosuge and Inagaki (1959) showed that two related components are found in capsicum extracts, both of which stimulate pungency. They showed that the ratio of the two components did not vary with the variety they studied, the degree of maturity, and harvest, and the term "capsaicinoids" for the mixture of related components was proposed. Bennett and Kirby (1968) showed that crystalline isolates from capsicum extract contained two major components, capsaicin (69%) and dihydrocapsaicin (22%), and three minor related components; nordihydrocapsaicin (7%), homocapsaicin (1%), and homodihydrocapsaicin (1%) (Fig. 41.1).

Within the genus *Capsicum*, numerous species and varieties are cultivated and the highest level of pungency is associated with *C. frutescens*, whereas *C. annuum* comprises rather mild varieties (Jurenitsch et al. 1979). Yazawa and others (1989) reported the presence of capsaicinoid-like substances (CLSs) in a cultivar named CH-19 Sweet of *C. annuum*. These CLS fractions have different resolution front (RF) values in TLC and are termed as CLS-A and CLS-B. Fraction A was known as capsiate and fraction B is known as dihydrocapsiate. Chemically, CLS-A was determined as 4-hydroxy-3-methoxybenzyl (E-B-methyl-6-nonenoate), whereas CLS-B was found to be a 6, 7-dihydro derivative of CLS-A (Kobata et al. 1998). The turnover of capsaicinoids during development, maturation, and senescence of chili peppers was related to peroxidase activity, and the content of capsaicinoids was found to decrease with an increase in peroxidase activity (Padilla and Yahia 1998). As such, the biosynthesis of capsaicinoids follows the cinnamic acid pathway, and the same had been described to occur in the placenta of the fruit (Fuzikawe et al. 1982). During the growth of chilis also, the turnover was found to be varied among different cultivars with a maximum buildup occurring at a specific stage of maturity followed by a decline (Iwai et al. 1979). Peroxidase causes the oxidative deterioration of capsaicin in the presence of hydrogen peroxide. Cellular disruption of *C. annuum* was found to result in significant levels of oxidative deterioration. Kirschbaum-Titze and others (2002) noted that the size of capsicum segments influenced the deterioration, and halving was found to restrict the degradation compared to smaller precut slices. Maintenance of minced fruits under nitrogen atmosphere was found to stabilize the capsaicinoid content. The stability of

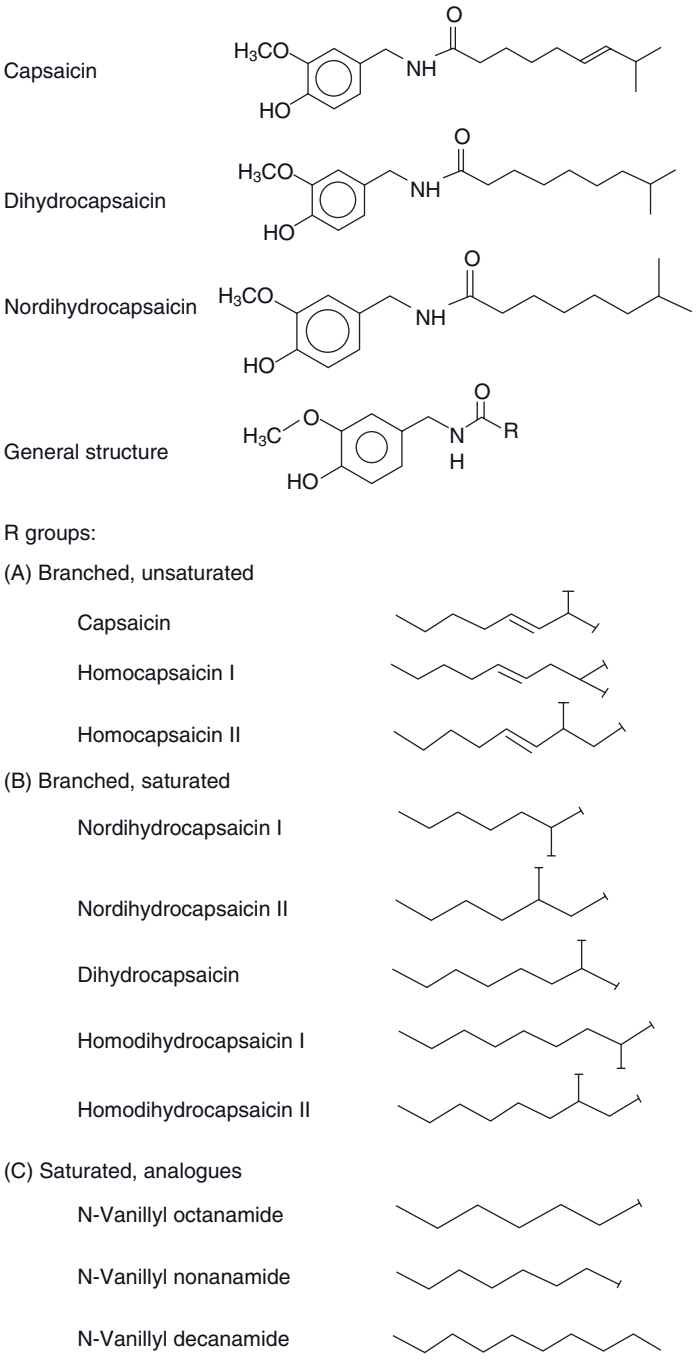

Figure 41.1. Pungent principles of chilis.

capsaicinoids during processing, extractions, and storage of flavor components is of critical importance in the commercialization of technologies concerning chili flavor (Govindarajan 1986).

BIOSYNTHESIS

The biosynthesis of capsaicinoids coincides with the synthesis of lignin-like material, and the two are the major end products of phenyl propanoid metabolism. It was known that the synthesis of these products depends on the supply of intermediates, and a strong competition was observed between lignification and capsaicinoid formation (Sukrasno and Yeoman 1993). Lignin formation could be linked with the development of seeds, and the capsaicinoids are synthesized in the placenta. The biosynthetic pathway of capsaicin follows a condensation process involving vanillyl amine and isocapryl–succinyl coenzyme A (SCoA). Phenylalanine is the precursor for vanillyl amine with the formation of intermediates such as cinnamic acid and coumaric acid. The condensed product of valine and acetyl-CoA results in the formation of isocapryl-CoA, which further condenses with vaniline amine to form capsaicin (Fig. 41.2). Active accumulation of cinnamyl glycosides and flavanoids both synthesized from phenylalanine before the onset of accumulation of capsaicinoids was found consistent with phenylalanine ammonia lyase (PAL) activity (Yeoman et al. 1989). The cinnamyl glycosides undergo transformation, resulting in synthesis of capsaicinoids. Vanillic acid glycoside was found to be present in the fruits of *C. frutescens* with close proximity to capsicum present in the placenta. As such, the biosynthesis of capsaicin was known to be associated with the cinnamic acid pathway, and the networking encompasses the formation of lignin, flavonoids, phenolic, and cinnamyl glycosides (Fig. 41.3).

EXTRACTION

Flavor extraction from different plant sources is an ever-evolving technology. Since flavors constitute a vast commercial market and food flavors are of the commodities industry, the cosmetic industry constitutes a highly competitive area of flavor technology. A food flavor could render aroma as well as taste, and in the general sense, the aroma gains dominance over the taste part. Cosmetic flavors form an amalgamation of trade formulations that are highly confidential and branded. The food flavors are open to a certain extent as the consumers need to know the nature of the flavor as they identify the available flavor with that of one which has a natural origin. Flavors could be of natural origin or synthetic in nature upon identification of critical chemical structures associated with natural flavor. In the industrial production of natural flavors, extraction plays an important role in obtaining the maximum recovery with minimal process losses of flavor. The plant tissue needs to be disrupted by mechanical, thermal, or enzymatic methods to allow the extraction of the flavor and aroma materials with high yields. The aroma materials as such could be distributed throughout the plant material or they could be localized in specialized plant structures such as the oil sacs that hold mint oils present on the underside of the mint leaves. Plant cell walls are basically three layers: the middle lamellae and the primary and secondary cell walls. The middle lamellae bind the cells and are mostly

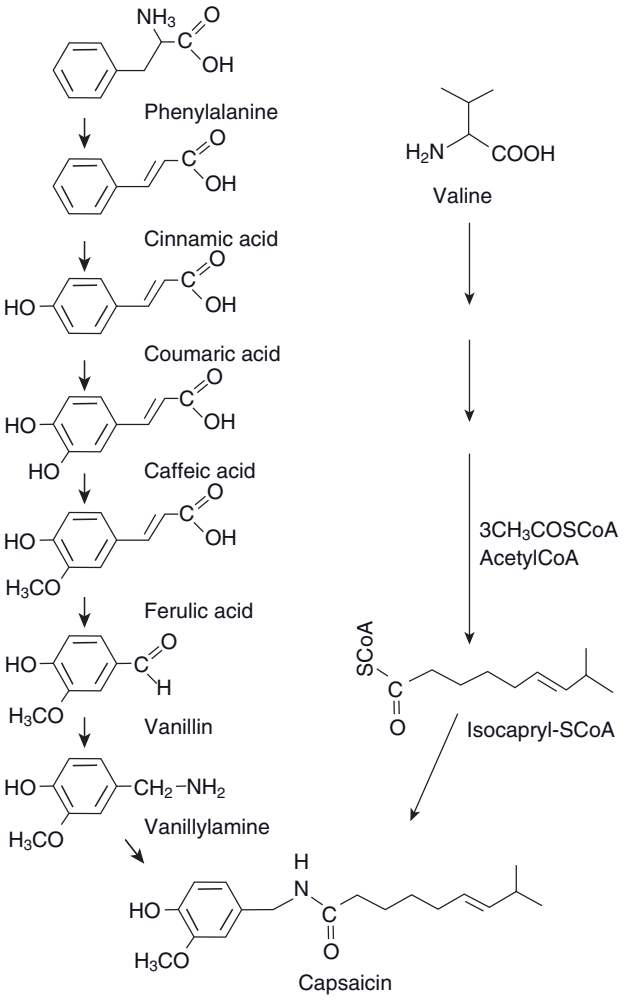

Figure 41.2. Biosynthetic pathway of capsaicin.

composed of pectin. The primary cell wall consists of cellulose fiber together with pectin, hemicelluloses, and proteins. The secondary cell wall contains lignin and pectin. These cellular structures need to be disrupted to release flavor and aroma chemicals contained with the cells. The enzymes commonly used in the manufacture of plant extracts include polygalacturonase, pectin or pectate lyases, esterases, cellulases, and hemicellulases.

The methods of flavor production are mainly categorized into four types:

1. by direct extraction from the natural source,
2. by compounded flavors as mixtures of chemically or naturally synthesized flavor molecules,
3. reaction flavors by compounding appropriate precursor molecules, and
4. enzyme- or fermentation-linked production of flavors.

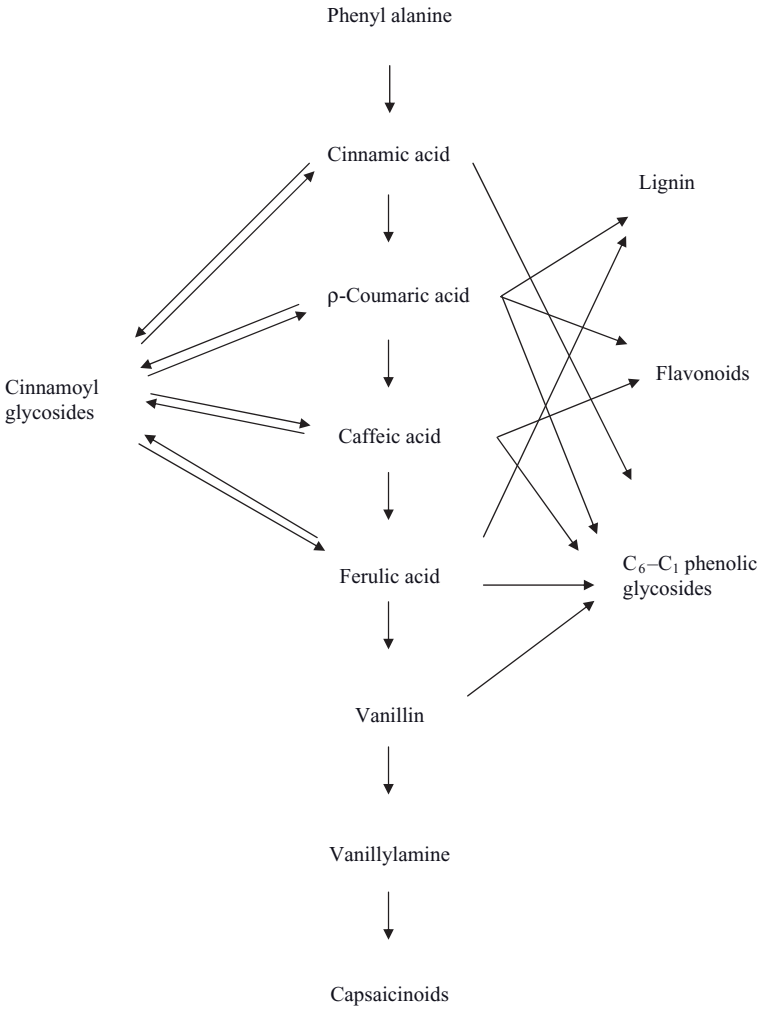

Figure 41.3. Biosynthetic relationship between phenolic compounds and capsaicinoids.

The direct extraction methods often include steam distillation and cold expression, and the terminology adopted for the product depends on the nature of the extracts, that is, essential oil, absolute extract, resinoid, and oleoresins. The oleoresins include many of the nonvolatile components that are otherwise not present in the corresponding essential oils. Capsicum flavors include oleoresins as a major product. The compounded flavors require a precise chemical analysis for synthesizing similar compounds followed by formulation as a fully compounded or semi-compounded product. Maillard reaction products are the major reaction flavors using sugars and amino acid sources besides sulfur-containing compounds followed by heating to accelerate the chemical reaction. The enzyme or fermentation-based reactions could be expensive initially, but the prices do get stabilized due to the emergence of demands.

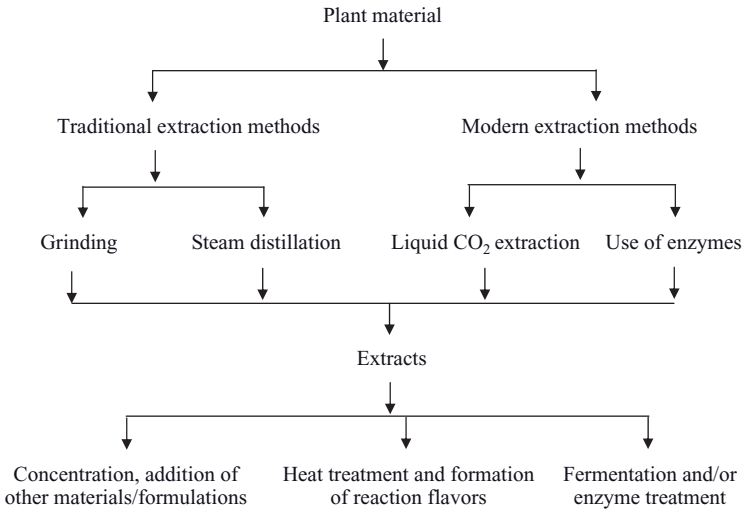

Figure 41.4. General methods of flavor production.

The directly extracted flavors offer a range of technological feasibility, and capsicum flavors could be cost effectively extracted using the standard procedures (Fig. 41.4).

The range of directly extracted flavors includes a variety of entrapment methods meant for extraction of exudates giving rise to resinoid, formation of concretes by nonpolar extraction, absorption into fat known as enflurage, or ethanol extractions to form tinchers. A newer method of flavor extraction aims at flavors drawn from glycosidic molecules (Spanier 1993).

EXTRACTION METHODOLOGIES

Solid–Liquid Extraction

The principle of solid–liquid extraction consists of adding liquid solvent to a solid matrix in order to selectively dissolve and remove the solute. The chosen extractants must be capable of preferentially dissolving the compound to be extracted. Solid–liquid extraction methods are applied to the industrial scale in the flavor industry apart from oils and fats. Red pepper is largely subjected to solid–liquid extraction methods apart from pepper, mint, vanilla, and licorice. The major characteristic of solid–liquid extraction lies in the fact that there is no defined distribution coefficient for the distribution of solutes in extracts and feeds (Gnayfeed et al. 2001). The equilibrium is never reached practically as the solid matrix still contains bound solute in the capillaries. It is advisable to carry out the practical measurement of moisture content, reduction ratio, and type and amount of solvent to be used to optimize the process. The polarity of the solvent also plays an important role in optimizing the extraction procedures.

Maceration Maceration is usually carried out by crushing, grinding, or cutting procedures. Maceration can be improved by agitating the extraction material with

mixtures, stirring devices, or homogenizers. The Use of acoustic waves also plays an important role, and a range between 25 and 1000 kHz has been found to be highly supportive in these aspects (Ziegler and Ziegler 1998). Similarly, electrical discharges with a broad frequency can be helpful in improving extraction. Changes in temperature and pH can also have an impact on the yield and quality of the extract.

Counter and Current Extraction The process is called percolation if the solid matrix is repeatedly extracted with fresh solvent. This process could be used for laboratory or industrial purposes. In the laboratory, the process could be carried out with Soxhlet extraction. Kurian and Starks (2002) developed single-stage quantitative extraction procedures for the extraction of capsaicin in dried and moist forms for HPLC analyses, which do not require extensive cleanup as compared to other methods. The countercurrent extraction could be continuous, discontinuous, or of absolute countercurrent methods. The differences in the extraction procedures depend on the movement of the solvent and the feed in terms of direction and mode of solvent application.

Liquid–Liquid Extraction

Liquid extraction is an important separation technique for laboratory use during which the aromatic compounds are obtained with glycolin and sulfolane. The principle of liquid–liquid extraction involves adding of liquid solvents to a mixture in order to selectively remove components by the formation of two immiscible liquid phases. The selected solvent shall be capable of preferentially dissolving the constituents to be extracted besides being immiscible or at least partially miscible to facilitate separation of layers subsequently. A number of factors including polarity and dielectric constants of the solvents play an important role in selecting appropriate solvents to optimize the process (Wooderck et al. 1994). The process could be single- or multistage extractions in continuous or discontinuous modes. The process could be limited to either single or multistage procedures. The equipment required for such extractions include a variety of extractions such as centrifugal extractors, extraction towers, mechanical agitators, and others.

Supercritical Fluid Extraction

Selection of solvents depends on a number of factors in conventional solvent extraction procedures, such as evaporation enthalpy–selectivity, specific heat capacity, combustibility–stability, flash point–reactivity, explosion limits–viscosity, maximum allowable working concentration–surface tension, and environmental relevance–boiling point.

The constraints are too many to select the appropriate solvent, and an ideal solvent meeting all the requirements besides the safety features is still at large. Another limitation of conventional solvent extraction is the formation of by-products, which have toxicological implications besides causing environmental hazards (Gnayfeed et al. 2001). These limitations lead to the invention in terms of use of gas-based solvents in a near-critical state. Supercritical fluid extraction, also referred to as dense gas extraction or near-critical solvent extraction, denotes the operational temperature of the process to be close to the critical temperature of the

solvent. Since the extraction of herbal raw materials requires nondrastic, gentle process temperatures, the choice of suitable near-critical solvents is limited to pure or partly halogenated C_1–C_3 hydrocarbons and carbon dioxide. Carbon dioxide is the most favored supercritical fluid extraction medium due to many reasons, such as adjustable selectivity, low dissolving power, high diffusion rates, low viscosity, exclusion of oxygen, restricted thermal stress, no solvent residues, easy solvent recycling, stable and inert behavior, and its being bacteriostatic, nonflammable, inexpensive, and environmentally safe (Tipsrisukond et al. 1998).

SALIENT FEATURES OF CO_2 EXTRACTION

CO_2 extracts are by nature lipophilic products. A number of volatile fractions, that is, monoterpines, phenyl propane derivatives, and sesqueterpines and oxygenated molecules like ethers, esters, ketones, lactones, and alcohols, are easily soluble, and all of them are typical components of essential oils. The solubility decreases with an increase in molecular weight and polarity. Therefore, oils, resins, steroids, alkaloids, carotenoids, and oligomers are less soluble. Supercritical carbon dioxide (SC CO_2) offers the possibility to change the solvent power within a wide range by adjusting the gas density. Liquified CO_2, in contrast, is more similar to normal solvents without the possibility to influence the dissolving power. Gnayfeed and others (2001) described SC CO_2-based extraction of ground paprika (*C. annuum* L.) and also subcritical propane at different conditions of pressure and temperature to estimate the yield and variation in carotenoids, tocopherols, and capsaicinoid contents and composition. The yield of paprika extract was affected by the extraction condition with SC CO_2 compared to subcritical propane. A maximum yield of oleoresin at 7.9% was reported. However, SC CO_2 was found suboptimal in the extraction of diesters of xanthophylls even at 400 bar and at 55°C. On the other hand, tocopherols and capsaicinoids were easy to extract at these conditions. A number of workers reported SC CO_2 as an effective mechanism to extract the pungent principles from spice paprika. Coenen and Kriegel (1983) described the use of supercritical gases on commodities such as spice paprika. Knez and others (1991) and Knez and Skerget (1994) highlight SC CO_2 as an effective extraction medium for capsicum flavors. Coenen and Hagen (1983) found SC CO_2 an effective solvent for the extraction of natural colorant from paprika. The paprika extracts with SC CO_2 were light red to red in color, and the extraction pressures from 200 to 400 bars showed positive correlation with the rate and yield of extraction along with the density of the extraction medium. The impact of extraction conditions on the solubilization of oleoresins from pungent paprika by SC CO_2 was similar to that noticed with non-pungent paprika (Illes et al. 1997). Galan-Jaren and others (1999) reported that oil and pigment extraction by SC CO_2 is pressure dependent. Skerget and Knez (1997) described the relative solubilities of β-carotene and capsaicin in high-density CO_2 on the basis of capsaicinoids extracted from a known quality of powder; the recovery of these compounds to significant levels could be achieved at 400-bar pressure at 55°C. However, the use of modifiers could not substantially increase the recovery of capsaicin (Yao et al. 1994). The other reports on capsaicin extraction from red peppers also describe SC CO_2 as an effective extraction medium (Yasumoto et al. 1994).

The extraction of capsaicin with SC CO_2 laid emphasis on relative solubilities of β-carotene and capsaicin, and model systems were worked out as binary or tertiary models. The data on binary systems were vast (Sakaki 1992). However, as a tertiary system with dense CO_2 as a component of the system and in comparison with liquid and supercritical forms, the results were highly interesting due to the establishment of equilibrium solubilities (Skerget and Knez 1997; Skerget et al. 1995). The extraction of paprika with organic solvents was known to cause some disadvantages causing extract denaturation. The process for production of paprika oleoresin has been described by many workers (Coenen et al. 1983). The various extraction models with regard to relative solubilities in SC CO_2 could be optimized with better recovery profiles and associated advantages with the addition of polar co-solvents such as 2-3, dimethyl phenolphthalein, and phenanthrine as described by Dobbs and others (1987).

QUANTIFICATION OF PUNGENCY

Pungency of chili flavors is an inseparable attribute that needs authentic quantification to determine the quality of the product. The nature of the pungency stimuli is extensively researched by Suzuki and Iwai (1984). The flavor profiles depend largely on the species as well as on varietal differences. In the case of *C. annuum* var. *annuum*, dihydrocapsaicin and capsaicin dominate the flavor, whereas nordihydrocapsaicin plays a secondary role toward the overall contribution to pungency. In the case of *C. frutescens*, the overall pungency is largely dominated by capsaicin alone with dihydrocapsaicin getting restricted to a secondary role in order to regulate the flavor profiles. Process optimization during extraction and also prevention of possible adulteration in authentic quantification schedule are highly essential. It is also known for a long time that synthetic nonoylvanylamide could be found in varying extents in commercial oleoresins from chilis. Therefore, it is highly essential to determine the upper limits of the natural occurrence of straight-chain analogues to determine the extent and type of adulteration.

ANALYTICAL METHODOLOGIES

The methods include subjective as well as objective methods. The subjective methodologies are time tested, and chili pungency is still widely reported as SHUs.

Scoville Test

Scoville test for chili flavor was reported by Scoville (1912). The subjective method was adopted and modified by American Spice Trade Association (ASTA 1968) and by the International Organization for Standardization (ISO 1983). In the Scoville test, samples were extracted as per the standard procedure described by ASTA methodology (21.0). In the sensory evaluation for pungency, six panelists could be used, three male and three female within the age group of 21–30 years, who were trained in pepper pungency evaluation trials. The tasters need to continue through a sequence of dilutions until a definite sensation was noted. The heat units for the

solutions for which the first three of the five panelists reported positive values are recorded. During the course of time, SHU testing underwent a series of changes. One of the foremost among them had been the conversion of objectively estimated capsaicinoid concentrations by making use of a coefficient for each compound (Todd et al. 1977). The coefficients reported were 16.1 for capsaicin and dehydrocapsaicin and 9.3 for nordihydrocapsaicin.

Gillette Method

Another popular method for subjective evaluation is the Gillette method. Although this method is not as popular as the Scoville test, positive correlations were obtained highlighting the usefulness of the test (Quinones-Seglie et al. 1989). In the Gillette test, a partially balanced incomplete block design could be used with four different samples per session amounting to nine sessions and four replications as such. The panelists score the samples on an intensity scale of 1–9 from no heat to extremely hot. The methodology as such involves dissolving 50 g of freeze-dried ground capsicum in 199.5 g of commercially available springwater at 90°C followed by extraction for 20 min. The extraction is followed by filtration with subsequent dilution in springwater to 10-fold. The testing procedure involves clearing of the mouth with springwater at 20°C. The samples are swallowed slowly and after 30 s are rated on the ballet followed by clearance of mouth with springwater at 20°C. Unsalted crackers are used for mouth clearance for 60 s followed by rinsing with springwater at 20°C.

LIMITATIONS OF SUBJECTIVE METHODS

The Scoville test method had been severely criticized but continues to be employed as an authentic method for capsicum pungency (Maga 1977; Suzuki et al. 1980). The specific problems associated with the Scoville test are buildup of heat, rapid test fatigue, ethanol bite, lack of statistical validity, lack of reference standards, a long extraction time of 16 h and poor reproducibility.

The Gillette method was considered by ASTM (ISO 1983) as the method that could restrict problems such as high taste fatigue and heat buildup. The increased threshold is accounted for by the use of standardized initial samples as well as a time-related rise between the samples. The extraction time is reduced from 16 h to 20 min in the Gillette method, and ethanol bite is avoided by the use of aqueous extraction.

OBJECTIVE METHODS

The objective methods could be grouped into four types depending on the cleanup and instrumental procedures as well as the involvement of chromogenic reactions or the use of chromatographic methods:

1. chromogenic reagents reacting with the phenolic hydroxyl group of the vanillyl fraction in the extracts;

2. removal of interference by natural pigments, that is, carotenoids, by a cleanup step before using chromogenic reagents;
3. improved chromatography cleanup and use of specific chromogenic or spectrophotometric measurement for the development of micro methods; and
4. use of HPLC methods for the separation of individual capsaicinoids.

The earlier methods included chromogenic reactions from crude extracts using vanidiumoxytrichloride or ammonium vanadate and hydrochloric acid to react with the phenolic hydroxyl group of the vannyl amide followed by measurement of the blue color. The presence of natural colors within the extract was compensated by use of natural carotenoids, synthetic colors, or inorganic mixtures of cupric nitrate and potassium dichromate (Suzuchi et al. 1957).

Kosuge and Inagaki (1959) determined capsaicinoids in ether-extracted concentrates taken in carbon tetrachloride, washed with acetic acid, and reacted with Folin–Ciocalteu reagent. The blue color was measured at 750 nm and was quantified using pure vanillin as standard and a factor of 2.15 to obtain capsaicinoid content. Though the method appears to yield very good results, a large number of determinations are required in breeding and selection programs.

METHODS BASED ON SEPARATION

Solvent Partition

Capsaicinoids could be separated from pigments by repeated partitioning between alkaline polar and nonpolar solvents. The capsaicinoids with only a trace of color and fat are estimated by reacting with phenolic reagents, that is, phosphomolybdic or phosphotungstic reagents followed by measurement of the blue color at 725 nm. Tirimanna (1972) used a more elaborated purification procedure with similar solvent partitioning to remove pigments and capsaicinoid phenolics by the judicious use of TLC methods.

Column Separation

Charcoal or alumina columns are widely used for absorption and elution of capsaicinoids. The purified capsaicinoids were quantified using crystalline capsaicinoids as standard by colorimetry involving reaction with diazobenzene sulfonic acid (Brawer and Schoen 1963). The column separation involved extraction followed by separation, elution, and measurement.

A joint committee (Anon. 1959) on methods for assay of crude drugs in the United Kingdom surveyed the methods and selected two methods for capsaicinoid separation:

1. acidic alumina column and illusion with absolute methanol and
2. ether-alkali partitioning extraction and quantification by spectrophotometric or colorimetric methods.

The recommendation also includes the use of diazo color reagents with Gibbs phenol reagent (2, 6-dichloro parabenzoquinone-4-chlorimine).

Chromatographic Micro Methods

This layer chromatography could give a new dimension in the separation and quantification of capsaicinoids. Dohman (1965) described a comprehensive method by TLC for the separation of capsaicinoids from the chloroform extracts of spice capsicum on silica gel G plates along with standard capsaicinoids. The plate is developed with chloroform–methanol–acetic acid (95:5:1 v/v/v) to a distance of 14 cm. The bands are marked as dark areas under UV light. The separated bands of capsaicinoids are detected with Folin–Denis reagent, and the capsaicinoid concentration is calculated spectrophotometrically at 725 nm.

Capsicum fruits in the maturing green stages are used regularly in the growing countries for making certain types of sauces and chutneys. There is also a growing report of green paprika stimulating mild pungency. These require a rapid quality control protocol for pungency evaluation. Isopropanol extracts of ground fresh capsicum fruit are treated with carbon to remove color then concentrated under vacuum and low temperature. The residue is dissolved in isopropanol to definite volume and absorption measured at 280 nm and quantified with respective pure capsaicinoid as reference (Gonzalez and Altamirano 1973).

Nagin and Govindarajan (1985) found acetone extraction of capsicum fruits resulting in poor extraction and mashy layer formation. Careful sun-drying of cut sections of green fruits to about 10% moisture allowed grinding of the samples and clean solvent extraction.

Gas chromatographic analysis of capsaicinoids also received major attention. Muller-Stock and others (1971) used 1% or 3% JXR on a Gaschrome Q (100–200 mesh) column using a linear temperature program run at 150–230°C at 6°C/min. The components were identified by a combined mass spectrophotometer and were quantified by an automated area integrator. The peak profile included a minor peak for nordihydrocapsaicin, a dominant peak for capsaicin, and an overlapping but distinct peak of dihydrocapsaicin.

Comprehensive literature exists with regard to the percentage of capsaicinoid component with emphasis on capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and octanyl vanillylamide in cultivars of *C. annuum*, *C. frutescens*, *C. baccatum* and *C. pubescens* (Govindarajan et al. 1986). The ratio of capsaicin to dihydrocapsaicin had been considered as characteristic of the species, variable but generally about 1:1 for *C. annuum*, 2:1 for *C. frutescens* and *C. baccatum*, and 0.7:1.0 for *C. pubescens*.

Modern HPLC technique dominated the scenario due to superior separation capabilities for closely related compounds, and the separation could be carried out at room temperature. Maillard and others (1997) reported a comprehensive account of 11 capsaicinoids by reversed-phase HPLC. More than 90 different capsicum varieties were evaluated using C-18 Hypersyl column eluted with ethanol–water–acetic acid (60:39:1 v/v/v). The maximum capsaicinoid content was reported for *C. frutescens* followed by *C. pubescens*, *C. baccatum*, and *C. annuum*. The fractions were coupled with nuclear magnetic resonance (NMR) analysis confirming the structural aspects of already known capsaicinoids. It was found that all the characterized capsaicinoid compounds possess a vanylamide structure and that the major differences were found to be in the alkanyl or alkanyl radicals of the acidic part of the molecule. The method was also applied to commercial oleoresins of capsicum, that is, cayenne

pepper and *C. frutescens*. The oleoresins were found to have equal amounts of capsaicin and dihydrocapsaicin (35%), and other components were found to be nordihydrocapsaicin (11.7% in *C. frutescens* and 8.4% in cayenne pepper) followed by octanyl vanyl amide (4–5%) and nonanylvanyl amide (4–5%).

Kurian and Starks (2002) described the HPLC separation of capsaicinoids from whole orange Habanero chili peppers. *C. chinense* (orange Habanero) pepper samples were analyzed, and capsaicin to an extent of 1250 ppm and dihydrocapsaicin to an extent of 540 ppm were reported on a fresh weight basis.

The above described analytical methods are extremely important in breeding and selection procedures to develop improved germplasm besides their role in quality control and inspection regimes of peppers and their oleoresins.

QUALITY OF CHILI FLAVORS

The quality of chili flavor has a lot to do with the principle of pungency alone. However, a number of other quality attributes such as size, shape, color, and firmness play an important role. Chili flavor, however, is linked with pungency; the flavor profile includes the aroma apart from the taste. Pungency is basically a mouthfeel, besides which also contributes to a strong aroma sensation. Weisenfelder and others (1978) gave a comprehensive account inclusive of subjective and objective quality attributes of jalapeño chilis. The authors reported a poor correlation between the total capsaicinoid content and pungency as such, and the capsaicinoid content covered a range of 0.60–1.65 mg/100 g. The correlations of sensory evaluation with instrumental measurement probably became flawed due to poorly defined scale descriptions and details of evaluation resulting in confused evaluation patterns.

The pungency testing by subjective means need to be defined appropriately in terms of direct biting, dilution to use level, or by threshold test. Direct testing may overpower the taste perception. On the other hand, aroma needs to be discriminated by comminution and sniffing under standard conditions. Determination of threshold pungency levels for optimal flavor evaluation received widespread acceptability. Rajpoot and Govindarajan (1981) reported sensory methodology using a pungency detection scale of 1–6 where a rating of 1 represents "definitely not detectable" and 6 represents "definitely detectable." The authors also recorded that Scoville value was not influenced by the interstimulus duration from 20 s to 4 min. The sugar concentration of the dilution medium ranged from 0% to 10%, and the swallow volumes varied from 2.5 to 10.0 mL. Suzuchi and others (1957) showed that 3% solution in place of 5% interferes less with detection and recognition of pungency. The method described by Gemert and others (1983) could be applied to chili oleoresins using the usual threshold experiment, which showed high correlation with total capsaicinoid content. Figen and others (2002) gave regression coefficient-based conversion formulae for the calculation of Scoville scores from capsaicinoid contents:

$$\text{Scoville score} = 76.8 \times (\text{capsaicin level, mg/100 g}) + 2691.0,$$

$$\text{Scoville score} = 88.2 \times (\text{dihydrocapsaicin level, mg/100 g}) + 3453.6, \text{ and}$$

$$\text{Scoville score} = 42.7 \times (\text{total capsaicinoid level, mg/100 g}) + 2935.1.$$

TABLE 41.2. Requirements of the U.S. Government Standards

Characteristic	Percentage on Material as Received				
	Paprika	Red Pepper			Chili Pepper
Moisture, not more than	12.0	10.0			—
Total ash, not more than	8.5	8.0			—
Acid insoluble ash, not more than	1.0	1.0			—
Extractable color (expressed as ASTA color units), not less than	110.0	—			70.0
Scoville pungency (expressed as pungency rating units)	—	30,000–55,000			—
Sieve test					
U.S. standard sieve size	No. 30	No. 4,	no. 8,	no. 20	—
Percent by weight required to pass through, not less than	95	95	85	—	—
Percent by weight retained, not less than	—	—	—	95	—

Source: U.S. Federal Specifications: spices, ground and whole, and spice blends, No EE-S-63H, June 5, 1975.

The regression coefficients for capsaicin, dihydrocapsaicin, and total capsaicinoid levels against Scoville scores were found to be 0.89, 0.85, and 0.91, respectively. An electronic nose was used to discriminate ground red pepper samples by headspace volatiles. The classification of ratings was carried out based on the electronic nose data for grouping.

The chances of flavor adulteration in case of oleoresins are high enough due to the availability of several capsaicin homologues and analogues. Todd and others (1977) described fine capsaicin homologues by synthesis under optimized conditions of trimethyl silylation, and all the fine homologues showed high correlation with pungency levels. These synthetic vanylamides could be separated by column chromatography and could be identified by gas or liquid chromatographic methods. The quality control methodology of capsicums shall include authentic quantification of pungency in fruits as such or in the oleoresins concerned. In the case of whole capsicums in fresh or dry forms and also in dry and powdered forms, the other quality attributes such as color, shape, size, and insoluble ash play an important role. Detection and quantification of adulterants is of major concern in powdered chilis and oleoresins in particular. The use of Sudan colors is of common occurrence apart from the use of fillers such as saw dust and wheat flour (Table 41.2).

U.S. STANDARDS FOR OLEORESIN CAPSICUM (AFRICAN CHILIES) (EOA NO. 244)

The product is obtained by the solvent extraction of the dried ripe fruits of *C. frutescens* L. or of *C. annuum* L. var. *conoides* Irish (a form of *C. frutescens*), with the subsequent removal of the solvent. The physical and chemical constants are specified as follows:

Appearance and odor:	A clear red, light amber, or dark red, somewhat viscid liquid with characteristic odor and very high bite
Scoville heat units:	480,000 minimum
Color value:	4000 maximum
Residual solvent in oleoresin:	Meets with the Federal Food, Drug, and Cosmetic Act regulations
Descriptive characteristic solubility:	Alcohol: partly soluble with oily separation and/or sediment. Benzyl benzoate: soluble in all proportions. Fixed oils: soluble in all proportions in most fixed oils Glycerin: insoluble Mineral oil: insoluble Propylene glycol: insoluble

FOOD APPLICATIONS

Chilis in fresh, dried, powdered, or paste forms are widely used in various food preparations including fermented foods, meat, cereal, millet, and vegetable products. The product range includes sauces, chutneys, culinary pastes, pickles, fermented beverages, and snack foods. The use of chilis could be to impart pungency, color, aroma, and aesthetic value as such due to the garnishing quality. The use of chilis depends on the geographic locations of the country concerned, which influences food habits and also cultural identity. India, Sri Lanka, Bangladesh, Pakistan, Nepal, and Bhutan use chilis to a greater extent. Some of the most pungent chilis are grown in Bhutan, in the northeastern states of India, and in Sri Lanka (Govindarajan et al. 1986). Dried red chili powder from *C. frutescens* is a major spice product, and the oleoresin extraction is a restricted practice.

ADVANTAGES OF CAPSICUM OLEORESINS

Oleoresins have many advantages over ground spices as flavor additives due to elimination of microbial contamination, uniformity of color and flavor strength, and optimal utilization. Usually, capsicum oleoresins are tailored to specific uses in terms of total color, pungency, and miscibility in oil or aqueous media. The ground spice equivalent of the oleoresins determined by sensory testing is also given as a guide. The use of chili oleoresins is relatively small and the products are gaining popularity over the years. Ground capsicums, due to their particulate nature, tend to render bursts of pungency when they come under the teeth compared to the uniform pungency of oleoresins throughout the mastication (Kirschbaum-Titze et al. 2002). It depends on the consumer taste in appropriating the relative use of ground capsicum and oleoresin. Perhaps the product nature is of crucial importance in deciding the choice. Salads, pickles, and Latin American recipes and also the Indian subcontinental recipes may prefer ground capsicum. However, sauces, meat preparations, certain types of flavored cheese, curry mixes, vegetable pastes, and sour beverages may be more compatible to the use of chili oleoresin.

TABLE 41.3. Classification of Herbs and Spices by Sensory Characteristics

Red pepper, fresh (1000)	Curry powder, blend (260)
Cayenne pepper, dried (900)	Mustard seed, dried (240)
Horseradish, fresh (800)	Coriander seed (230)
Mustard powder (800)	Turmeric, fresh (220)
Pickling spice, dried (700)	Turmeric, dried (200)
Clove, dried (600)	Peppermint, dried (150)
Garlic fresh (500)	Cardamom, dried (125)
Bay leaf, dried (500)	Spearmint, dried (100)
Ginger, dried (475)	Poppy seed, dried (90)
Black pepper, dried (450)	Thyme, dried (85)
Cinnamon, dried (400)	Parsley, dried (75)
Onion, fresh (390)	Sweet basil, dried (70)
Mace, dried (340)	Onion, dried (60)
Celery seed, dried (300)	Paprika, dried (50)
Cumin seed (290)	Saffron, dried (40)
Fennel seed, dried (280)	Sesame seed, dried (25)

Chili flavors come under the class of pungent and hot flavors, and other flavors in the same category are ginger, horseradish, mustard, and pepper. Table 41.3 shows the classification of herbs and spices by sensory characteristics.

USE OF CHILI FLAVORS IN VARIOUS ETHNIC FOOD PREPARATIONS

Chili flavor or ground capsicum as such is preferred in hot and pungent food products, and the countries include the Indian subcontinent, Latin American countries, oriental countries, China, Southeast Asian countries, Middle East, and the Central African countries. The products include meat and baked foods, soups, curries, gravies, seafoods, fermented foods, and beverages. Kimchi is a popular oriental fermented food and the pungency factor is an important quality aspect. Irradiated red pepper powder was widely reported to render optimal sensory quality without causing microbial contaminations (Lee 2004). Irradiation was found to cause certain variations in the volatiles involving formation of butyl benzene derivatives, which is not desirable (Lee et al. 2004). Therefore, the use of oleoresins derived from capsicum and colorant in the form of paprika with residual flavor has an increasing commercial utility in the preparation of several products including kimchi and sauerkraut. Acuka sauce, a famous Turkish product, makes use of hazelnut and pepper paste (Ozcan 2002). The product could be an ideal one for the use of chili oleoresins as ground pepper paste could make the texture susceptible besides hindering the spreadability of the product. Kochuzang is another important traditional oriental product. It is available in sweet or savory taste. The commercial product is basically a decomposed product fortified with flavor, and chili flavor could be an appropriate substitute for ground chilis traditionally used in flavoring the product (Park et al. 2003).

The Indian culinary makes use of ground chilis, chili paste, and precut green chilis as a major source of pungent flavoring. The products are formulated in the form of vegetable, meat, poultry, and seafood-based curries, chutneys, pickles, and sauces. The traditional use of chilis tends to pose a number of problems with regard to microbial spoilage, and the fungal spores could be a major source of concern. Most of these products are thermally processed to varying extents, and the pasteurized products depend on rendering medium to high acid levels to the product to ensure food safety. The lack of commercial sterilization in these products causes a latent incidence of fungal spores as in the case of ketchups, stabilized chutneys, and other products. Chilis could be a major source of spore contamination in these products and in precut green chilis; the acidic pH tends to discolor the chilis, which is not desirable in terms of color attributes. The use of chili oleoresins in some of these products could be beneficial in restricting the latent infections, which otherwise may cause health hazards.

The Middle East countries use a number of chili-flavored products including meat and baked products apart from soups, sauces, and juice cocktails. Chilis and extracts of *tejpat* (*Cinnamomum tamala*) are widely used in these preparations apart from black pepper. Salami preparations, sausages, and minced meat preparations such as barbecues, meat and fish *tikkas*, *kababs*, and patties require pungent flavor in abundance, and ground spice-based *masalas* are widely used across these countries (Coon 2003). Chili oleoresins are suitable for these products due to their uniform pungency and ready miscibility with oil or aqueous media. Certain wines are also used, which could be flavored with chili or pepper oleoresins. The popularity of health drinks based on vegetable and herbs is on the increase including *Aloe vera*-based products. These beverages are usually prepared in salted or spiced forms to restrict the calorie output, which otherwise has to depend on nonnutritive sweeteners. Chili oleoresins could be used on unsaponified or saponified extracts of red pepper depending on the nature of the product. Some products could be suitable for the use of paprika extract, such as fried boiled eggs or meat preparations, and the residual pungency flavor meets the flavor requirements where color quality is predominant rather than the pungency level of the extracts. Several cereal-based products such as *biryanies* could be subjected to the use of chili oleoresins or paprika extracts. Among the baked or extruded products, cookies and crunchy extruded products are the ideal ones for the application of chili flavors to enhance the sensory attributes.

Among the popular Middle East recipes, *kibbeh* is one of the most sought after products. *Kibbeh* is a dish made with lamb meat or beef along with cracked wheat and pine nuts. The dish is popular in Lebanon, Israel, and Syria. It can be eaten in fried or baked form. Chilis, along with cumin and cinnamon, are used as spices in the preparation of the dish, and chili oleoresins could be used to render uniform pungency within the product. Other meat dishes such as *sambaosak*, lamb *shishlik*, and *al-motubug* could also be spiced with chili flavor or chili oleoresins. As such, the Middle East cuisine includes a number of spices including chilis. Historically, Middle East was either the source or the transit point for spice trade between Asia to Europe. Middle Eastern foods are usually eaten with "pita bread," and moderate pungency levels in the various foods, either meat based or salads, are preferred (Giese 1994). Chili oleoresins could play a major role in meeting these requirements.

SAFETY OF CHILI FLAVORS

Flavors in general represent a class of food additives with comparatively less legislation. This is probably due to the relatively large number of ingredients used in flavorings, the very small quantities involved, and the difficulty involved in regulating added substances. The legislation includes positive and negative approaches with the positive list including permitted flavor agents and the negative list including nonpermissible substances. The Generally Recognized as Safe (GRAS) list of the Food and Drug Administration (FDA) and the Flavor and Extract Manufacturing Association (FEMA) complement each other in regulating flavors in the United States. The Joint Expert Committee on Food Additives (JECFA) as a nodal organization for Codex Alimentarius has evaluated around 300 flavoring substances (Munro et al. 1999). The Council of Europe Expert Committee (CEEC) laid down certain guidelines (Council of Europe 1995) to regulate the production of processed flavorings, ingredients, and process conditions. The guidelines include various aspects, such as ingredients added prior to processing, ingredients added after processing, process conditions, purity criteria, and safety evaluation.

In the case of chili flavors, adulteration with synthetic analogues is one of the major problems, and appropriate analytical techniques were described to identify the adulteration. The physiological, pharmacological, and toxicological aspects of capsaicin were described in detail by Kati-Coulibaly and others (1998). The LC_{50} and LD_{50} tests in toxicological experiments using capsaicin at various doses showed lung, thorax, skin, as well as seizers as the observable effects when the feeding is carried out by different modes such as inhalation and oral or intraperitoneal applications. However, the doses are high ranging from 0.25 to 10.0 g/kg body weight.

GENETICALLY MODIFIED CHILI

The genetic modifications in capsicums, as such, are to improve disease resistance of capsicum plants as the crops are highly susceptible to insects and to bacterial or fungal attacks. The other approach is to make the crop tolerant to temperate conditions and also to induce osmotolerance and cold adaptations. Being a member of the Solanaceae family, the crop is highly conducive for genetic manifestations. Yamakawa and others (1998) described the transformation of chili pepper with the PAL gene, which has ramifications in terms of disease resistance as well as biosynthesis of capsaicinoids. However, efforts were made to increase the capsaicin content by biotechnological methods. Sudhakar-Johnson and Ravishankar (1998) described a biotransformation method to regulate the capsaicinoid pathway to result in enhanced yields of capsaicin. Immobilized placental tissues of capsicum frutescence were treated with L-phenylalanine, cinnamic acid, coumaric acid, caffeic acid, ferulic acid, and vanyl amine in combination with L-valine to obtain promotive effects on capsaicinoid yield. However, it is pertinent to note that climatic conditions also show significant effects on capsaicinoid yield (Harvell and Bosland 1997). The genetic control of pungency in *C. chinense* was reported through the Pun1 locus and modification of the same was found to result in modifications in the pungency levels (Stewart et al. 2007). The future areas of research in genetic modifications of capsicums include regulation of capsaicin content and also the sweeter varieties in terms

of improvement in palatability. The chili oleoresin-, paprika-, and condiment-based industries would like to make full use of these improved varieties. Colored and sweet capsicums, on the other hand, form an important aspect of marketing for salads, and they would also be beneficial for the improvements in the germplasm of capsicums.

CONCLUSION

The botanical and agronomic aspects of chilis and the geographic distribution of different chili varieties and cultivars enable appreciation toward the novelties of different cultivars grown in different agro-climatic zones. Extraction, isolation, and characterization of capsaicinoids are ultimately directed toward the development of highly sensitive analytical methods. The chemistry and biosynthetic pathway gives a comprehensive account of the various intricacies in the development of pungency within chilis. The industrial methods of extraction such as SC CO_2 extraction methods need to be applied to a greater extent to improve the production and trade concerned with chili flavors, that is, chili oleoresins. The correlations drawn between the subjective and objective methodologies make it possible to draw conversion equations for the benefit of quality control procedures. The subjective methods such as Scoville heat testing have specific sensory evaluation protocols, and the latest modifications could be helpful in improving the dependability of such tests. The HPLC methodologies provide a greater degree of safety consciousness to detect use of synthetic analogues in the quality control regimes. The toxicological aspects highlight the necessity to adopt appropriate allowed daily intake (ADI) levels for various chili-based flavorings. The genetic modifications paved the way for the development of cultivars with controlled pungency levels for the benefit of different consumer groups. Certain highly pungent cultivars such as Bhut Jolokia and Naga Jolokia need to be commercially exploited for the development of value-added products and flavorings. The marketing of chilis is restricted to a greater extent to powdered preparations, which needs further inroads in the form of oleoresins besides paprika extracts, which hold a significant demand in the international market.

REFERENCES

Anon. 1959. Joint Committee (PS/SAC). Recommended methods of analysis of crude drugs. The determination of capsaicin content of capsicum and its preparations I. *Analyst* 84:603–4.

ASTA. 1968. *Official Methods of Analysis*, 2nd Ed. Englewood Cliffs, NJ: American Spice Trade Association, pp. 43–4.

Bennett DJ, Kirby GW. 1968. Constitution biosynthesis of capsaicin. *J Chem Soc* C:42–446.

Brawer O, Schoen J. 1963. Determination of flavour constituents of paprika. *Angew Bot* 36:25; *Chem Abstr* 58:111660.

Coenen H, Hagen R. 1983. Natural colorant from paprika with gas extraction. *Gordian (Hamburgo)* 9:1964–170.

Coenen H, Hazen R, Knuth M. 1983. Method for obtaining aromatics and dyestuffs from bell peppers. EEUU Patent 4400398.

Coenen H, Kriegel E. 1983. Application of extraction with supercritical gases in food industry. *Chem Ing Tech* 11:890–1.

Coon D. 2003. Chile peppers: Heating up Hispanic foods. *Food Technol.* 57(1):39–43.

Council of Europe. 1995. *Guidelines on the Production of Thermal Process Flavorings*. Strasbourg, France: Council of Europe Publishing.

Dobbs JM, Wong JM, Lahiene RJ, Johnston KP. 1987. Modification of supercritical fluid phase behaviour using polar cosolvents. *Ind Eng Chem Res* 26:56–65.

Dohman K. 1965. Thin layer chromatography. *Lab Pract* 14:808–9.

Ferrari JP, Aillaud G. 1971. Bibliographical du genre capsicum. *J Agric Trop Bot Appl* 18:385–479.

Figen K, Neniman B, Murat OB, Yasar H. 2002. Grand red peppers: Capsaicinoids content, Scoville scores and discrimination by an electronic nose. *J Agric Food Chem* 50:3257–61.

Fuzikawe H, Suzuki T, Iwai K. 1982. Intracellular distribution of enzymes and intermediates involved in the biosynthesis of capsaicin and its analogs in capsicum fruits. *Agric Biol Chem* 46:2685–9.

Galan-Jaren M, Nienaber U, Schwartz SJ. 1999. Paprika (*Capsicum annuum*) oleoresin extraction with supercritical carbon dioxide. *J Agric Food Chem* 47:3558–64.

Gemert LJ, Nigssen LM, de Bie ATMJ, Maarse M. 1983. Relation between the capsaicinoids content and the Scoville index of capsicum oleoresins. In Williams AA (ed.), *Sensory Quality in Foods and Beverages*. Bristol: Ellis Horwood, pp. 260–4.

Giese J. 1994. Spice and seasoning blends: A taste for all seasons. *Food Technol* 48(4):87–90, 92, 94–95, 98.

Gnayfeed MH, Daood HG, Illes V, Biacs PA. 2001. Supercritical CO_2 and subcritical propane extraction of pungent paprika and quantification of carotenoids, tocopherols and capsaicinoids. *J Agric Food Chem* 49:2761–6.

Gonzalez AT, Altamirano CW. 1973. New method for determination of capsaicin in capsicum fruits. *J Food Sci* 38:342–5.

Govindarajan VS. 1986. Capsicum production, technology, chemistry and quality III. Chemistry of the colour, aroma and pungency stimuli. *Crit Rev Food Sci Nutr* 24(3):245–355.

Govindarajan VS, Rajalakshmi D, Nagin C. 1986. Capsicum production, technology, chemistry and quality. Part IV. Evaluation of quality. *Crit Rev Food Sci Nutr* 25:185–282.

Harvell KP, Bosland PW. 1997. The environment produces a significant effect on pungency of pungency of chiles (Chillies). *HortScience* 32(7):1292–6.

Haymon LW, Aurand LW. 1971. Volatile constituents of Tabasco peppers. *J Agric Food Chem* 19:1131–4.

Heiser CB. 1969. Systematics and origin of cultivated plants. *Taxon* 18:36–45.

Heiser CB, Pickersgill B. 1975. Names for the Bird's peppers (capsicum-Solanaceae). *Baileya* 19:151–6.

Heiser CB, Smith PG. 1953. The cultivated capsicum peppers. *Econ Bot* 7:214–26.

Illes V, Szali M, Then M, Dacod HG, Perneczki S. 1997. Extraction of hiprose fruit by supercritical CO_2 and propane. *J Supercrit Fluids* 10:209–18.

Irish HC. 1898. Revision of the genus capsicum. *Ann Mo Bot Gard* 9:53–110.

ISO. 1983. *Spice and Condiments*. Geneva: International Organization for Standardization.

Iwai K, Susuki T, Fuziwaki H. 1979. Formation and accumulation of pungent principle of hot pepper fruits, capsaicin and its analogues, in *Capsicum annuum* var. *annuum* cv. Karayat subusa at different growth stages after flowering. *Agric Biol Chem* 43:2493–8.

Jurenitsch J, Kubelka W, Jentzsch K. 1979. Identification of cultivated taxa of capsicum. Toxonomy, anatomy and composition of pungent principle. *Planta Med* 35:174–83.

Kati-Coulibaly S, Coxam V, Barlet JP. 1998. Physiological, pharmacological and toxicological interests of chillies and of their active principle: Capsaicin. *Med Nutr* 34(6):236–45.

Kirschbaum-Titze P, Hiepler C, Mueller-Seitz E, Petz M. 2002. Pungency in paprika (*Capsicum annuum*). 1. Decrease in capsaicinoid content following cellular disruption. *J Agric Food Chem* 50:1260–3.

Knez Z, Posel F, Hunek J, Golob J. 1991. Extraction of plant materials with supercritical CO_2. In McHugh M (ed.), *Proceeding of the 2nd International Conference on Supercritical Fluids*. Baltimore, MD: John Hopkins University, pp. 101–4.

Knez Z, Skerget M. 1994. Application of supercritical CO_2 for food processing. In Yane T, Matsuno R, Nakamura K (eds.), *Proceeding of the 6th International Congress on Engineering and Food*. Chiba: Blackie Academic & Professional, pp. 826–8.

Kobata K, Todo T, Yazawa S, Iwai K, Watanabe T. 1998. Novel capsaicinoid like substances, capsiate and dihydrocapsiate from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (*Capsicum annuum* L.). *J Agric Food Chem* 46(5):1695–7.

Kosuge S, Inagaki Y. 1959. Studies on pungent principles of red pepper. III. Determination of pungent principles. *Nippon Nogei Kagakukaishi* 33:470–2.

Kurian AL, Starks AN. 2002. HPLC analysis of capsaicinoids extracted from whole orange Habanero chili peppers. *J Food Sci* 67:956–62.

Lee JH, Sung TH, Lee KT, Kim MR. 2004. Effect of gamma irradiation on colour, pungency and volatiles of Korean red pepper powder. *J Food Sci* 69(8):C585–92.

Lee SC. 2004. Effect of irradiated red pepper powder as kimchi quality during fermentation. *J Food Sci Nutr* 9(3):218–21.

Maga JA. 1977. Capsicum. *Crit Rev Food Sci* 6:177–99.

Maillard MN, Giampaoli P, Richard HMJ. 1997. Analysis of eleven capsaicinoids by reversed phase high performance liquid chromatography. *Flav Frag J* 12:409–13.

Muller-Stock A, Joshi RK, Buchi J. 1971. Studies on the components of capsicum II. Quantitative gas chromatographic determination of individual homologues and analogues of capsaicin in mixtures from natural sources and of vanillyl pelargonic amide as adulteration. *J Chromatogr* 63:281–7.

Munro K, Kennepohl E, Kroes R. 1999. A procedure for the safety evaluation of flavouring substances. *Food Chem Toxicol* 37:207–32.

Murray KE, Whitfield FB. 1975. The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J Sci Food Agric* 26:973–86.

Nagin C, Govindarajan VS. 1985. Paper chromatographic determination of total capsaicinoids in green chillies (*Capsicum annuum*). *J Food Sci Technol* 22:285–9.

Ozcan M. 2002. A new flavour: Acuka sauce. *Obst Gemuese ond Kartoffelverarbeitung* 87(6):12–13.

Padilla MC, Yahia EM. 1998. Changes in capsaicinoids during development, maturation and senescence of chile peppers and relation with peroxidase activity. *J Agric Food Chem* 46:2075–9.

Park HK, Bogim G, Jong K. 2003. Characteristics of taste components of commercial Kochujang. *Food Sci Biotechnol* 12(2):119–21.

Pickersgill B. 1969. The domestication of chilli peppers. In Ucko PJ, Dimbleby GW (eds.), *Domestication and Exploitation of Plants and Animals*. London: Duckwork, pp. 270–8.

Purseglove JW, Brown EG, Green CL, Robbins SRJ. 1981. *Spices*. Essex: Longman Group.

Quinones-Seglie CR, Burns EE, Villalon B. 1989. Capsaicinoids and pungency in various capsicums. *Lebensm Wiss Technol* 22:196–8.

Rajpoot NC and Govindarajan VS. 1981. Paper chromatography determination of total capsaicinoids in capsicum and their oleoresins with precision reproducibility and validation through correlation with pungency in Scoville units. *J Assoc Anal Chem* 64(2):311–4.

Sakaki K. 1992. Solubility of β-carotene in dense carbon dioxide and nitrous oxide from 308 to 323 K and from 6 to 30 MPa. *J Chem Eng Data* 37:249–51.

Scoville WL. 1912. Note on capsicum. *J Am Pharm Assoc* 1:453–4.

Skerget M, Knez Z. 1997. Solubility of binary mixture of β-carotene-capsaicin in dense CO_2. *J Agric Food Chem* 45:2066–9.

Skerget M, Knez Z, Habulin M. 1995. Solubility of β-carotene and oleic acid in dense CO_2 and data correlation by a density based model. *Fluid Phase Equilib* 109:131–8.

Spanier AM. 1993. Molecular approaches to the study of food quality. In Spanier AM, Okai H, Tamura M (eds.), *Food Flavour and Safety: Molecular Analysis and Design*. Vol. 528. Washington DC: American Chemical Society, pp. 1–7.

Stewart C, Mazourek M, Stellari GM, Cornell MO, Jahn M. 2007. Genetic control of pungency in *C. chinense* via the *Pun*1 locus. *J Exp Bot* 58(5):979–91.

Sudhakar-Johnson T, Ravishankar GA. 1998. Precursor biotransformation in immobilized placental tissues of *Capsicum frutescens* Mill. II. Influence of feeding intermediates of the capsaicinoids pathway in combination with L-valine as capsaicin and dihydrocapsaicin accumulation. *J Plant Physiol* 153:240–3.

Sukrasno N, Yeoman MM. 1993. Phenyl propanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochemistry* 32(4):839–44.

Suzuchi JI, Tausig F, Morse RE. 1957. Some observations on red pepper. I. A new method for the determination of pungency in red pepper. *Food Technol* 11:100–4.

Suzuki T, Iwai K. 1984. Constituents of red pepper species: Chemistry, biochemistry, pharmacology and food science of the pungent principle of capsicum species. In Brossi A (ed.), *The Alkaloids, Chemistry and Pharmacology*, Vol. 23. Orlando, FL: Academic Press, pp. 227–99.

Suzuki T, Kavada T, Iwai K. 1980. Effective separation of capsaicin and its analogues by reverse phase high performance thin layer chromatography. *J Chromatogr* 198:217–23.

Thresh LT. 1946. Isolation of capsaicin. *Pharm J* 6:941–3.

Tipsrisukond N, Fernando N, Clarke AD. 1998. Antioxidant effects of essential oils and oleoresins of black pepper from supercritical carbon dioxide extractions in ground pork. *J Agric Food Chem* 46:4329–33.

Tirimanna ASL. 1972. Quantitative determination of the pungent principle capsaicin of Ceylon Chillies. *Analyst* 97:372–4.

Todd P, Bensinger M, Bigtu T. 1977. Determination of pungency due to capsicum by gas liquid chromatography. *J Food Sci* 42:660–5.

van Ruth SM and Roozen JP. 1994. Gas chromatography/sniffing port analysis and sensory evaluation of commercially dried bell peppers (*Capsicum annuum*) after rehydration. *Food Chem* 51:165–70.

Weisenfelder AE, Huffuman VV, Villalon B, Burns EE. 1978. Quality and processing attributes of selected jalapeño pepper cultivars. *J Food Sci* 43:885–8.

Wooderck SH, Jaeho HH, Jinbong H, Youngjung N. 1994. Effective separation and quantitative analysis of major heat principles in red pepper by capillary gas chromatography. *Food Chem* 49:99–103.

Yamakawa T, Sekigichi S, Kodama T, Smith SM, Yeoman MM. 1998. Transformation of chilli pepper (*Capsicum frutescens*) with a phenyl alanine ammonia lyase gene. *Plant Biotechnol* 15(4):189–93.

Yao J, Nair MJ, Chandra A. 1994. Supercritical carbon dioxide extraction of Scotch Bannet (*Capsicum annuum*) and quantification of capsaicin and dihydrocapsaicin. *J Agric Food Chem* 42:1303–5.

Yasumoto M, Inakuma T, Okazak T, Kobayash T. 1994. Extraction of capsaicin from red pepper with liquid carbondioxide. *J Jpn Soc Food Sci* 41:565–7.

Yazawa S, Suetome N, Okamoto K, Namiki T. 1989. Content of capsaicinoids and capsaicinoid like substances in fruit of pepper (*Capsicum annuum* L.) hybrids made with 'CH-19 Sweet' as a parent. *J Jpn Soc Hortic Sci* 58:601–7.

Yeoman MM, Holden MA, Hall RD, Holden PR, Holland SS. 1989. Regulation of capsaicin synthesis in cultured cells of *Capsicum frutescens*. In Kurz WGW (ed.), *Primary and Secondary Metabolism of Plant Cell Cultures*, Vol. II. Berlin: Springer-Verlag, pp. 162–73.

Ziegler E, Ziegler H. 1998. *Flavourings. Production, Composition, Applications, Regulations*. Weinheim, New York: Wiley-VCH Verlag GmbH.

CHAPTER 42

Corn Flavor

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INTRODUCTION

Corn is a large part of the modern diet through sweeteners, oil, processed foods, and animal-derived foods. In addition, corn is eaten directly in bread and in cereal-type foods, snack foods, and foods made from masa flour. Corn gluten meal is a by-product of grain processed by wet milling. Although primarily used as animal feed, its use as human food is being investigated. Specialty-type corns eaten directly include popcorn and sweet corn. Depending on its preparation, popcorn can be a healthy whole-grain snack food. Sweet corn contains recessive alleles that alter carbohydrate composition in the kernel endosperm. As opposed to other food corns utilized as grain, sweet corn is eaten immature as a vegetable either fresh or processed by freezing or canning.

PROCESSED CORN PRODUCTS

Extrusion

Cooking corn by extrusion involves heating a corn product to high temperatures under pressure, then forcing the product through a series of pores. In this rapid cooking method, moisture is reduced, leaving a finished product such as breakfast cereal or snack food. However, the texture and flavor of these foods is not equal to the quality of traditional baked products for several reasons, including the speed at which the cooking occurs and the loss of water at the end of the process. Flavor must be added separately or through a cooking method like toasting (Maga 1989). Because previous studies have reported over 220 volatile compounds generated during cereal extrusion, Bredie and others (1998a) determined those generated during corn extrusion by using flour made of a mixture of dent and flint endosperm varieties that was extruded under eight different combinations of temperature, moisture level, and residence time in the extruder. Eighty volatile compounds were

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identified, including 20 sulfur-containing compounds. Some had not previously been identified in extruded cereals. The compounds were primarily associated with products from lipid degradation in lower temperature and higher moisture extrusions, with more compounds associated with the Maillard reaction occurring with increased temperature, reduced moisture, or longer residence time. The relative contribution of volatiles to the aroma of extrudates was greater when extrusion occurred under higher temperatures and lower moisture levels.

Bredie and others (1998b) then characterized the flavors produced though changing the conditions of extrusion cooking by using corn grits of mixed dent and flint varieties. Sensory evaluation reduced 167 descriptive terms to 17 attributes describing aroma qualities. Altering the extrusion cooking conditions significantly influenced the intensity and character of the aroma quality of extruded maize grits. Higher temperature and lower moisture increased attributes of burnt, toasted, puffed wheat, sugar puffs, nutty, and bran-like. Porridge-like cooked milk and cooked vegetable were more important in exudates using lower temperatures and higher moistures. For the intermediate temperature and moisture, the popcorn attribute had the highest intensity score. Burnt sugar puffs and puffed wheat notes were dominant under the most severe conditions. The Maillard reaction promoted by lower moisture and higher temperature conditions increased popcorn, toasted, puffed wheat, and burnt aromas. For extrusion condition changes, temperature and moisture combinations had a greater effect on aroma generation than retention time.

Masa Flour Products

Masa flour is made from dried corn soaked in lime (calcium hydroxide) solution. Adding lime to the soak water softens the grain pericarp, allowing easy removal without impairing the food value of the grain. The resulting product, hominy, can be eaten or ground to a dough called masa. Masa is used to make numerous Latino foods such as tortillas, tamales, and empanadas, and for making snack foods such as corn and tortilla chips (Pollak and White 1995).

Buttery and Ling (1995) established that many of the volatile compounds of masa flour and its products were similar to those previously reported by Karahadian and Johnson (1993) and to those identified in popcorn by Schieberle (1991). However, a component not previously identified, 2-aminoacetophenone, was described as important to the character of the aroma and flavor of corn masa products (Buttery and Ling 1994). It comes from the alkaline degradation of tryptophan, a result of the treatment of corn with calcium hydroxide to make masa flour. Apparently, the compound is formed during commercial preparation when corn heated briefly to boiling with water containing lime is then allowed to steep for up to 24 h. It has long been known by processors of masa products that alkaline treatment is important to developing the traditional desired flavor, but the mechanism was not known previous to this work.

Many volatiles occurred in masa and its products in common, but taco shells and chips contained additional compounds resulting from the heat of the frying process as well as compounds apparently derived from the frying oils (Buttery and Ling 1995). No notable differences were found between the volatiles of products made with white and yellow corn. Relatively significant concentrations of important

popcorn aroma compounds were found in the fried taco shell and chip products but not in the flour or tortillas. The compound 2-aminoacetophenone was found in all products.

In 1998, Buttery and Ling studied tortilla chips. In contrast to their previous study, volatile compounds were isolated with a small amount of water to allow identification of highly water-soluble volatiles together with the more easily isolated poorly water-soluble volatiles. A "cracker" or "popcorn" character was given to tortilla chips by 2-acetyl and (2-propionyl) nitrogen heterocyclic compounds previously identified in popcorn by Schieberle (1991). They are only found in chips moistened at some point, indicating that they are released by moisture. Aliphatic aldehydes gave a "deep fried" character to aroma and flavor. They are responsible for that character in many other fried foods and likely originate from cooking oils. Alkylpyrazines and furans also probably originate from cooking oils. The furans were relatively potent; thus, in moderate concentrations, they are likely to contribute to the total aroma and flavor. Other compounds likely contributing include sulfur, benzene derivatives, and free aliphatic acids. Fried foods such as tortilla chips contain very low concentrations of water, the liquid medium being primarily vegetable oil. When chips are chewed, the saliva gives a water medium along with the chip oil medium so the atmospheric concentration of volatiles is somewhat between oil and water.

Starch Pastes

Starch is widely used in processed foods to provide thickness and/or texture. When starch is heated in the presence of water, it gelatinizes to a paste with physical properties desirable in many food products. It is also used as an inert material that can absorb moisture or can help in product dispersion (White and Pollak 1995). Because corn is the leading cereal grain produced in the United States and much of production is processed into other products, corn is by far the most widely used starch.

The amylose portion of starch can have nonspecific interactions with aroma ligands to form reversible complexes during gelatinization or subsequent cooling. The complexes are insoluble in water (Arvisenet et al. 2002a). Because of the interactions between the food and aroma compounds, perception of the aroma is directly related to the aroma concentration in the headspace above the food (Arvisenet et al. 2002b). These compounds are difficult to study because the concentrations are low and it is difficult to make conclusions on more than one compound at a time. Interactions between the aroma compounds and other food ingredients affect flavor perception of the food. Because starch is an important ingredient in foods, understanding of aroma–starch interactions is useful for improving food flavor (Boutboul et al. 2002).

Arvisenet and others (2002a) measured the retention of three aroma compounds (isoamyl acetate, ethyl hexanoate, and linalool) with four different cornstarch-based food matrices: high amylose, standard, unmodified waxy, and modified (stabilized and cross-linked) waxy. Linalool and ethyl hexanoate had more retention overall, but for the same aroma compound, retention depended on the starch. For unmodified starches, the retention of linalool and ethyl hexanoate was significantly higher in matrices with amylose-containing starches. These two aroma compounds were

shown to be able to form complexes with amylose, demonstrating the existence of aroma compound–amylose complexes, and the complexes can be formed at concentrations important for foodstuff. High-amylose cornstarch hybrids have relatively more fatty acid than normal or waxy starches, and lipids are known for their aroma retention ability, so they could have a role in the retention of aroma compounds. Aroma retention was about 20–30% less in waxy cornstarch matrices than in amylose-containing matrices. Nevertheless, the aroma compound mostly retained in waxy starch matrices was linalool followed by ethyl hexanoate and isoamyl acetate. The authors suggested the possibility of interactions between linalool or ethyl hexanoate and amylopectin because external amylopectin chains could behave like amylose and could interact in the same way with small ligands. Isoamyl acetate does not form complexes with amylose but could interact with amylopectin by another mechanism.

The texture of the starchy matrices had an important influence on aroma retention. For all aroma compounds, the retention was significantly higher in modified waxy cornstarch matrices than in unmodified waxy starch matrices. This could be explained by interaction between aroma compounds and chemically modified chains or by the effect of the modifications on the texture of the food. If true, chemical starch modifications could be used to influence the retention of aroma compounds as well as texture.

Arvisenet and others (2002b) then showed that the flavoring rates (the ratio of the quantity of the aroma compound introduced in the system before cooking to the quantity of the aroma compound measured after cooking) were different for each of the above three compounds. In systems with a blend of two aroma compounds, the retention of isoamyl acetate was drastically decreased, probably because linalool and ethyl hexanoate could form complexes with amylose. The authors suggested that in standard cornstarch gels, interactions occurred mainly with amylose because the small concentration of the aroma compound might not favor additional binding with amylopectin. In waxy starch pastes, linalool and ethyl hexanoate could bind with amylopectin. Isoamyl acetate was equally retained by normal and waxy cornstarch pastes due to a higher affinity of this compound for amylopectin than for amylose. Since standard cornstarch has nearly 74% amylopectin, amylopectin-binding sites were available for isoamyl acetate in both cornstarch pastes. Isoamyl acetate, which is unable to form complexes with amylose, was less retained in starch matrices when compounds that are able to interact with amylose were added, because the formation of complexes modified the structure of amylose (from double to single helices) and thus the structure of the matrix.

Boutboul and others (2002) looked at aroma compounds (1-hexanol, octanal, ethyl hexanoate, and D-limonene) and high-amylose cornstarch and found that aroma retention with high-amylose cornstarch mainly resulted from an adsorption phenomenon. Water facilitated the diffusion of aroma compounds into the starch granules and allowed partitioning of the aroma compounds between the gas phase and the water phase with weak energy bonds to the granules. For more polar compounds (1-hexanol, octanal, and ethyl hexanoate), hydrogen bonds and dipole–dipole interactions were likely to be involved, but for ethyl hexanoate, steric hindrance of the molecule prevented hydrogen bonds, so only dipole–dipole interactions had to have been involved. Apolar D-limonene cannot develop polar inter-

actions with starch, so hydrophobic interactions with noncomplexed lipids may cause retention.

Wulff and others (2005) found that volatile flavors, especially those with low boiling points, can be expected to be stable in complexes with amylose under freeze-drying conditions. Storage at or below 30°C did not result in significant loss of flavor. In mixed complexes of the compounds hexanol and guaiacol, there was no detectable difference in the stability during freeze-drying and during storage compared to complexes of pure compounds. It was also possible to obtain a mixed complex from three different guest molecules (the two above plus (*E*)-2-nonetal). Mixed complexes of different guest molecules could be prepared, but the ratio and the content could not be precisely calculated in advance. The guest molecules influenced the complexing behavior of the mixture as well as did the type of amylose used (native potato, native wrinkled pea, and 70% amylose corn). Long, linear amylose chains showed the best binding. Because of the inability to predict the guest molecule content, it was advisable to mix pure complexes instead of performing mixed complexing.

Conde-Petit and others (2006) reviewed starch–flavor complexation in food systems. In summary, in a neutral aqueous solution, amylose has the characteristics of a random coil but may have some helical regions. Water promotes spontaneous association of linear starch segments to form double helices. In the presence of suitable guest molecules, the amylose adopts the configuration of a single-helix conformation stabilized by hydrogen bonds. The outside of the helix is hydrophilic, whereas the inside is hydrophobic and suited to accommodate suitable guest molecules of appropriate dimensions. This is known as an amylose inclusion complex, which is largely stabilized by hydrophobic forces. Inclusion complexation is a non-covalent binding of small molecules such as flavor compounds like alcohols, aldehydes, lactones, and terpenes to starch where the guest molecule is bound by inclusion into the helical cavity of amylose. Both amylose and amylopectin are able to bind flavor compounds, but the exact nature of flavor binding to amylopectin remains unknown. There is a broad knowledge of the structural aspects of starch–flavor complexation in contrast to the rather poor understanding of the relationships between starch complexation and overall flavor performance in foods. This is largely due to the numerous components and the complex nature of food where flavor quality is the result of various superimposed mechanisms of flavor retention and release.

Tapanapunnitikul and others (2008) used high-amylose maize starch, with and without native lipid, to make inclusion complexes with flavor compounds for investigating the effect of water solubility of flavor compounds on inclusion complex formation. Two pairs of terpenes, of high (thymol and menthone) and low (limonene and cymene) water solubility, were used. Water solubility of the flavor compound was related to the extent of inclusion complexation. For higher water solubility flavor, starch yield and flavor entrapment were higher. Complex formation with low-solubility flavor was most effective in the presence of native lipid. Lipid in native high-amylose starch may enhance complexation with low-solubility compounds by forming ternary coinclusion complexes of starch–lipid–flavor. Native high-amylose starch with native lipid can form inclusion complexes with flavor compounds of both low and high water solubility. It is still necessary to determine the impact of native lipids of high-amylose starch on flavor release and flavor quality.

Commercially Objectionable Odors

Determining "off-odors" or commercially objectionable odors in grain grading is important for two reasons. First, if grain is graded as sample grade due to commercially objectionable odors, it is severely discounted. Second, odors can be carried through into the ultimate product and thus cannot be used for food (Dravnieks et al. 1973).

Because this type of classification depends on human judgment, a study to determine the amount of agreement between human judgments and data from gas chromatography (GC) analysis of volatiles in the headspace over corn was conducted by Dravnieks and others (1973). Pooling samples into two or three groups (good, musty, and sour, or good and bad) using discriminate analysis on the area of GC peaks of odor compounds and then using a variety of transformations significantly classified the samples into groups distinguished by United States Department of Agriculture (USDA) grain inspectors.

Products from Corn Germ

Lipoxygenase (LPO) is considered to be responsible for off-flavors and lipid oxidation in food. In corn, products of LPO oxidation, fatty acid hydroperoxides, do not accumulate.

Gardner and Inglett (1971) found that the amount of enzyme activity in corn germ before roll cooking was dependent on the postharvest history of the corn. Heated grain drying significantly inactivated enzymes. Cooking germ by heated rolls completed inactivation except for peroxidase (POD). If germ was stored at high humidity, mold developed and there was increased oxidative rancidity as measured by the peroxide value of the extracted oil. Low peroxide values of oil from relatively unprocessed grain occur because linoleic acid hydroperoxide isomerase catalyzes the conversion of hydroperoxide to products not measured by the peroxide value.

Eight sensory attributes (grainy, corn-like, musty, brown, sweet, green, sour, and chemical [10% ethanol]) were established to differentiate aroma among four corn germ protein flours treated to improve flavor quality by using relationships between sensory and GC data (Huang and Zayas 1996). Corn germ protein flour was characterized mainly by grainy, brown, corn-like, and sweet notes. In general, treated samples had less grainy, brown, corn-like, and sweet notes than untreated samples. There were more green, musty, and chemical notes in the treated samples. Changes in the distribution of volatile compounds also occurred in treated and untreated samples.

Food flavors are composed of mixtures of volatiles; therefore, the use of multivariate statistics is necessary to find essential aroma compounds that are related to sensory responses. Most vegetable protein products have undesirable flavors, which have been the greatest barrier to increased usage of such proteins in foods. Off-flavors are the limiting factors in the utilization of corn germ protein flour.

Corn Gluten Meal

Phillips and Sternberg (1979) investigated the flavor aspects of the corn protein concentrate by-product of the saccharification of the starch in corn flour, made from

the corn gluten by-product of dextrose manufacture. Flavor and odor components are removed in its production. A slurry in water at room temperature had essentially no taste, while a slight corn flavor developed upon heating.

Wu and others (2001) substituted corn gluten meal for semolina or farina in making spaghetti. The meal was a high-protein by-product of corn wet milling. The flavor score decreased with increasing additions of corn gluten meal because of the higher intensity of fermented flavor. An acceptable quality was obtained with spaghetti with a treatment of 5% water/ethanol to wash the corn gluten meal. Corn gluten meal does not taste good but had a better flavor at pH 7–8 than at pH 4. A water wash gave a less undesirable flavor, and a further ethanol washing of the water washed meal improved flavor even more. Wu and others (2006) identified volatile components in extracts as a step to identify the odor components of corn gluten meal.

SPECIALTY CORN

Popcorn

Popcorn can be distinguished from other types of corn because the kernel endosperm consists primarily of tightly packed starch granules giving the kernels a glassy, hard appearance. When heated, the kernels have the ability to explode and to produce large, puffed flakes (Ziegler 2003). The heat produces popcorn's characteristic flavor and aroma, a reason why it has become a popular snack food item.

Walradt and others (1970) identified volatile compounds from popcorn popped in oil and microwaved without oil in order to identify what gave popcorn its distinctive flavor, often used as an attribute in describing other food flavors. Thirty-six compounds were identified that contribute to the flavor, with 20 other possibilities. Compounds they believed to make important contributions to the flavor and aroma of popcorn are pyrazines, furans, pyrroles, carbonyls, and substituted phenols. Microwave corn exhibited a typical popcorn flavor but lacked some of the tactile characteristics of oil-popped corn. The primary difference in their volatile compound profiles was more 2-pentyl furan and short-chain *n*-aldehydes in oil-popped corn. In summary, there was predominance of furfural and 2-acetyl pyrazine. Alkyl-substituted pyrazines probably provide the predominant nutty character. Furfurals appear to add a slightly burned character. Strecker aldehydes could also provide malty or burned aroma notes. N-furfuryl pyrrole, a green hay-like aroma, may also contribute. Ferulic acid leads to vanillin notes, and the pleasant clove-like aroma of 4-vinyl-2-methoxy phenol and the strong, smoky aroma of 4-vinyl phenol probably contribute to the background flavor.

Schieberle (1991) examined freshly prepared hot-air popcorn to determine if the sweet-roasty flavor notes of heat-processed cereal foods described as "popcorn-like" are present in popcorn. The compound 2-acetyl-1-pyrroline was important, also reported as responsible for the roasty, popcorn-like odor note of cooked rice (Buttery and Ling 1982) and bread crust (Schieberle and Grosch 1985). Acetyl pyrazine, reported by Walradt and others (1970) as important in popcorn, had a minor contribution to the overall flavor of the 23 identified compounds. Other compounds (2-acetyltetrahydropyridine and 2-propionyl-1-pyrroline) were also associated with roasty odors.

Schieberle (1995) then determined amounts of the four roast-smelling popcorn odorants in fresh popcorn (2-acetyl-tetrahydropyridine, 2-propionyl-1-pyrroline, 2-acetyl-1-pyrroline, and acetyl pyrazine). The compound 2-acetyl-tetrahydropyridine had the highest concentration followed by 2-acetyl-1-pyrroline. These two compounds were determined to be the key contributors to the roasty popcorn odor. These two primary compounds and 2-propionyl-1-pyrroline decreased to about one-third after storage for 7 days in a sealed polyethylene bag, indicating that the flavor of fresh popcorn is not stable. Lower concentrations of 2-acetyl-tetrahydropyridine were obtained by popping in a pan than for fresh hot-air popping, but acetyl pyrazine increased by a factor of 3. This finding implies that longer heating leads to degradation, especially of the most labile roast odorant 2-acetyl-tetrahydropyridine, but based on its high odor activity value, 2-acetyl-tetrahydropyridine was also the most odor-active compound.

Most studies dealing with aroma attempt to identify the compounds important for flavor, but Shen and Hoseney (1995) assumed that GC profiles would be similar for various cereals since chemical compositions are very similar. Compounds responsible for differences in aroma among cereals should stand out as different peaks. They examined GC profiles of extracts from popcorn, rice, sorghum, and dent corn to find characteristic peaks that might be related to popcorn aroma. Two compounds, N-furfuryl-pyrrole (green hay-like aroma according to Walradt et al. 1970) and N-furfuryl-2-formyl pyrrole (no aroma description found), were present in popcorn, but were present only in trace amounts in dent corn and other cereals. This suggests that these two compounds may be important for popcorn aroma. The GC profiles of popcorn extracts with and without germ were essentially the same, indicating that the germ does not play an important role in popcorn aroma. The popping mechanism (internal increase in temperature and pressure) was not important in aroma production. The two pyrroles described above and certain other heat-induced aroma compounds found in popcorn extracts were not present in an extruded cornmeal extract. This appears to account for the lack of aroma in extruded cornmeal. A GC profile comparison of the extracts from dent corn and of those from dent corn supplemented with hydroxyproline showed that the peak areas of N-furfuryl-pyrrole and N-furfuryl-2-formylpyrrole in the supplemented corn increased 130 and 20 times, respectively.

Flavor and aroma volatiles isolated from microwave oven-produced popcorn were studied by Buttery and others (1997), who also wanted to develop a practical method that could be used to quantitatively analyze the aroma component of new breeding genotypes. The fact that the aroma of volatiles produced during popping was important to consumers, along with volatiles involved in eating popcorn, was recognized by industry. Therefore, the authors used dry and wet methods of isolation (dry includes volatiles produced during popping). The dry method involved popping in a microwave and isolating the volatiles, while the wet involved blending the popped corn with water, sodium chloride, and sodium carbonate and isolating the volatiles.

Unlike many other foods, popcorn differs in being eaten quite dry. Assuming there are no added materials, it contains a very low concentration of water (about 2.5%). However, the eating and chewing of popcorn involves saliva, and the concentrations of volatiles in the atmosphere of the mouth and reaching the olfactory system retronasally are important. Buttery and others (1997) stated that popcorn is

similar to coffee and chocolate in that a large number of potent aroma compounds must blend to give an integrated aroma and flavor perception. The effect must also be somewhat different depending on whether it is (1) the aroma perceived during popping (where hydrogen sulfide [H_2S] and dimethyl sulfide [DMS] must also contribute), (2) the aroma of the dry popcorn, or (3) the olfactory part of flavor perceived retronasally while eating the popcorn. Relatively water-soluble compounds such as furaneol, 2-acetyl-1-pyrroline, and 2-acetyltetrahydropyridines would be expected to be more effective for (1) and (2).

Results from Buttery and others (1997) confirmed findings from previous studies and some additional new compounds including DMS, dimethyl di- and trisulfides, 3-methylindole (skatole), α- and β-ionones, 2-methyl-3-hydroxypyran-4-one (maltol), 2,3-dihydro- 3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, 5-methyl-4-hydroxy-3(2*H*)-furanone (norfuraneol), geranyl acetone, among others. H_2S was a major component of the volatiles emitted during popping.

Six popcorn hybrids (two white butterfly flakes, two yellow butterfly flakes, and one yellow mushroom flake) from hot-air-popped samples were evaluated by Park and Maga (2006) with GC/mass spectrophotometry (MS) and sensory panel. GC/MS found 195 total volatiles, and 51 were positively identified. The compound 2-acetylpyridine contributed to the overall popped popcorn aroma quality favorably or not adversely. Total peaks per hybrid ranged from 159 to 187. Six other compounds were important but contributed negatively to the characteristic popped popcorn odor. Numerous other compounds may be responsible for typical popcorn aroma characteristics. Some may be responsible not only for background popcorn aroma but also for aroma quality differences among hybrids. Other volatiles differed in quantity among hybrids, although they were low in concentration, but would not necessarily impact odor greatly because threshold values are different. Of 18 selected volatiles of each hybrid, half were not significantly different among hybrids, while others ranked differently among hybrids. Their results indicate that selection for improved aroma and flavor may be successful in popcorn breeding.

Sweet Corn

Sweet corn is one of the most popular vegetables in the United States and is becoming increasingly popular in other countries. Although any type of corn can be eaten when kernels are immature, sweet corn cultivars contain recessive alleles that alter the carbohydrate composition of the endosperm and are specifically bred for their use as a vegetable. The primary recessive alleles used in sweet corn cultivars include *sugary1*, *shrunken2*, and *sugary enhancer1* (Marshall and Tracy 2003).

Identification of Aroma and Flavor Compounds Early studies on sweet corn aroma and flavor were reviewed by Wiley (1985). Quality aspects of sweet corn include sweet flavor, tenderness, and aroma. Raw sweet corn has little or no odor, with typical corn aromas occurring as the kernels are heated. The aroma that arises upon cooking is highly volatile and fleeting. Plant breeders have been successful at developing cultivars with a tender pericarp and sweet flavor over a long time period. Improving the typical sweet corn aroma would be beneficial to increased consumption, especially in stored and processed corn.

Gould and others reported quick objective measurements of maturity and tenderness for fresh, frozen, and canned sweet corn in 1951. Studies on aroma and flavor came later. Early studies implicated DMS and H_2S as the major components of cooked corn aroma (Wiley 1985). Bills and Keenan (1968) found high but widely variable concentrations of DMS among commercially canned and frozen corn samples, even though DMS can contribute to flavor at concentrations of a few parts per billion. The flavor of one sample with low DMS (0.3 ppm) resembled pumpkin or squash. Acetaldehyde was noted during GC above the flavor threshold in most samples and increased upon heating. The high concentrations of DMS indicated that it is one of the important flavor constituents of sweet corn. Varietal differences in DMS indicate that breeding and selection for this compound and other flavor compounds may be successful and may lead to cultivars with wider commercial acceptance.

Flora and Wiley (1974a) found that DMS and H_2S as measured by GC in cooked corn headspace and colorimetrically, respectively, varied significantly due to planting date and cultivar. They used five commercially grown cultivars, four processing and one fresh market, and two planting dates. DMS decreased with increasing maturity, while H_2S did not change. DMS was highest in canned corn, then fresh corn, then frozen corn. H_2S was highest in frozen corn, then canned corn, then fresh corn. However, sensory panelists often did not respond to these differences. Panelist results indicated that although DMS and H_2S are important aroma components, there are more components in the total complement of flavor volatiles that are important.

Evaluating five commercial cultivars harvested at fancy to standard maturity, then processed frozen and canned, Flora and Wiley (1974b) detected seven distinct odor notes by GC. They included "rotten egg" (H_2S), an unpleasant fecal sulfurous odor (methanethiol), fruity (acetaldehyde and ethanol), sulfur (ethanethiol), strong but pleasant characterized as "corny" (DMS), and grainy or musty (not identified) and only detected in canned samples. Most aroma components were present in the low-boiling headspace volatile fraction. Compounds present in the high-boiling volatile fraction also contributed to the flavor of sweet corn but appeared to be of secondary importance. Flora and Wiley (1974b) observed that sulfur-containing compounds contribute most to the aroma of cooked sweet corn, and that in pure states, they are repulsive in small concentrations, and in combination, they can be mild and pleasant. The sulfur-containing compounds are heat processed and are not present in raw corn. DMS appears to be the dominant character of cooked sweet corn because of its high concentration and odor mostly related to that of sweet corn.

In Flora and Wiley's (1974b) study, sensory panels indicated that sweetness and texture were the most important attributes in overall flavor and that aroma played only a small part. But panelists may have had trouble scoring for aroma. Texture and sweetness have essentially equal importance in fresh corn, but sweetness and aroma gain more importance in processed corn. The increased importance of aroma in processed corn is probably due to its prominence in canned corn and the greater likelihood of off-odors.

Buttery and others (1994) confirmed identification of components in major cooked sweet corn products, their concentration, and the degree of their contribution to the aroma and flavor of sweet corn. Many volatiles previously identified in fresh uncooked corn were below detection in cooked corn, likely lost by steam vaporization in the cooking process. The compound 2-acetyl-1-pyrroline (found in

aromatic rice), which contributes to the popcorn-like aroma, was found, as was 2-acetyl-2-thiazoline, first reported in meat volatiles. Neither compound had previously been reported in sweet corn. None of the other compounds reported in popcorn were found. In general, the concentrations of volatiles found in the canned products were many times higher than those found in the fresh and frozen products. This is probably due to the higher temperature used in can sterilization, which can help produce many of these compounds. Some of the volatile formations might also arise during the normal long storage of canned products at room temperature. The major volatile compounds that were common to all three products included DMS, 1-hydroxy-2-propanone, 2-hydroxy-3-butanone, and 2,3-butanediol. Additional major components in canned corn but minor in fresh and frozen corn included pyridine, pyrazine, alkylpyrazines, and 2-acetylthiazole. The compounds most important to canned sweet corn aroma included DMS, 2-acetyl-1-pyrroline, 2-ethyl-3, 6-dimethylpyrazine, acetaldehyde, 3-methylbutanal, 4-vinylguaiacol, and 2-acetylthiazole. Alkylpyrazine and 3-methylbutanal were less important to fresh sweet corn aroma, whereas 2-acetyl-2-thiazoline was important.

The compound 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DHF or furaneol), a well-known component of some fruits, was identified in canned corn in higher levels and in frozen and fresh cooked sweet corn in lower levels by Buttery and Ling (1997). The compound was also identified in tortilla chips and taco shells. Previously, it had been identified in popcorn (Schieberle 1991; Walradt et al. 1970). Highest concentrations were found in products that were heated well above 100°C in a dry state during manufacture. It was not found in tortillas because they are manufactured in a relatively moist state while heated compared to chips and shells. Also, the use of calcium hydroxide in the preparation of tortilla flour may have an adverse effect on furaneol because furaneol is less stable under alkaline conditions. Furaneol was found in canned sweet corn in high concentration because of the decomposition of sugar phosphates, as canned sweet corn has added sugar, and high canning temperatures. Furaneol has a sweet aroma important in fruits but may also enhance the flavor and aroma of corn products. Furaneol does not seem to be released from these foods until they come into contact with moisture, such as saliva, as they are placed in the mouth.

Off-Flavors Because off-flavor and off-aroma catalyzed by LOX, a corn germ enzyme, are common in unblanched or underblanched frozen stored sweet corn, Theerakulkait and Barrett (1995) compared LOX activity in frozen sweet corn germ fraction with that in the degermed fraction. Activity in the germ fraction was three times greater than in the degermed fraction. They concluded that LOX may be more appropriate than POD (another corn germ enzyme) to use as an index for blanching in the frozen sweet corn industry.

Sensory analysis of unblanched frozen stored sweet corn described higher undesirable characteristics than from blanched corn according to Theerakulkait and others (1995). By adding crude enzyme and purified LOX and POD to blanched corn, LOX increased "painty" and "stale/oxidized" off-odor and lowered "sweet" and "corn descriptors." POD was not important in off-odor formation, so as in the previous study, the authors concluded that LOX is more appropriate as a blanching indicator. Other enzymes in the germ may also be involved in off-odor formation, especially a "cooked cabbage" odor.

The mass of a corn cob means that surface corn is blanched longer than the typical time used for cooking. Processed corn loses much of the natural texture and original aromatic flavor. Boyes and others (1997) found that corn blanched for shorter times resulted in off-flavors following storage at –18°C. Likely, the major enzyme responsible for off-flavor was LOX. The percentage of linoleic acid did not change in unblanched and blanched sweet corns, suggesting that enzyme-controlled breakdown by LOX did not occur during frozen storage, but adding LOX to blanched samples reproduced the odor of unblanched corn. LOX in corn catalyzes breakdown of both linoleic and linolenic fatty acids, so it is difficult to clearly understand the role of LOX in off-flavor formation from these studies.

Barrett and others (2000) found that cultivars differed in LOX enzyme activity, leading to differences in the amount of time required for blanching to deactivate LOX. Because LOX activity may result in the formation of short-chain alcohols that confer a grassy flavor and aroma, blanching results in a rapid loss of volatiles and in a detectable change in aroma.

Plant Breeding Studies Evidence for the potential to improve cultivars for DMS came from a study by Williams and Nelson (1973). They determined the DMS potential of 21 sweet corn hybrids and observed the effects of blanching and blending of hybrids on their DMS potential while processing. No DMS was found in raw controls. There were significant DMS potential differences among hybrids. Those with high DMS potentials were fresh market hybrids, mostly of early maturity, while those with low DMS potential were processing hybrids. Others were new or used for both fresh market and processing markets. Blending hybrids with different DMS potentials give a DMS potential equal to the mean of the individual DMS levels when blended 1:1. They suggested that DMS potential should be added to the list of quality selection criteria used by sweet corn breeders, which includes uniform maturity, yield, color, sweetness, and pericarp tenderness. DMS potential could be improved by genetic selection for an increased level of its methionine analogue, thus leading to increased DMS levels resulting from its thermal decomposition. Another result from their study was that increasing the time of blanching reduced DMS potential.

Another study by Dignan and Wiley (1976) using five cultivars harvested over a wide range of maturities in 1 year also found that DMS was related to cooked sweet corn odor. Its concentration measured by GC varied significantly with cultivar, maturity, blanching time, and packaging. DMS decreased as maturity decreased. Corn blanched on the cob had more DMS than when blanched as whole cut kernels. Similar to the previous study, differences were significant but were not important according to sensory panels.

Reyes and others (1982) examined a *shrunken2* (high sugar) variety and a normal *sugary1* sweet corn variety at two levels of commercial harvest maturity after freezing a corn-on-the-cob product. The less mature sample had more sugar, but sugar level was not significant within variety. Sensory evaluation showed no consistent preference for higher sweetness or overall quality of shrunken samples (considered overly sweet). Sensory perception of sweetness was most highly correlated with sucrose content.

Azanza and others (1996a) found significant differences between supersweet (*shrunken2*) inbred lines and commercial supersweet corn cultivars for sweet corn aroma, texture, and flavor. Sweetness was strongly correlated with sweet corn flavor,

sugar, and sucrose content, while grassy flavor and aroma correlated with DMS content.

Statistical analysis of the quality attributes of F_3 families from a cross between two sweet corn lines that differed in kernel chemical and physical characteristics related to eating quality suggested that sweetness and tenderness appeared to be primarily under genetic control (Azanza et al. 1996b). These attributes have been considered the most important in eating quality. Results suggested that plant breeders could select for lines with higher levels of sweetness and tenderness for superior eating quality. In contrast to Flora and Wiley (1974b), other attributes such as aroma were not as easily defined genetically, thus selection for improved aroma would be more difficult.

Corn aroma was negatively associated with grassy aroma and grassy flavor by Azanza and others (1996b). Flavor and texture were mainly uncorrelated with aroma, suggesting that aroma could be improved without a negative effect on other attributes. All attributes were influenced by the carbohydrate composition of the kernel endosperm (phytoglycogen, water-insoluble starch, and total starch concentration). Coefficients of variation were large for aroma and even larger for tenderness, suggesting substantial variation among families for eating quality. Most sensory attributes related to eating quality were significantly correlated with kernel chemical and physical characteristics. There was a wide range of variation for each volatile compound (DMS and two unknowns). No association was found between DMS concentration and aroma. Grassy aroma and grassy flavor were correlated and related to a higher concentration of the two unknown volatiles. Higher DMS families also had higher volatiles associated with increased grassy flavor. Sensory panel evaluation indicated that sweet corn taste (mainly sweetness) and texture were the most important quality attributes. Even with sweet corn aroma third in importance, it should be considered when breeding for superior eating quality.

Extensive genetic variation among endosperm alleles and among genetic backgrounds within the endosperm groups for most kernel characteristics associated with eating quality (moisture, tenderness, sugars, phytoglycogen, and DMS) was found by Azanza and others (1996a). Most attributes were uncorrelated, indicating that selection to improve specific eating quality characteristics can be conducted simultaneously. The amount of genetic variation suggested that improvement for eating quality should be successful using plant breeding and genetic selection.

In another study, Azanza and others (1996c) used restriction fragment length polymorphism (RFLP) markers to find the chromosomal location and genetic effect of quantitative trait loci (QTLs) associated with eating quality in sweet corn. Eating quality characteristics were evaluated, and the genetic basis for phenotypic correlations between kernel characteristics and panelist perceptions of different sensory attributes was studied by using kernel chemical determinations and taste panel evaluations. They used $F_{2:3}$ lines from a cross between an inbred line with poor eating quality and an inbred line with good eating quality. Kernel traits (tenderness, starch content, phytoglycogen content, sucrose content, and DMS concentration) were correlated with various sensory attributes (corn aroma, grassy aroma, sweetness, starchiness, grassy flavor, crispness, tenderness, and juiciness). Seventy-four unlinked chromosomal regions that influence the different characteristics associated with sweet corn eating quality were detected. Significant QTLs were found for all traits and accounted for 3% to 42% of the total phenotypic variation. Some markers for

sensory factors were also found to be associated with kernel characteristics. Over all the traits, 24% showed genetic effects that were primarily additive; 31% had genetic effects with partial dominance effects; 8% showed primarily dominance effects; and 37% had primarily overdominance effects. These results suggest a model for quantitative inheritance of eating quality traits and sensory attributes where some individual QTLs have major effects on the phenotype and others have nearly undetectable effects. This research showed that molecular markers in combination with phenotyping can be used to identify QTL and suggests that the QTL could be a tool used in plant breeding improvement of eating quality.

Yousef and Juvik (2001) compared the efficiency of molecular marker-assisted selection (MAS) and phenotypic selection to improve kernel sucrose concentration, kernel tenderness, and taste panel preference. For sucrose concentration and tenderness, both breeding methods were effective, but MAS was superior. For multiple traits including seedling emergence, superiority of either method was not consistent, and sometimes, there were negative selection gains. But in most cases, MAS provided for simultaneous improvements for multiple traits. MAS was especially effective when traits required laboratory evaluation and were difficult and expensive to characterize.

In sweet corn breeding, maintaining or improving specialty traits diverts breeding and testing resources and genetic pressure from field performance. In addition, specialty traits are expensive and difficult to measure and thus cannot often be measured until advanced inbred lines or hybrids are developed and significant field resources have been already allocated. In some cases, genetic components of specialty traits can conflict with agronomic performance; therefore, it is difficult to conduct simultaneous selection for both, especially when specialty traits are measured later in the breeding process. Genetic control of flavor compounds is largely unknown and the number of genes controlling tenderness is also unknown, although progress has been made by genetic selection (Tracy 2001). Johnson (2001) described a successful marker-assisted breeding strategy for sweet corn breeding in the industry, which allowed for marker-based selection conducted earlier and was cheaper than phenotypic selection for processing and consumer quality traits such as flavor and tenderness.

CONCLUSION

Many compounds affecting flavor have been identified in major classifications of corn used as food. Flavor can be affected by factors such as cultivar, growing conditions, and processing method. Improvement of eating quality including flavor by plant breeding should be successful if fast and easy screening methods exist. MAS may be a useful tool in improving flavor. Corn has a vast reservoir of genetic variability in its germplasm resources that has not been characterized for flavor. These resources could be valuable sources for new and improved flavors.

REFERENCES

Arvisenet G, Le Bail P, Voilley A, Cayot N. 2002a. Influence of physicochemical interactions between amylase and aroma compounds on the retention of aroma in food-like matrices. *J Agric Food Chem* 50:7088–93.

Arvisenet G, Voilley A, Cayot N. 2002b. Retention of aroma compounds in starch matrices: Competitions between aroma compounds toward amylase and amylopectin. *J Agric Food Chem* 50:7345–9.

Azanza F, BarZur A, Juvik JA. 1996a. Variation in sweet corn kernel characteristics associated with stand establishment and eating quality. *Euphytica* 87:7–18.

Azanza F, Klein BP, Juvik JA. 1996b. Sensory characterization of sweet corn lines differing in physical and chemical composition. *J Food Sci* 61:253–7.

Azanza F, Tadmor Y, Klein BP. 1996c. Quantitative trait loci influencing chemical and sensory characteristics of eating quality in sweet corn. *Genome* 39:40–50.

Azanza F, Juvik JA, Klein BP. 1994. Relationships between sensory quality attributes and kernel chemical composition of fresh-frozen sweet corn. *J Food Qual* 17:159–72.

Barrett M, Garcia EL, Russell GF, Ramirez E, Shirazi A. 2000. Blanch time and cultivar effects on quality of frozen and stored corn and broccoli. *J Food Sci* 65:534–40.

Bills DD, Keenan TW. 1968. Dimethyl sulfide and its precursor in sweet corn. *J Agric Food Chem* 16:643–5.

Boutboul A, Lenfant F, Giampaoli P, Feigenbaum A, Ducruet V. 2002. Use of inverse gas chromatography to determine thermodynamic parameters of aroma-starch interactions. *J Chromatogr A* 969:9–16.

Boyes S, Chevis P, Perera C. 1997. Peroxidase isoforms of corn kernels and corn on the cob: Preparation and characteristics. *Lebensm Wiss Technol* 30:192–201.

Bredie WLP, Mottram DS, Guy RCE. 1998a. Aroma volatiles generated during extrusion cooking of maize flour. *J Agric Food Chem* 46:1479–87.

Bredie WLP, Mottram DS, Hassell GM, Guy RCE. 1998b. Sensory characterisation of the aromas generated in extruded maize and wheat flour. *J Cereal Sci* 28:97–106.

Buttery RG, Ling LC. 1982. 2-Acetyl-1-pyrroline: An important component of cooked rice. *Chem Ind* 958–9.

Buttery RG, Ling LC. 1994. Importance of 2-aminoacetophenone to the flavor of masa corn flour products. *J Agric Food Chem* 42:1–2.

Buttery RG, Ling LC. 1995. Volatile flavor components of corn tortillas and related products. *J Agric Food Chem* 43:1878–82.

Buttery RG, Ling LC. 1997. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone in corn products. *J Agric Food Chem* 45:1306–8.

Buttery RG, Ling LC. 1998. Additional studies on flavor components of corn tortilla chips. *J Agric Food Chem* 46:2764–9.

Buttery RG, Ling LC, Stern DJ. 1997. Studies on popcorn aroma and flavor volatiles. *J Agric Food Chem* 45:837–43.

Buttery RG, Stern DJ, Ling LC. 1994. Studies on flavor volatiles of some sweet corn products. *J Agric Food Chem* 42:791–5.

Conde-Petit B, Escher F, Nuessli J. 2006. Structural features of starch-flavor complexation in food model systems. *Trends Food Sci Technol* 17:227–35.

Dignan DM, Wiley RC. 1976. DMS levels in the aroma of cooked frozen sweet corn as affected by cultivar, maturity, blanching and packaging. *J Food Sci* 41:346–8.

Dravnieks A, Reilich JG, Whitfield J, Watson CA. 1973. Classification of corn odor by statistical analysis of gas chromatographic patterns of headspace volatiles. *J Food Sci* 38:34–9.

Flora LF, Wiley RC. 1974a. Influence of cultivar, process, maturity, and planting date on the dimethyl sulfide and hydrogen sulfide levels in sweet corn. *J Agric Food Chem* 22:816–9.

Flora LF, Wiley RC. 1974b. Sweet corn aroma, chemical components and relative importance in the overall flavor response. *J Food Sci* 39:770–3.

Gardner HW, Inglett GE. 1971. Food products from corn germ: Enzyme activity and oil stability. *J Food Sci* 36:645–8.

Gould WA, Krantz FA, Mavis J. 1951. Quality evaluation of fresh, frozen and canned yellow sweet corn. *Food Technol* 5:175–9.

Huang CJ, Zayas JF. 1996. Aroma characteristics of corn germ proterin flours. *J Food Sci* 61:258–61.

Johnson, L. 2001. Marker assisted sweet corn breeding: A model for specialty crops. *Proceedings of the 56th Annual Corn and Sorghom Research Conference*, Chicago, Dec. 5–7, pp. 25–30.

Karahadian C, Johnson KA. 1993. Analysis of headspace volatiles and sensory characteristics of fresh corn tortillas made from fresh masa dough and spray-dried masa flour. *J Agric Food Chem* 41:791–9.

Maga JA. 1989. Flavor formation and retention during extrusion. In Mercier C, Linko P, Harper JM (eds.), *Extrusion Cooking*. St. Paul, MN: AACC, pp. 387–98.

Marshall SW, Tracy WF. 2003. Sweet corn. In White PJ, Johnson LA (eds.), *Corn: Chemistry and Technology*, 2nd Ed. St. Paul, MN: AACC, pp. 537–69.

Park D, Maga JA. 2006. Identification of key volatiles responsible for odour quality differences in popped popcorn of selected hybrids. *Food Chem* 99:538–45.

Phillips RD, Sternberg M. 1979. Corn protein concentrate: Functional and nutritional properties. *J Food Sci* 44:1152–5.

Pollak LM, White PJ. 1995. Corn as a food source in the United States: Part I. Historical and current perspectives. *Cereal Foods World* 40:749–53.

Reyes GR, Varseveld GW, Kuhn MC. 1982. Sugar composition and flavor quality of high sugar (shrunken) and normal sweet corn. *J Food Sci* 47:753–5.

Schieberle P. 1991. Primary odorants in popcorn. *J Agric Food Chem* 39:1141–4.

Schieberle P. 1995. Quantitation of important roast-smelling odorants in popcorn by stable isotope dilution assays and model studies on flavor formation during popping. *J Agric Food Chem* 43:2442–8.

Schieberle P, Grosch W. 1985. Identification of the volatile flavour compounds of wheat bread crust-comparison with rye bread crust. *Z Lebensm Unters Forsch* 180:474–8.

Shen G, Hoseney RC. 1995. Comparisons of aroma extracts of heat-treated cereals. *Lebensm Wiss Technol* 28:208–12.

Tapanapunnitikul O, Caiseri S, Peterson DG, Thompson DB. 2008. Water solubility of flavor compounds influences formation of flavor inclusion complexes from dispersed high-amylose maize starch. *J Agric Food Chem* 56:220–6.

Theerakulkait CC, Barrett DM. 1995. Lipoxygenase in sweet corn germ: Isolation and physicochemical properties. *J Food Sci* 60:1029–33.

Theerakulkait CC, Barrett DM, McDaniel MR. 1995. Sweet corn germ enzyme affect odor formation. *J Food Sci* 60:1034–40.

Tracy WF. 2001. Sweet corn. In Hallaurer AR (ed.), *Specialty Corns,* 2nd Ed. Boca Raton, FL: CRC Press, pp. 155–97.

Walradt JP, Lindsay RC, Libbey LM. 1970. Popcorn flavor: Identification of volatile compounds. *J Agric Food Chem* 18:926–8.

White PJ, Pollak LM. 1995. Corn as a food source in the United States: Part II. Processes, products, composition, and nutritive values. *Cereal Foods World* 40:756–62.

Wiley RC. 1985. Sweet corn aroma: Studies of its chemical components and influence on flavor. In Pattee H (ed.), *Evaluation of Quality of Fruits and Vegetables*. Westport, CT: AVI, pp. 349–66.

Williams MP, Nelson PE. 1973. Effect of hybrids and processing on the dimethyl sulfide potential of sweet corn. *J Food Sci* 38:1136–8.

Wu YV, Hareland GA, Warner K. 2001. Protein-enriched spaghetti fortified with corn gluten meal. *J Agric Food Chem* 49:3906–10.

Wu YV, Payne-Wahl KL, Vaughn SF. 2006. Corn gluten meal odorants and volatiles after treatment to improve flavor. *Cereal Chem* 83:228–34.

Wulff G, Avgenaki G, Guzmann MSP. 2005. Molecular encapsulation of flavours as helical inclusion complexes of amylase. *J Cereal Sci* 41:239–49.

Yousef GG, Juvik JA. 2001. Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. *Crop Sci* 41:645–55.

Ziegler KE. 2003. Popcorn. In White PJ, Johnson LA (eds.), *Corn: Chemistry and Technology*, 2nd Ed. St. Paul, MN: AACC, pp. 783–809.

CHAPTER 43

Olive and Olive Oil

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INTRODUCTION

The olive tree, which is one of the oldest known cultivated trees, is the symbol of friendship and peace besides playing other social and religious roles in Greek mythology and in the Old Testament.

Nowadays, productive olive trees are spread out over 7 million hectares, with pedoclimatic conditions such as those prevailing in the Mediterranean basin, which account for not less than 97% of the world's production (Table 43.1). Olive oil consumption is also concentrated in these producing countries and reflects the culinary traditions of their inhabitants.

There are not one but several olive oil designations. Olive oil is marketed in accordance with the designations of the International Olive Council (IOC) trade standards (IOC 2006) and regulation of the Commission of the European Communities (CEC 1991). Virgin olive oil (VOO) must be obtained by only mechanical or other physical means under conditions, particularly thermal, which do not lead to alterations in the oil. This oil has not undergone treatment other than washing, decantation, centrifugation, and filtration. Extra-virgin olive oil (EVOO) and VOO are different edible grades of VOO. Lampante VOO is not fit for consumption and is intended for refining or for technical purposes. Refined olive oil (ROO) is the oil refined by methods that include neutralization, decolorization with bleaching earth, and deodorization. Olive oil is an edible blend of VOO and ROO. Olive-pomace oil is obtained by solvent extraction of the olive milling by-products; its triacylglycerol composition is similar to that of VOO, but some of the non-saponifiable compounds (e.g., waxes) may differ significantly, requiring oil to be winterized before refining. Olive-pomace oil designation means a mixture of refined olive-pomace oil with virgin olive oil.

Olive oil chemical composition is clustered, by a very simple classification, into major and minor compounds. The set of major compounds is made up primarily of

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TABLE 43.1. Geographic Distribution of World Olive Oil and Table Olive Production

	Olive Oil Production		Table Olive Production	
Country	Tn[a]	%	Tn[a]	%
European Union	2144.0	76.0	712.0	38.9
Spain	1095.6	38.9	465.0	25.4
Italy	630.0	22.3	80.0	4.4
Greece	370.0	13.1	147.0	8.0
Portugal	35.0	1.2	10.4	0.6
France	4.7	0.2	2.0	0.1
Other EU countries	8.7	0.3	7.6	0.4
Tunisia	130.0	4.6	15.0	0.8
Turkey	140.0	5.0	240.0	13.1
Morocco	80.0	2.8	100.0	5.5
Syria	154.0	5.3	200.0	10.9
Algeria	40.0	1.4	90.0	4.9
Argentina	14.0	0.5	55.0	3.0
United States	1.0	>0.1	45.0	2.4
Others	117.0	4.1	375.5	20.5
Mediterranean countries	2659.9	94.3	1370.5	74.8
Total	2820.0	100.0	1832.5	100.0

Source: The International Olive Council, prevision of crop, 2006/2007.

[a]1000 tons.

triacylglycerols, a set of glyceridic compounds such as mono- and diacylglycerols, and free fatty acids, while other fatty acid derivatives such as phospholipids, waxes, and esters of sterols are traditionally classified into the set of minor compounds. The set of minor compounds is constituted by those already cited: the chemical compounds from the unsaponifiable matter, which have the common characteristic of being obtained by saponification, and an ample and heterogeneous group of compounds constituted by pigments, phenols, and volatile compounds. This set of minor compounds plays an important role in purity issues, studies of authentication and genuineness, and, more recently, in olive oil traceability. Concerning sensory quality, volatile and phenolic compounds are responsible for VOO flavor.

CHEMICAL COMPOUNDS RESPONSIBLE FOR VOO FLAVOR

VOO is appreciated by consumers not only for its health benefits but also for its fragrant flavor. Volatile compounds are responsible for its aroma, while phenolic compounds are related to its taste. Odor and taste joined to somatosensory information gather the complex perception of flavor. Food flavor is directly related to quality and decisively influences the recognition, selection, and acceptability of the food, influencing consumers' preferences. The result is that flavor plays an important role in human nutrition and determines, to a certain degree, the quality of life of consumers (Rogers 1996).

The presence of these compounds contributes to the particular VOO flavor, characterized by a series of positive sensory attributes that produce a correct balance

of green, fruity, bitter, and pungent sensory notes, which makes it a distinctive edible oil.

Many volatile compounds have been described as components of VOO. They comprise compounds with diverse molecular weights and chemical nature, although they are present at very low concentrations. The total content of volatiles in VOO is variable depending on the olive oil designation and quality. ROO contains a scarce amount of total volatiles; they are removed in the deodorization step in the refining process, which is related to olive oil oxidative degradation. VOO has a higher amount of total volatiles, which are produced from the olive fruit, and they are direct metabolites produced in plant organs by intracellular biogenic pathways and oxidative processes, all of them being responsible for sensory attributes appreciated by consumers. On the contrary, low-quality VOOs have complex profiles composed of a large number of volatiles that are responsible for off-flavors. The amount of volatiles depends on sensory defects (Morales et al. 2005).

Different functional groups can be found in the volatile compound molecules. Aldehydes, alcohols, ketones, and esters are the major compounds, but there are also furans, hydrocarbons, acids, and aromatic compounds, though in lower amounts. The influence of each volatile on the sensory quality depends not only on its concentration but also on its odor threshold.

The volatile content of VOO, from a qualitative and quantitative point of view, depends on different factors such as genetic characteristics, geographic origin, pedoclimatic conditions, olive ripening, processing conditions, and olive oil storage (Aparicio and Morales 1998; Aparicio et al. 1996a; Luna et al. 2006a; Morales and Aparicio 1999; Morales et al. 1997). Table 43.2 shows the volatile compounds identified in different categories of VOO by several authors.

Concerning the taste, the bitter perception depends on the phenolic composition (Artajo et al. 2006; Favati et al. 1995; Gutiérrez et al. 1992, 1989), although the contribution of each individual phenol to this sensory note has not been clearly defined (Siliani et al. 2006). Phenolic compounds also affect VOO stability (Aparicio et al. 1999) as they have antioxidant properties and play a beneficial role in human health (Blekas and Boskou 2006). Phenols are multifunctional antioxidants and can act as oxidative enzyme inhibitors, metal chelators, and free radical scavengers. Radical scavenging is considered the main antioxidant mechanism of phenols both *in viv*o and *in vitro* (Tsimidou et al. 2006).

VOO contains many phenolic compounds (Table 43.3), in both free and bound forms, which play a role in the resistance of this product to oxidation (Carrasco-Pancorbo et al. 2005). A linear relationship has been demonstrated between the phenolic content and the oxidative stability of VOO (Shahidi 1997). The phenolic content of olive oil depends on a number of factors such as cultivar, origin of plantation, olive ripeness, extraction procedure, and olive oil storage (Abaza et al. 2005; Baccouri et al. 2007; Ben Temime et al. 2006; Kharazi 2008).

The polyphenol content in VOOs varies from a few milligrams per kilogram to more than 1000 mg/kg oil, expressed as caffeic acid equivalents. VOOs with concentrations that exceed 300 mg/kg have a pronounced bitter taste. Phenols are also found in olive leaves, and it has been suggested to supplement ROOs with phenolic extracts to increase their shelf life and to give them both a green color and a bitter taste.

The effect of parameters such as olive variety, olive ripeness, and olive oil extraction on the individual concentration of the phenols has been the object of many

TABLE 43.2. Volatile Compounds Identified in Different Categories of VOO by Several Researchers

Aldehydes	Alcohols	Esters	Hydrocarbons	Ketones
Acetaldehyde	Methanol	Methyl acetate	2-Methylbutane	2-Butanone
2-Methylbutanal	Ethanol	Ethyl acetate	2-Methylpentane	3-Methyl-2-butanone
3-Methylbutanal	2-Methyl-1-butanol	Butyl acetate	3-Methylpentane	3-Pentanone
2-Methyl-2-butenal	3-Methyl-1-butanol	2-Methylbutyl acetate	Hexane	4-Methyl-2-pentanone
Pentanal	2-Methyl-3-butenol	Isopentyl acetate	Hexene	1-Penten-3-one
(E)-2-Pentenal	2-Methyl-1-propanol	Hexyl acetate	Heptane	2-Hexanone
(Z)-2-Pentenal	1-Pentanol	2-Hexenyl acetate	Octane	2-Heptanone
Hexanal	3-Pentanol	3-Hexenyl acetate	1-Octene	6-Methyl-5-hepten-2-one
2-Hexenal	1-Penten-3-ol	(Z)-3-Hexenyl acetate	Nonane	2-Octanone
(E)-2-Hexenal	(Z)-2-Pentenol	Octyl acetate	Tridecene	3-Octanone
(Z)-2-Hexenal	3-Hexen-1-ol	2-Ethylphenyl acetate	Pentene dimers	2-Nonanone
3-Hexenal	(E)-3-Hexen-1-ol	Benzyl acetate	Methylbenzene	Acetophenone
(Z)-3-Hexenal	(Z)-3-Hexen-1-ol	Phenethyl acetate	Styrene	
2,4-Hexadienal	2-Hexen-1-ol	Ethyl propanoate	Ethyl benzene	Sulfur Compounds
Heptanal	(E)-2-Hexen-1-ol	Propyl propanoate	Limonene	3-Isopropenylthiophene
(E)-2-Heptenal	(Z)-2-Hexen-1-ol	Ethyl 2-methylpropanoate		2,5-Diethylthiophene
(Z)-2-Heptenal	4-Hexen-1-ol	Propyl 2-methylpropanoate	Phenols	2-Ethyl-5-hexylthiophene
2,4-Heptadienal	1-Hexanol	Methyl butanoate	Anisole	
Octanal	1-Heptanol	Ethyl butanoate		Furans
(E)-2-Octenal	1-Octanol	Methyl 2-methylbutanoate	Ethers	Ethylfuran
Nonanal	1-Octen-3-ol	Ethyl 2-methylbutanoate	Diethyl ether	2-Propylfuran
(E)-2-Nonenal	2-Octen-1-ol	Methyl 3-methylbutanoate	1,8-cineole	3-Propylfuran
2,4-Nonadienal	1-Nonanol	Ethyl 3-methylbutanoate		3-Methyl-2-pentylfuran
(E)-2-Decenal	1-Decanol	Butyl 3-methylbutanoate	Acids	2-Propyldihydrofuran
Decanal	Lavandulol	Methyl pentanoate	Acetic acid	3,4-Methyl-3-pentenylfuran
2,4-Decadienal	Linalool	Methyl hexanoate	Butanoic acid	
(E)-2-Undecenal	Benzyl alcohol	Ethyl hexanoate	Pentanoic acid	
	2-Phenylethanol	Methyl heptanoate	Propanoic acid	
	α-Terpineol	Methyl octanoate	Hexanoic acid	
	1,3-Butanediol	3-Methyl-2-butenylacetate	Heptanoic acid	

Source: Morales and Tsimidou (2000).

TABLE 43.3. Phenolic Compounds Identified in VOO by Several Researchers

Oleuropein	Caffeic acid
Hydroxytyrosol	Ferulic acid
Tyrosol and derivatives	Gallic acid
4-Acetoxy-ethyl-1,2-dihydroxybenzene	Homovanillic acid
1-Acetoxy-pinoresinol	*p*-Hydroxybenzoic acid
Pinoresinol	Protocatechuic acid
Apigenin	Sinapic acid
Luteolin	Syringic acid
1-(3′-Methoxy-4′-hydroxy-phenyl)-6,7-dihydroxy-isochroman	*o*-Coumaric acid
1-Phenyl-6,7-dihydroxy-isochroman and derivatives	*p*-Coumaric acid

Source: Boskou (2006), Young and others (2007).

TABLE 43.4. Concentration (mg/kg) of Phenolic Compounds in Spanish VOO Varieties

	Virgin Olive Oil Variety			
Phenolic Compound	Arbequina	Cornicabra	Hojiblanca	Picual
Hydroxtyryosol	40.8	57.3	88.5	110.1
Hydroxytyrosol acetate	63.0	3.5	56.0	38.0
Hy-EDA	235.2	227.3	274.6	133.7
Hy-EA	23.4	129.1	196.2	264.7
Tyrosol	32.8	93.6	51.9	97.0
Ty-EDA	414.6	795.6	272.4	326.1
Ty-EA	28.0	167.9	76.0	199.5
1-Acetoxypinoresinol	119.9	18.2	71.2	13.9
Pinoresinol	106.0	110.1	51.7	103.8
Others	15.4	5.6	15.0	9.8
Total polyphenols	1083.4	1610.5	1159.3	1299.9

Source: García and others (2003).

Note: Hy-EDA, dialdehydic form of decarboxymethyl oleuropein aglycon; Hy-EA, oleuropein aglycon; Ty-EDA, dialdehydic form of decarboxymethyl ligstroside aglycon; Ty-EA, ligstroside aglycon.

studies (García et al. 2003; Morales and Tsimidou 2000). Table 43.4 shows the concentration of several phenols in four VOO Spanish varieties reported by García and others (2003), in which quantitative differences can be observed.

Polar phenolic compounds also have biological properties that have received much attention lately. They have been thoroughly investigated with the aim of establishing a relationship between dietary intake and the risk of cardiovascular disease or cancer (Kampa et al. 2006; Vita 2005). The pharmacological effects of some individual phenols quantified in VOO are well-known. Thus, oleuropein is a coronary vasodilatador and has antispasmodic, antihypertensive, and antiarrhythmic effects; (-)-oleocanthal inhibits COX-1 and COX-2; caffeic acid exhilarates and scavenges reactive oxygen species; *p*-coumaric acid has anti-hyperlipidemic effects; and hydroxytyrosol and 1-(3′-methoxy-4′-hydroxy-phenyl)-6,7-dihydroxy-isochroman inhibit platelet aggregation (García-González et al. 2008a).

Biogenesis of VOO Volatile Compounds

VOO contains two kinds of biogenic volatile compounds: the volatiles present in the intact olive fruit as direct metabolites produced in the plant organs by intracellular biogenic pathways, and other volatiles known as "secondary volatiles" formed very quickly during disruption of the cell structure due to enzymatic reactions in the presence of oxygen. The secondary volatiles are the main factors responsible for the "green" aroma of VOO (Morales and Tsimidou 2000).

The main precursors for volatile compound formation in VOO are fatty acids (linoleic and α-linolenic) and amino acids (leucine, isoleucine and valine) that are involved in several biochemical pathways responsible for VOO aroma biogenesis.

Besides the biochemical pathways, autoxidation plays an important role in olive oil volatile compound production; several volatile compounds, especially aldehydes, can be formed by this mechanism. The different pathways that lead to the formation of volatile compounds are summarized in Table 43.5.

The lipoxygenase pathway is the best-studied biochemical pathway directly involved in the formation of the major volatile compounds of VOO. It is responsible for the "secondary" volatile compounds. The presence of the lipoxygenase (LOX) pathway in olives has been discussed and the characterization of the different enzymes involved in the pathway has been studied in recent years (Olías et al. 1993; Salas 1998). The main precursors are linoleic and α-linolenic polyunsaturated fatty acids. Figure 43.1 shows the main volatile compounds of VOO produced through this pathway. It is well-known that the formation of C_6 aliphatic volatile compounds from the 13-hydroperoxides of linoleic and α-linolenic acids is promoted in olives, while the formation of C_9 compounds is practically absent.

E-2-hexenal, hexanal, and Z-3-hexenal are the major aldehydes found in VOOs. Z-3-hexenol, E-2-hexenol, and hexan-1-ol are usually found at high concentrations, and differences between their ratios depend on the variety and the stage of ripeness of the harvested olives (Aparicio and Morales 1998). Other C_6 volatile compounds such as Z-2-hexenal, E-3-hexenal, Z-2-hexenol, and E-3-hexenol can also be produced through this pathway and are usually present in VOO at low concentrations. The concentrations of the esters, which contribute to a fruity sensory perception, depend on the olive variety as well.

The final nuances of the aroma of VOOs are related to the contribution of the different pathways. If the most active pathway is the LOX cascade, the aroma of the oil will not show any defect; on the contrary, sensory defects will be present if, among major volatile compounds, some of them derive from possible fermentations or amino acid conversions, from enzymatic activities of molds, or from oxidative processes (Angerosa 2002; Morales et al. 2005).

Biogenesis of Olive Oil Phenolic Compounds

The phenolic fraction of VOO derives mainly from hydrolysis products of oleuropein and ligstroside, aglycones. As previously mentioned, they are responsible for some of the better known health benefits of olive oil (Covas 2007) and have a great impact on the sensory assessment (Morales and Tsimidou 2000) and shelf life of VOOs (Morales and Przybylski 2000). Phenols identified in VOO originate from the fruit. The main one is oleuropein, an heterosidic ester of elenolic acid with

TABLE 43.5. Main Chemical and Biochemical Routes for the Formation of Volatile Compounds in VOO

Precursor	Chemical/ Biochemical Route	Effect	Main Volatile Compound Produced
Lipids: fatty acids	Lipoxygenase pathway	C_6-aldehyde volatile compounds are formed from 13-hydroperoxides of linoleic and α-linolenic fatty acids. Further transformation into alcohols and esters by the action of alcohol dehydrogenase and alcohol acyltransferase	Hexanal, hexanol, hexyl acetate, Z-3-hexenal, E-2-hexenal, Z-3-hexenol, E-2-hexenol, Z-3-hexenyl acetate, E-2-hexenyl acetate
	Cleavage reaction of 13-hydroperoxide of linolenic acid	Reaction mediated through the LOX pathway originates volatile compounds; further activation of alcohol dehydrogenase enzyme produces C_5 compounds	Pentene dimers, C_5 carbonyl compounds 1-penten-3-ol, 2-penten-1-ol
	Fatty acid metabolism	Production of volatile compounds from fatty acids during olive ripening	Alcohols, ketones, and esters
	Autoxidation of unsaturated fatty acids	Formation of hydroperoxides and production of secondary products by a breakdown reaction	Aldehydes, ketones, alcohols, and acids
Amino acids: valine, leucine, and isoleucine	Biochemical transformation of branched-chain amino acids	Transformation into branched aldehydes later transformed to alcohols and esters by the action of alcohol dehydrogenase and alcohol acyltransferase	2-Methylpropanal, 2-methylbutanal, 3-methylbutanal, corresponding alcohols and ester derivatives

Source: Morales and others (1999), Morales and Tsimidou (2000), Yangkyo and others (1995).

3,4-dihydroxyphenethylalcohol (hydroxytyrosol), followed by hydroxytyrosol and their glucosidic forms (Abaza et al. 2005; Mateos et al. 2001), tyrosol, and several other compounds clustered around phenolic acids, phenolic alcohols, flavonoids, hydroxy-isocromans, secoiridoids, and lignans (Carrasco-Pancorbo et al. 2005) (Table 43.3).

Phenolic acids were the first group of phenols observed, and they are present as minor components in VOO (Carrasco-Pancorbo et al. 2005). They have a basic chemical structure of C6-C1 (benzoic acids) and C6-C3 (cinnamic acids) such as

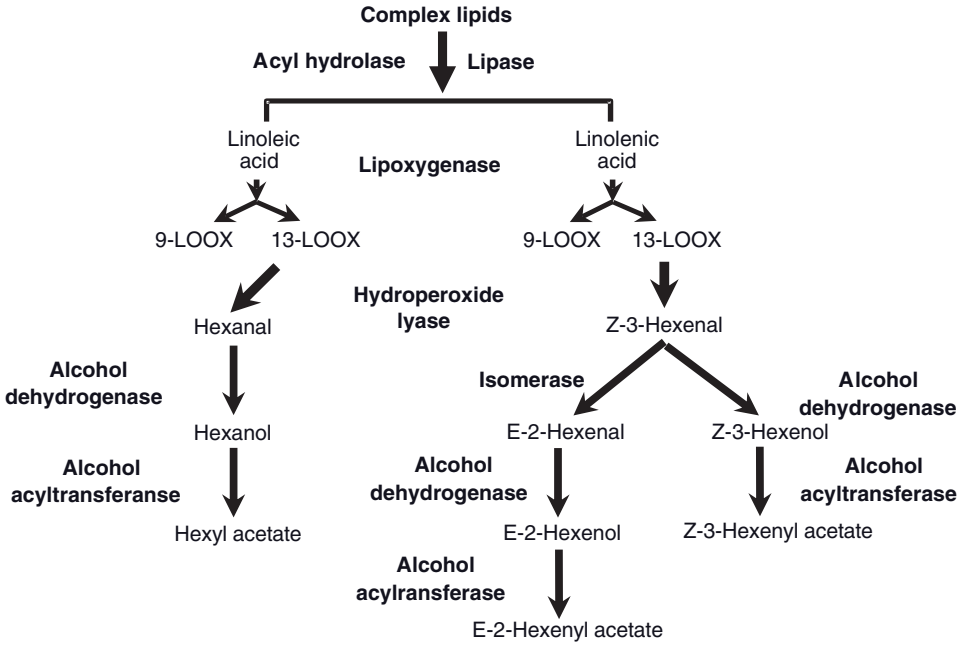

Figure 43.1. Lipoxygenase pathway in olives.

caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechic, sinapic, and p-hydroxybenzoic acid (Montedoro et al. 1992).

The most prevalent in VOO phenols are, however, secoiridoids. They are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure. These compounds (e.g., oleuropein, demethyloleuropein, and ligstroside) are derivatives of the secoiridoid glucosides of olive fruits. Breakdown products of two major phenolic constituents of the olive fruit, oleuropein and ligstroside, form the majority of the phenolic fraction. The most abundant secoiridoids of VOO are the dialdehydic form of oleuropein aglycon, the dialdehydic form of ligstroside aglycon, ligstroside aglycon and oleuropein aglycon (Montedoro et al. 1992). Several authors have reported that flavonoids such as luteolin and apigenin are also phenolic components of VOO. Thus, (+)– taxifolin, a flavanonol, has been found in seven Spanish VOOs (Rovellini et al. 1997).

Lignans are also found as prevalent in VOO phenolic compounds. Some authors have isolated and characterized 1-acetoxy pinoresinol, pinoresinol, and 1-hydroxypinoresinol as the most frequent lignans (Owen et al. 2000). A new class of phenolic compounds, hydroxy-isocromans, has been found in different samples of EVOO. Thus, Young and others (2007) have identified and quantified 1-phenyl-6,7-dihydroxy-isochroman and 1-(3′-methoxy-4′-hydroxy) pheny1-6,7-dihydroxy-isochroman.

Production of VOO Sensory Defects

High-quality olive oils have a profile of volatile compounds—mainly constituted by aldehydes, esters, alcohols, and ketones—that is responsible for a balanced flavor of

green and fruity sensory perceptions. The intrinsic pleasant flavor of VOOs can be altered by processes, such as lipolysis and oxidation, that give rise to unpleasant sensory notes or off-flavors. Lipolysis usually starts before the olive processing, while oxidation begins after olive oil has been obtained (Kochhar 1993). Thus, sensory defects can be due to the storage of olive fruits in piles before oil extraction. Olives transpire during the storage so that the temperature of the pile increases. Furthermore, if olives are stored for a long time under conditions of high humidity, fungi and yeasts will proliferate, and the microflora will change the flavor (Schnürer et al. 1999) of the extracted olive oil to make it inedible (Springett 1993).

The current olive oil official regulations (CEC 1997; IOC 1996) classify the most frequent off-flavors into four groups: winey–vinegary, fusty, humidity–mustiness, and rancid. The winey–vinegary defect is produced by lactic acid (*Lactobacillus*) and acetic acid bacteria, among other microorganisms, which induce a fermentative process in the olives, giving rise to the production of ethyl acetate and acetic acid, which are responsible for this defective sensory attribute (Morales et al. 2005). Fustiness is the characteristic flavor of oils obtained from olives in an advanced stage of fermentation after having been stored in piles. The Enterobacteriaceae genera *Aerobacter* and *Escherichia* have been found at the beginning of storage, while the genera *Pseudomonas*, *Clostridium*, and *Serratia* are the most significant after a long time of storage (Morales et al. 2005). The presence of these microorganisms increases the concentrations of esters and acids. Humidity–mustiness is the characteristic flavor of oils obtained from olives piled under humid conditions for several days with the consequence of development of various kinds of fungi. VOO obtained from those olives are characterized by a low concentration of volatiles and the presence of volatiles with seven and eight atom carbons. Rancidity is a common sensory characteristic of all the oils and fats that have undergone a process of autoxidation caused by a prolonged contact with air. Rancid oils are characterized by volatiles coming from the oxidation of unsaturated fatty acids, mainly aldehydes (e.g., pentanal, hexanal, heptanal, octanal, and nonanal) and acids (e.g., acetic, butanoic, hexanoic, and heptanoic). The first three defects are due to inadequate fruit preservation before olive oil processing, while the last is produced during the olive oil storage process.

When oils reach high intensities of sensory defects, they are classified as lampante VOOs and must undergo refining before being consumed. From an economical point of view, it is important to prevent the presence of off-flavors since the classification of an olive oil as lampante means lower incomes for farmers.

OLIVE OIL FLAVOR ANALYSIS

The analysis of olive oil flavor can be performed by two kinds of analysis: chemical analysis, including both volatile and phenolic compound analyses, and sensory assessment by trained assessors.

Volatile Compound Analysis

Some considerations should be taken into account before studying the aromatic fraction of any food from an analytical point of view (Morales and Tsimidou 2000;

Morales et al. 1992): (1) the concentration of volatiles is normally low, so that a process of concentration can be necessary; (2) the volatile fraction comprises components with diverse molecular weights and chemical nature; (3) there is no direct relationship between the incidence of each compound in VOO aroma and its concentration since the thresholds can vary greatly among the volatiles; and (4) the formation of artifacts must be avoided during analysis since they produce incorrect results.

It has been determined that the first step in volatile compound analysis is the isolation and preconcentration of the volatile compounds, so a sample preparation step is of great importance in volatile compound analysis. The selection of the sample preparation method should be carefully chosen. The methods used in olive oil volatile compound analysis have changed through the years according to the technical and instrumental development; they can be split into two groups: methods not involving preconcentration and methods involving preconcentration. Table 43.6 describes the main sample preparation methodologies used for quantifying VOO volatile compounds together with their advantages and disadvantages.

Methods Not Involving Preconcentration

Direct Injection This is the simplest technique, but is of low sensitivity, and degradation products can appear if the temperature is too high. It consists of placing the sample at the chromatograph injector inlet. The volatiles are purged by the carrier gas for chromatography. It has been scarcely employed in VOO analysis.

Static Headspace (SHS) This is a simple way to analyze the volatile fraction. An aliquot from the vapor phase in equilibrium with the sample, in a sealed vial subjected to a determined temperature, is sampled by a syringe and injected in the chromatograph. It requires strict control of temperature and sampling. The technique was used to study VOO volatiles responsible for sensory defects (Del Barrio et al. 1983; Gutiérrez et al. 1975), to study the relationships between the contents of volatiles and fatty acids in thermoxidized olive oils (Dobarganes et al. 1986), and to study halogenated volatiles in VOOs (Mariani et al. 1990).

Methods Involving Concentration

Simultaneous Distillation–Extraction Flath and others (1973) carried out an exhaustive study of VOO volatiles with this technique in the early 1970s. Other authors have used a procedure consisting of a vacuum co-distillation of oil with diethyl ether, collected in three traps cooled with liquid N_2 and then in a "cold finger" cooled with running water; the last fraction was extracted with ether and combined with the ether extracts collected in the cold traps; finally, the extract is concentrated by distillation and micro-distillation (Guth and Grosch 1989).

Dynamic Headspace (DHS) This technique (Swinnerton et al. 1962) consists of purging the volatiles of the sample, subjected to a determined temperature, with an inert gas at a controlled flow, and passing them through a trap where they are

TABLE 43.6. Traditional Common Procedures Employed for Sample Preparation in VOO Volatile Compound Analysis

Procedure	Description	Advantages	Disadvantages
Without preconcentration			
Direct injection	The sample is placed in a tube fitted to a GC injector. Volatiles are purged by a carrier gas into the column.	Rapid. Simple.	Very low sensitivity. Degradation products.
Static headspace	The sample is deposited in a sealed vial. An aliquot of the vapor phase is injected in a GC.	Fine with multiple extractions. Appropriate for volatiles with high molecular weight.	Poor sensitivity and reproducibility. Artifacts. Inappropriate for trace analysis. Leaks during syringe filling.
With preconcentration			
Distillation–extraction	The vapor from distillation is condensed on a refrigerant or trapped in different cryogenic traps or adsorbent materials and is later injected in a GC.	Small amount of solvents. Rapid concentration process. Low thermal degradation of volatiles.	Not appropriate for thermolabile volatiles. Lack of solute by co-evaporation.
Dynamic headspace, Tenax traps, thermal desorption and cool trap injection	An inert gas (e.g., N_2) sweeps the sample headspace that is stirred or bubbled. Volatiles are trapped in Tenax TA. The trap is desorbed by GC.	High adsorption capacity. Useful for almost all kinds of volatiles. Good recovery factors. No artifacts.	Less sensitive to some acids. Temperature and flow rate must be controlled. An analysis per sample. Expensive.
Supercritical fluid extraction	The sample is placed in an extraction cell. A supercritical fluid passes through the cell and extracts the volatile compounds	Easy to apply. Detection of volatiles in oil and olives. Adequate for off-flavors. All the steps in a single process	Leaks. Selective with some volatiles (oxygenated compounds and by molecular weight and polarity) A concentration step is necessary (e.g., Tenax).
Solid phase microextraction	An SPME fiber is exposed to a sample vapor phase. Volatiles adsorbed on the fiber are desorbed in the GC injection port.	Automatic, rapid, cheap, easy to use. All the steps in a single process. Various kinds of fibers. Good repeatability.	Differences in quantification of low-weight molecules. Lower number of volatiles at low concentrations. Some of the disadvantages of static headspace.

Source: Morales and Tsimidou (2000).

retained. Volatiles are later desorbed and injected into the chromatograph for their separation. There are two methods within this technique. The first is the true DHS, which consists of sweeping the sample surface with the inert gas under stirring, while the second, known as the purge-and-trap technique, consists of bubbling the gas through the sample. It can be carried out in two different ways: in open circuit, where the gas passes through the sample and trap and is then voided, or in closed circuit, where the gas is recycled through the sample and trap.

Although various factors affect the process (diameter and length of the traps, size and shape of the container, and absorbent particle size), the three fundamental variables are temperature, time, and purge flow. The latter two should be fixed beforehand, bearing in mind that too low values may lead to a defective purge and too high values to loss of volatiles. The temperature depends on the types of compounds to be analyzed. If the working temperature is low (maximum 60°C), the volatiles are those really present in the sample at the moment of analysis. But if the temperature is higher (up to 160°C), there will also be volatiles formed by thermal degradation.

As said before, this method needs a concentration step using traps made of adsorbent materials and/or cryogenic traps. Adsorbent traps are made of a tube filled with sorbent material. When the inert gas with the volatiles passes through the trap, the volatiles are retained. Various materials have been used: activated carbon and graphitized sorbents, Tenax, Porapak, Chromosorb, and Amberlite. Cryogenic traps are made of a capillary tube that is cooled to a very low temperature with liquid nitrogen. Compounds passing through it condense inside. This kind of trap has been extensively used to analyze VOO volatiles (Morales and Tsimidou 2000; Morales et al. 1994).

Desorption of the trapped volatile compounds is usually carried out with one of two procedures: desorption with solvents or thermal desorption. Desorption with solvents is accomplished using a small volume of organic solvent. Trapping on activated carbon with desorption using CS_2 or diethyl ether as solvent has been used to analyze VOO volatiles (Dobarganes et al. 1980; Gutiérrez et al. 1981; Ranalli and Serraiocco 1996; Solinas et al. 1988). Thermal desorption consists of heating the trap at high temperatures while a flow of inert gas, passing through, sweeps the volatiles inside the chromatographic column.

The complete process constituted by DHS as the concentration method of the volatiles, using Tenax TA traps as adsorbent material, a condensation of the volatiles on a cryogenic trap, and, finally, a thermal desorption, has been widely applied in the analysis of VOO samples. This method has been used to study the volatile fraction of VOOs (Morales et al. 1994), to establish the relationship between volatile compounds and sensory attributes (Aparicio et al. 1996b; Morales et al. 1995), to characterize different European varieties (Aparicio et al. 1997; Luna et al. 2006a; Morales and Aparicio 1993), to elucidate the importance of the extraction systems (Aparicio et al. 1994; Morales and Aparicio 1999) and the degrees of ripeness (Aparicio and Morales 1998; Morales et al. 1996) in the virgin olive sensory quality, and to study changes in olive oil quality during the storage (Luna et al. 2006b).

Microwave desorption has also been applied to the analysis of olive oil volatile compounds. The quantification of the olive oil hydrocarbons (Almarcha and Rovira 1994) and other components of VOO aroma (Bocci et al. 1992) has been carried out by using this method.

Supercritical Fluid Extraction (SFE) The use of supercritical fluids for analytical extractions provides a powerful alternative to traditional extraction techniques, although it has been scarcely applied to edible oils. VOO and olive fruits have been analyzed using supercritical CO_2 to isolate the volatile compounds (Morales et al. 1998) that were then collected in a Tenax TA trap. Different profiles of volatiles were obtained when different extraction conditions (temperature and pressure) were applied. Softer conditions allowed obtaining the volatiles responsible for VOO flavor, while harder conditions allowed obtaining VOO off-flavors.

Solid Phase Microextraction (SPME) Previous methods using solvents are time-consuming, labor-intensive, and need multistage operations. Each step can introduce errors and losses. Waste disposal of solvents is an additional problem, adding cost to the analytical procedure, adverse impact on the environment, and health hazards to the laboratory personnel.

A more recent and very successful new approach to sample preparation is SPME. It was invented by Pawliszyn and coworkers (Arthur and Pawliszyn 1990) in an attempt to redress limitations inherent in solid phase extraction (SPE) and in liquid–liquid extraction (e.g., it is time-consuming and there is loss of volatiles).

The SPME device looks like a modified syringe consisting of a fiber holder and a fiber assembly, the latter containing a 1- to 2-cm-long retractable SPME fiber. The SPME fiber is a thin fused silica optical fiber coated with a thin polymer film conventionally used as a coating material in chromatography. In VOO volatile compound analysis, the fiber is used for sampling the headspace after being interred through the septum of a vial containing the VOO sample; the needle protecting the fiber is retracted, and the fiber is exposed to the environment. The polymer coating acts like a sponge, concentrating the volatile analytes by absorption/adsorption processes. After sampling, the fiber is retracted into the metal needle, and the next step is the transfer of the analytes to the chromatograph (Vas and Vékey 2004).

The extraction principle is based on the general rules of the gas–liquid equilibrium. Extraction efficiency and time to reach equilibrium depend on several variables—the fiber thickness and the diffusion coefficient of the analyte molecule in the sample being the most important—though equilibrium can be reached before with sample agitation. Less important are the fiber geometry and analyte diffusion coefficient in the fiber.

There are different kinds of fiber coatings: polydimethylsiloxane (PDMS), PDMS-divinylbenzene (DVB), polyacrylate (PA), Carboxen-PDMS, Carbowax-DVB, Carbowax-templated resin (TPR) and DVB-Carboxen-PDMS (Vas and Vékey 2004), the latter being the most commonly used in VOO volatile compound analysis.

The sensitivity of SPME is sufficient for most applications, but occasionally, it is limited by the small amount of coating material on the needle (typically less than 0.5 μL), which results in low extraction efficiency in comparison with the DHS traps. An approach has been introduced using a short bed packed with PDMS. The packed PDMS bed contains approximately 300 μL of PDMS polymer, which is a marked increase (about 600 times more) compared with the amount present in in-tube capillary systems (0.25–0.5 μL) of SPME fiber.

Thus, SPME integrates sampling, extraction, concentration, and sample introduction into a single process. The technique has been routinely used in combination with gas chromatography (GC) and gas chromatography–mass spectrometry

(GC-MS), though it has also been coupled to high-performance liquid chromatography (HPLC) and HPLC-MS to analyze thermally labile compounds. The SPME-HPLC interface is equipped with a special desorption chamber, utilized for solvent desorption prior to chromatographic separation. Furthermore, a new SPME-HPLC system, known as in-tube SPMS, has recently been developed using an open-tubular fused-silica capillary column instead of SPME fiber (Vas and Vékey 2004). In-tube SPME not only shortens analysis times but often provides better accuracy.

In summary, the main advantage of SPME is its simplicity and low cost, which explains the exponentially increasing number of publications on VOO volatile analysis in the past 10 years. SPME has been applied to study the relationship between VOO volatiles and cultivars, geographic origin, oxidation, quality control, adulteration, and so on (Jimenez et al. 2004, 2006; Mildner-Szkudlarz and Jelen 2008; Tena et al. 2007; Vichi et al. 2003a–c).

Table 43.6 shows the main sample preparation treatments employed considering the main advantages and disadvantages of each. In recent years, the most commonly employed methodologies have been DHS and SPME, which are considered in depth.

The following analysis is usually carried out by a GC of the isolated volatiles to separate the different compounds constituting the VOO aroma. Mass spectroscopy is the most frequently used technique in volatile compound identification.

Other methodologies not requiring the isolation and separation of volatile compounds have been applied to the evaluation of VOO aroma; sensors (metal oxide semiconductor [MOS], polymers, surface acoustic wave [SAW]) have been applied to classify olive oil categories and to characterize olive oil varieties or qualities (Aparicio et al. 2000; García-González and Aparicio 2002a,b, 2003, 2004; García-González et al. 2004); systems based on multistep direct thermal desorption-comprehensive GC–time-of-flight (TOF)–MS using a programmed temperature vaporizing injector have also been applied to the characterization of olive oil volatiles (Koning et al. 2008).

Phenolic Compound Analysis

Phenolic compound analysis can be carried out following different methodologies, but in all the cases, the phenolic components have to be extracted previously from the oil matrix. Sample preparation involves isolation of the polar fraction from the oil by liquid–liquid extraction or by SPE, with or without further purification of the extract (Morales and Tsimidou 2000).

First, sample preparation procedures are based on liquid–liquid extraction commonly allowing obtaining the polar fraction of olive oil with water–methanol mixtures using different solvent ratios (Montedoro and Cantarelli 1969; Vázquez Roncero et al. 1976). Various extraction schemes aimed at adjusting the sample preparation procedure for high-performance liquid chromatographic analysis of phenols have been proposed in the past decades using different solvents (Angerosa et al. 1995; Cortesi et al. 1995; Montedoro et al. 1992; Ragazzi and Veronese 1973a,b; Solinas and Cichelli 1981; Solinas et al. 1978; Vázquez Roncero et al. 1973, 1976, 1978). Extract cleanup procedures are often needed to eliminate interfering components (Montedoro 1972; Montedoro and Cantarelli 1969; Solinas 1987; Solinas and Cichelli 1981; Vázquez Roncero et al. 1976).

SPE has become a successful alternative method for the isolation of the phenolic fraction from the olive oil matrices prior to further analysis (Cortesi et al. 1995; Favati et al. 1994, 1995; Gutiérrez et al. 1989; Mannino et al. 1993). The first applications included cartridges packed with C_{18}, amino-modified C_{18}, or polyvinylopyrollidone (PVPP) phases. C_{18}-bonded silica is a general-purpose material stable at a wide range of solvents and pH values. It yields extracts less pure compared with those obtained using more selective packing materials. Selective retention of phenols was achieved on PVPP phases, and selective separation of non-acidic phenols was achieved with BondElut SAX amino-modified C_{18} cartridges (Andreoni and Fiorentini 1995). The use of SPE with diolcartridges has also been described (Rios et al. 2005).

GC and HPLC coupled with UV detection have been the techniques of choice for the separation and characterization of individual phenolic compounds. Many studies have been carried out to determine the total amount of phenolic compounds in olive oils by spectrophotometric analysis and to characterize their phenolic patterns by capillary gas chromatography (CGC) and, mainly, by reversed-phase high-performance liquid chromatography (RP-HPLC). However, colorimetric procedures are still the most suitable means for the estimation of total phenols or of total *o*-diphenols. On the other hand, NMR and MS have been applied to assist in the identification of the most complex compounds (Christophoridou et al. 2005).

Capillary electrophoresis has recently been applied to the analysis of phenolic compounds of olive oil and has opened up great expectations, especially because of its high resolution, reduced sample volume, and the duration of the analysis. In recent years, several methods based on the use of capillary zone electrophoresis have been developed, and HPLC-TOF-MS has been described for the identification and quantification of phenolic compounds (Carrasco-Pancorbo et al. 2006a,b, 2007).

Isocratic reversed-phase capillary electrochromatography using liquid–liquid extraction as a previous sample extraction procedure has also been recently applied to the analysis of the main phenolic compounds of EVOO. Results have demonstrated that it can be successfully employed for the separation of polar compounds with high precision, linearity, and sensitivity (Aturki et al. 2008).

SENSORY ASSESSMENT OF VOO FLAVOR

VOO sensory quality represents its acceptability and desirability by consumers, and the assessment should be performed for any of five reasons (Angerosa 2000): (1) to establish a basic quality by checking the absence or the presence and strength of sensory defects; (2) to have a particular sensory assessment for VOO designations from a denomination of controlled origin or a denomination of protected origin; (3) to point out possible modifications of sensory profiles in relation to variety, geographic origin, extraction technology, and shelf life of VOOs; (4) to find critical sensory characteristics for consumer preference; and (5) to evaluate, in sensory terms, the differences between different kinds of consumers (habitual vs. potential).

Collaborative international studies, supported for years by the IOC, pointed out numerous sensory attributes qualifying the diverse VOO designations. The result was a strict methodology for VOO sensory assessment (IOC 1987)—the so-called

TABLE 43.7. Virgin Olive Oil Designation Defined According to Defect and Fruity Median Values

Median of Defects (M_d)	Robust Coefficient of Variation (%)	Median of Fruity Aroma (M_f)	Robust Coefficient of Variation (%)	Virgin Olive Oil Designation
$M_d = 0$	≤20	$M_f > 0$	≤10	Extra-virgin
$0 < M_d \leq 2.5$	≤20	$M_f > 0$	≤10	Virgin
$2.5 < M_d \leq 6.0$	≤20			Ordinary
$M_d > 6.0$	≤10			Lampante

Panel Test—that was approved by the Commission of the European Communities (CEC 1991) and later amended (CEC 2008; IOC 1996) to avoid problems of reproducibility of the first method as well as to underline the presence of sensory defects in VOOs.

According to the official sensory method, samples are evaluated in booths of an environmentally isolated sensory panel room at 21 ± 1°C by 8–12 assessors. The oil samples (15 mL each) are presented in covered blue glasses (diameter: 70 mm; volume: 130 mL) and are warmed at 28 ± 2°C. The cover is removed from the warmed glass, and the sample is smelled and then tasted by each assessor in order to judge its flavor. Samples are unmarked and presented in a random way, and never in numbers high enough to lead to sense organ fatigue.

Assessors note the intensity of each attribute according to an unstructured scale of 100 mm. This new scoring system underlines the defective sensory attributes (fusty, humidity–mustiness, muddy sediment, winey–vinegary, metallic, and rancid notes) that are the most commonly detectable in the VOOs. The intensity of the sensory attributes, according to the assessors' perception, is statistically processed to calculate the median. Table 43.7 shows the values of the medians of the sensory perceptions for each VOO designation.

RELATIONSHIP BETWEEN SENSORY PERCEPTION AND CHEMICAL COMPOSITION

The relationship between sensory perception and chemical composition is the most difficult problem to solve when the complex aroma of VOO is studied. First, not all the volatiles identified and quantified in VOO headspace contribute to flavor. It is accepted that the odor activity value (OAV)—the ratio between the concentration of the volatile in the sample and its odor threshold—is the parameter to determine if a volatile compound contributes to the sensory perception (or sensory quality). Thus, volatiles with OAV < 1.0 do not contribute to VOO aroma, while volatiles with OAV > 1.0 do.

A complement rather than an alternative is the GC–sniffing technique that was widely used to study the independent sensory impact of the volatiles on VOO aroma attributes (Morales and Tsimidou 2000). An interesting approach is the statistical sensory wheel (SSW) that represents the global flavor matrix of EVOO after com-

piling information on dozens of sensory attributes evaluated by assessors from several European trained VOO sensory panels (Aparicio and Morales 1995). The resulting information is a circle divided into seven sectors, each sector representing a particular sensory perception of olive oil (Aparicio et al. 1996b): bitter-pungent-astringent, green, sweet, fruity, ripe fruit, ripe olives, and undesirable, plus four miscellanies. A mathematical procedure ordered them in such a way that each sector resembles the main sensory perception in VOO obtained from olives harvested at successive ripening levels. Miscellanies are small sectors of the circle where there are diverse sensory attributes that cannot be clustered under a common sensory designation. They also mean that the transition between qualified sectors is not sudden but progressive and that there is room for intermediate or eclectic sensory perceptions (Aparicio et al. 1996b).

Figure 43.2 shows the green (from fruity-green to bitter-green) sector of SSW. This sector includes green and olive fruity notes such as cut grass, green olives, and banana skin. Most of the compounds placed in this sector are qualified with a green or/and fruity odor when they are evaluated by GC–sniffing. The green odor is mainly associated with C6-aldehydes, alcohols, and their corresponding esters. The SSW obtained with this procedure allowed the acquisition of a deep knowledge about the volatiles that are responsible for the desirable sensory attributes as well as those volatiles that are responsible for unpleasant perceptions. The later volatiles were detected at trace levels or at low concentration in EVOO and VOO. Table 43.8 summarizes volatiles associated with some SSW sectors and individual sensory attributes.

Figure 43.2. Green sector of statistical sensory wheel (SSW). *Source*: Aparicio and others (1996b).

TABLE 43.8. Some of the Relationships Found between Volatiles and Sensory Attributes of VOO According to the Statistical Sensory Wheel (SSW)

Sensory Perception	Compounds
Sectors	
Green fruity	Z-3-hexen-1-ol, Z-3-hexenal, hexyl acetate, 3-hexenyl acetate, hexanal
Sweet	1-Penten-3-one
Undesirable	1-Octen-3-ol, nonanal, propanoic, and butanoic acids
Bitter, pungent, astringent	Phenols, 6-methyl-5-hepten-2-one, ethyl benzene, E-2-hexenal
Individual sensory attributes	
Green banana	2-Penten-1-ol
Almond	E-2-hexenal[a]
Butter	3-Methyl-2-butanal, 2-methyl-butanal
Artichoke	E-3-hexenal
Green tomato	2-Heptanone, 2-nonanone, 1-hexanol, E-2-pentenal

[a]E-2-hexenal also contributes to twig attribute (García-González et al. 2008).

FLAVOR COMPOUNDS IN OLIVE OIL TRACEABILITY

The European Union (EU) General Food Law Regulation defines traceability as "the ability to trace and follow a food, feed, food-producing animal or substance through all the stages of production, processing and distribution." In the case of VOO, traceability is of great importance to ensure that only authentic products with enough quality reach the consumer.

The traceability of the VOO chain has been developed at different levels that concern fruit, processing, authenticity, and alterations in VOO storing and distribution. To implement the traceability, it is necessary that adequate analytical methodologies are available to determine possible markers at each level described in Figure 43.3.

Minor compounds are the most remarkable markers of VOO traceability. Alkenes, alkanes, and sesquiterpene hydrocarbons, together with 4-monomethylsterols and terpenic alcohols, among others, have been used to determine the geographic origin and cultivar of unknown olive oil samples as well as to authenticate them (Aparicio 2000; García-González et al. 2008b). However, the volatiles are noticeable markers of the traceability steps related to cultivation, processing, storing, and distribution. But the main contribution of volatile compounds to VOO traceability is in the evaluation of manufacturing practices, as the presence of sensory defects can mean inadequate processing of the olives. Table 43.9 shows how abnormal concentrations of some volatile compounds (OAV > 1.0), due to a poor implementation of good manufacturing practices in farms and in olive mills, are good markers of VOO sensory defects defined in the sensory assessment of VOO (IOC 1996).

Under optimal extraction conditions, using healthy and mature olive fruits, EVOO is always produced whichever the olive variety processed. Only the olives attacked by pests or those that fell to the ground before harvesting produce

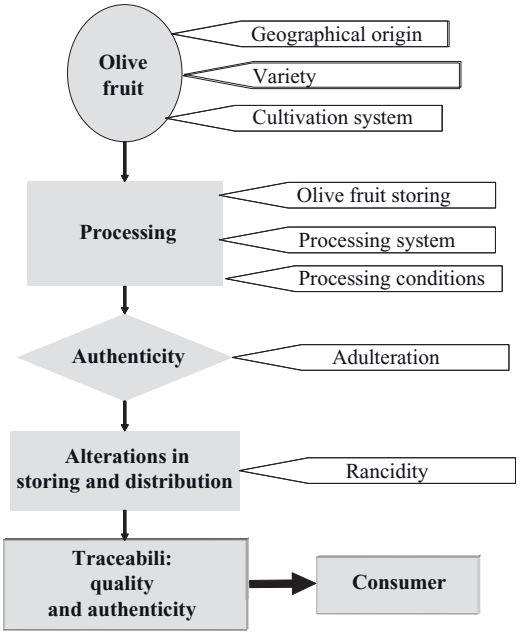

Figure 43.3. Scheme of the main stages of VOO traceability.

TABLE 43.9. Volatile Compounds That Can Be Used as VOO Traceability Markers

Volatiles	Cause	Defect
High concentrations of ethanol and some acids	Harvested olives were infested by *Dacus oleae* or *Prays*, or they were dried too much.	Grubby Haywood
High concentration of heptan-2-ol	Olives were processed under unclean conditions.	Muddy Earth
Abnormal concentrations of butan-2-ol, 2-methyl-butan-1-ol, 1-octen-3-ol, 1-octen-3-one, and pentanoic, butanoic, and acetic acids	Processed olives were left in piles for too long and became fermented, or they still contained fungi and yeasts.	Fusty Winey Humidity Mustiness
High simultaneous concentrations of the aldehydes pentanal, hexanal, and nonanal	The malaxation temperature was excessive ($T \geq 35°C$) or prolonged its heating time ($t > 60$ min).	Heated
High concentrations of ethyl acetate and ethanol	Olive oil was in contact with wastewater for a long time.	Muddy sediment Vegetable water
High concentrations of long-chain aldehydes and acids	Olive oil was inadequately stored.	Rancid
High concentration of 2,6-nonadienal	Olive oil was hermetically stored in plastic bottles for a long time.	Cucumber

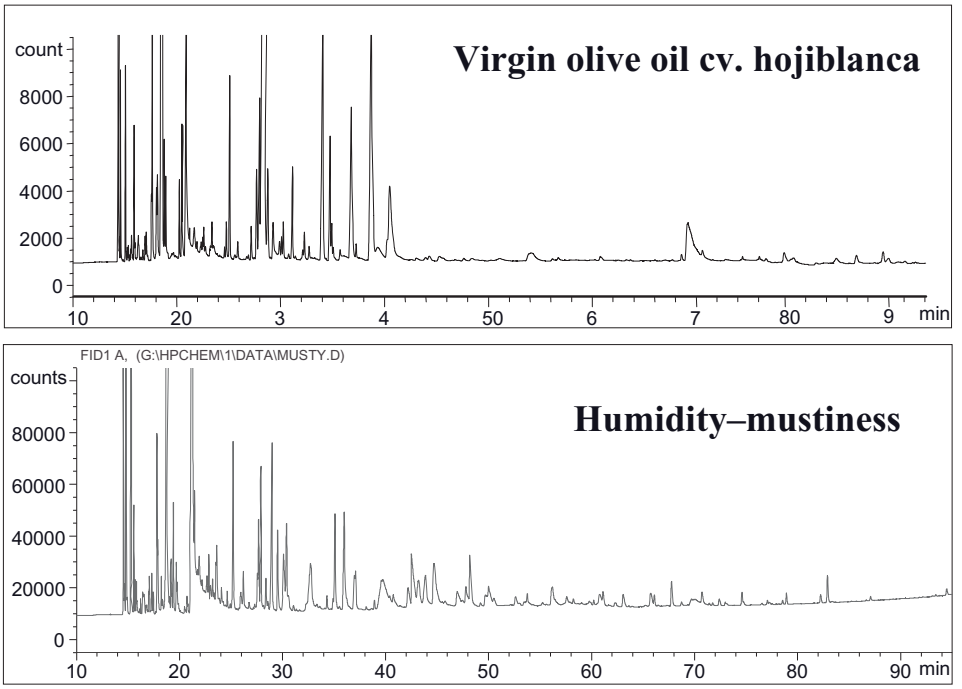

Figure 43.4. Chromatograms of volatile compounds of an extra-virgin olive oil and lampante virgin olive oil characterized by the humidity–mustiness sensory defect.

off-flavors; the rest of the defective sensory notes in the olive oils are due to an inadequate harvesting or processing or olive oil preservation (Alba 2001). Figure 43.4 shows the chromatograms of volatiles of an EVOO (cv. Hojiblanca) and a lampante VOO characterized by the humidity–mustiness sensory defect (score 8/10). The profiles of both chromatograms are quite different from both qualitative and quantitative points of view. Table 43.10 shows the main volatile compounds that have been proposed as traceability markers of the main sensory defects. Most of them are not present or are present only at very low concentrations in VOOs of high quality, whereas the concentration in the defective olive oils is significantly higher.

The current studies of VOO aroma are mainly focused on the development of new, rapid methods that allow detecting defects in VOO, which is in increasingly high demand by producers and sellers (García-González and Aparicio 2007). Further, work should contribute more information on chemical compounds, which, being present at very low concentration, influence VOO acceptability by consumers as well as predict VOO quality by analyzing the olive paste (García-González et al. 2007).

Consumer awareness of the importance of traceability in food safety and quality assurance has increased the interest in new methods. In addition to consumer interest in safety and quality, the high number of VOO protected designations of origin (PDO) is also a topic of concern to producers and consumers. Thus, future work will

TABLE 43.10. Volatile Compound Characteristics of the Main Off-Flavors Quantified in VOO and Responsible for VOO Sensory Defects

Volatile Compound	Sensory Description	Odor Threshold (mg/kg)	Concentration Range in VOO (mg/kg)	Mean Concentration in Defective VOO (mg/kg)	Sensory Defect
Propanoic acid	Sour, strong	0.72	nd–0.19	15.63	Fusty
1-Octen-3-ol	Mold, earthy	1×10^{-3}	nd	0.25	Humidity
Acetic acid	Vinegary, sour	0.50	nd–0.37	6.21	Vinegary
Nonanal	Fatty, waxy	0.15	nd–0.30	7.12	Rancid
Alternative information from					
Butanoic acid	Fusty, cheese	0.65	nd–tr	11.52	Fusty
1-Octen-3-one	Mold, mushroom	1×10^{-2}	nd–0.07	0.13	Humidity
Hexanal	Fatty	0.32	7×10^{-3}–1.05	33.8	Rancid

nd, not detected; tr, trace.

also focus on geographic traceability, with an olive oil map of the most representative olive oils from each productive region characterized by chromatographic, spectroscopic, and isotopic techniques.

REFERENCES

Abaza L, Taamalli W, Ben Temime S, Daoud D, Gutiérrez F, Zarrouk M. 2005. Natural antioxidant composition as correlated to stability of some Tunisian virgin olive oils. *Riv Ital Sost Grasse* 82:12–8.

Alba J. 2001. Elaboración de aceite de oliva virgen. In Barranco D, Fernández-Escobar R, Rallo L (eds.), *El Cultivo del Olivo*. Madrid: Ediciones Mundi-Prensa, pp. 551–87.

Almarcha M, Rovira J. 1994. Microwave-guide thermal desorption in food analysis. *Técnicas de Laboratorio* 16:699–703.

Andreoni N, Fiorentini R. 1995. Determinazione di composti fenolici in oli di oliva. *Riv Ital Sost Grasse* 72:163–4.

Angerosa F. 2000. Sensory quality of olive oils. In Harwood J, Aparicio R (eds.), *Handbook on Olive Oil: Analysis and Properties*. Gaithersburg, MA: Aspen, pp. 355–92.

Angerosa F. 2002. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *Eur J Lipid Sci Technol* 104:639–60.

Angerosa F, D'Alessandro N, Konstantinou P, Di Giacinto L. 1995. GC-MS evaluation of phenolic compounds in virgin olive oil. *J Agric Food Chem* 43:1802–7.

Aparicio R. 2000. Characterization: Mathematical procedures for chemical analysis. In Harwood J, Aparicio R (eds.), *Handbook on Olive Oil: Analysis and Properties*. Gaithersburg, MA: Aspen, pp. 285–354.

Aparicio R, Calvente JJ, Alonso MV, Morales MT. 1994. Good control practices underlined by an on-line fuzzy control database. *Grasas Aceites* 45:75–81.

Aparicio R, Calvente JJ, Morales MT. 1996a. Sensory authentication of European extra-virgin olive oil varieties by mathematical procedures. *J Sci Food Agric* 72:435–47.

Aparicio R, Morales MT, Alonso MV. 1996b. Relationship between volatile compounds and sensory attributes by statistical sensory wheels — An application to virgin olive oil. *J Am Oil Chem Soc* 73:1253–64.

Aparicio R, Morales MT. 1995. Sensory wheels: A statistical technique for comparing QDA panels. Application to virgin olive oil. *J Sci Food Agric* 67:247–57.

Aparicio R, Morales MT. 1998. Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J Agric Food Chem* 46:1116–22.

Aparicio R, Morales MT, Alonso V. 1997. Authentication of European extra-virgin olive oils by their chemical compounds, sensory attributes and consumers' attitudes. *J Agric Food Chem* 45:1076–83.

Aparicio R, Rocha SM, Delgadillo I, Morales MT. 2000. Detection of rancid defect in virgin olive oil by electronic nose. *J Agric Food Chem* 48:855–60.

Aparicio R, Roda L, Albi MA, Gutierrez F. 1999. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J Agric Food Chem* 47:4150–5.

Artajo LS, Romero MP, Morello JR, Motilva MJ. 2006. Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index. *J Agric Food Chem* 54:6079–88.

Arthur CL, Pawliszyn J. 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal Chem* 62:2145–8.

Aturki Z, Fanali S, D'Orazio G, Rocco A, Rosati C. 2008. Analysis of phenolic compounds in extra virgin olive oil by using reversed-phase capillary electrochromatography. *Electrophoresis* 29:1643–50.

Baccouri B, Ben Temime S, Campeol E, Cioni PL, Daoud D, Zarrouk M. 2007. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. *Food Chem* 102:850–6.

Ben Temime S, Taamalli W, Baccouri B, Abaza L, Daoud D, Zarrouk M. 2006. Changes in olive oil quality of Chétoui variety according to origin of plantation. *J Food Lipids* 13:88–99.

Blekas G, Boskou D. 2006. Antioxidant phenols in vegetable oils. In Boskou D (ed.), *Natural Antioxidant Phenols: Sources, Structure-Activity Relationship, Current Trends in Analysis and Characterization*. Kerala: Research Signpost, pp. 15–27.

Bocci F, Frega N, Lercker G. 1992. Preliminary research on the volatile component of extra virgin olive oil. *Riv Ital Sost Grasse* 69:611–13.

Boskou D, Tsimidou M, Blekas G. 2006. Polar phenolic compounds. In Boskou D (ed.), *Olive Oil: Chemistry and Technology*, 2nd Ed. Champaign: AOCS Press, pp. 73–92.

Carrasco-Pancorbo A, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A. 2006a. Capillary electrophoresis-electrospray ionization-mass spectrometry method to determine the phenolic fraction of extra-virgin olive oil. *Electrophoresis* 27:2182–96.

Carrasco-Pancorbo A, Gomez-Caravaca AM, Cerretani L, Bendini A, Segura-Carretero A, Fernandez-Gutierrez A. 2006b. A simple and rapid electrophoretic method to characterize simple phenols, lignans, complex phenols, phenolic acids, and flavonoids in extra-virgin olive oil. *J Sep Sci* 29:2221–33.

Carrasco-Pancorbo A, Cerretani L, Bendini A, Segura-Carretero A, Gallina-Toschi T, Fernandez-Gutierrez A. 2005. Analytical determination of polyphenols in olive oils. *J Sep Sci* 28:837–58.

Carrasco-Pancorbo A, Neususs C, Pelzing M, Segura-Carretero A, Fernandez-Gutierrez A. 2007. CE- and HPLC-TOF-MS for the characterization of phenolic compounds in olive oil. *Electrophoresis* 28:806–21.

CEC. 1991. Regulation No. 2568/91: On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Official Journal of the European Commission, No. L248.

CEC. 1997. Official Journal of the Commission of the European Communities, Regulation No. 2472/97, L341, December 12, 1997.

CEC. 2008. Commission Regulation (EC) No. 640/2008 of 4 July 2008 amending Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

Christophoridou S, Dais P, Tseng LH, Spraul M. 2005. Separation and identification of phenolic compounds in olive oil by coupling high-performance liquid chromatography with postcolumn solid-phase extraction to nuclear magnetic resonance spectroscopy (LC-SPE-NMR). *J Agric Food Chem* 53:4667–79.

Cortesi N, Azzolini M, Rovellini P. 1995. Dosaggio dei componenti minori polari (CMP) in oli vergini di oliva. *Riv Ital Sost Grasse* 72:333–7.

Covas MI. 2007. Olive oil and cardiovascular system. *Pharmacol Res* 55:175–86.

Del Barrio A, Gutiérrez F, Cabrera J, Gutierrez R. 1983. Aplicación de la cromatografía gas-líquido, técnica de espacio de cabeza, al problema del atrojado de los aceites de oliva. II. *Grasas Aceites* 34:1–6.

Dobarganes MC, Olías JM, Gutiérrez R. 1980. Componentes volátiles en el aroma del aceite de oliva virgen. III. Reproducibilidad del método utilizado para su aislamiento, concentración y separación. *Grasas Aceites* 31:317–21.

Dobarganes MC, Rios JJ, Perez-Camino MC. 1986. Relaciones entre la composición de aceites vegetales y los componentes volátiles producidos durante su termoxidación. *Grasas Aceites* 37:61–7.

Favati E, Caporale G, Bertuccioli M. 1994. Rapid determination of phenol content in extra virgin olive oil. *Grasas Aceites* 45:68–70.

Favati F, Caporale G, Monteleone E, Bertuccioli M. 1995. Rapid extraction and determination of phenols in extra virgin olive oil. In Charalambous G (ed.), *Food Flavors: Generation, Analysis and Process*. Amsterdam: Elsevier Science, pp. 429–52.

Flath RA, Forrey RR, Guadagni DG. 1973. Aroma components of olive oil. *J Agric Food Chem* 21:948–52.

García A, Brenes M, García P, Romero C, Garrido A. 2003. Phenolic content of commercial olive oils. *Eur Food Res Technol* 216:520–5.

García-González DL, Aparicio R. 2002a. Detection of vinegary defect in virgin olive oils by metal-oxide sensors. *J Agric Food Chem* 50:1809–14.

García-González DL, Aparicio R. 2002b. Detection of defective virgin olive oils by metal-oxide sensors. *Eur Food Res Technol* 215:118–23.

García-González DL, Aparicio R. 2003. Virgin olive oil quality classification combining neural network and MOS sensors. *J Agric Food Chem* 51:3515–9.

García-González DL, Aparicio R. 2004. Classification of different quality virgin olive oils by metal-oxide sensors. *Eur Food Res Technol* 218:484–7.

García-González DL, Aparicio R. 2007. Olive oil research: Current challenges. *INFORM* 18:286–8.

García-González DL, Aparicio-Ruiz R, Aparicio R. 2008a. Olive oil. In Kamal-Eldin A, Moreau R (eds.), *Gourmet and Health-Promoting Oils*. Champaign, IL: AOCS Press, pp. 1–40.

García-González DL, Tena N, Morales MT, Aparicio, R. 2008b. Geographical traceability of olive oil. 99th AOCS Annual Meeting and Expo, May 20, Seattle, WA.

García-González DL, Barié N, Rapp M, Aparicio R. 2004. Analysis of virgin olive oil volatiles by a novel electronic nose based on miniaturized SAW sensor array coupled with SPME enhanced headspace enrichment. *J Agric Food Chem* 52:7475–9.

García-González DL, Tena N, Aparicio R. 2007. Caracterization of olive paste volatiles to predict the sensory quality of virgin olive oil. *Eur J Lipid Sci Technol* 109:663–72.

Guth H, Grosch W. 1989. 3-Methylnonane-2,4-dione: An intense odour compound formed during flavour reversion of soya-bean oil. *Fat Sci Technol* 91:225–30.

Gutiérrez F, Albi MA, Palma R, Ríos JJ, Olías JM. 1989. Bitter taste of virgin olive oil: Correlation of sensory evaluation and instrumental HPLC analysis. *J Food Sci* 54:68–70.

Gutiérrez F, Perdiguero S, Gutiérrez R, Olías JM. 1992. Evaluation of the bitter taste in virgin olive oil. *J Am Oil Chem Soc* 69:394–5.

Gutiérrez R, Dobarganes MC, Gutiérrez F, Olías JM. 1981. Componentes volátiles en el aroma del aceite de oliva virgen. V. Aceites obtenidos de frutos atrojados. *Grasas Aceites* 32:299–303.

Gutiérrez R, Olías JM, Gutiérrez F, Cabrera J, Del Barrio A. 1975. Los métodos organolépticos y cromatográficos en la valoración de las características aromáticas del aceite de oliva virgen. *Grasas Aceites* 26:21–31.

IOC. 1987. International Olive Oil Council Doc T.20/n.3, June 18, Madrid.

IOC. 1996. International Olive Oil Council Doc T.20/n. 15/Rev.1, November 20, Madrid.

IOC. 2006. International Olive Council Trade Standard applying to olive oil and olive-pomace oil. COI/T.15/NC n°3/Rev. 2, Madrid.

Jimenez A, Aguilera MP, Beltran G, Uceda M. 2006. Application of solid-phase microextraction to virgin olive oil quality control. *J Chromatogr A* 1121:140–4.

Jimenez A, Beltran G, Aguilera MP. 2004. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils. *J Chromatogr A* 1028:321–4.

Kampa M, Nifli AP, Notas G, Alexaki VI, Hatzoglou A, Castanas E. 2006. Antioxidant plant phenols and cancer cell grow. In Boskou D (ed.), *Natural Antioxidant Phenols: Sources, Structure-Activity Relationship, Current Trends in Analysis and Characterization*. Kerala: Research Signpost, pp. 195–242.

Kharazi PR. 2008. Does the amount of phenolic compounds depend on olive varieties? *J Food Agric Environ* 6:125–9.

Kochhar SP. 1993. Oxidative pathways to the formation of off-flavours. In Saxby MJ (ed.), *Food Taints and Off-Flavours*. London: Blackie Academic & Professional, pp. 150–201.

Koning S, Kaal E, Janssen HG, van Platerink C, Brinkman UATh. 2008. Characterization of olive oil volatiles by multi-step direct thermal desorption-comprehensive gas chromatography-time-of-flight mass spectrometry using a programmed temperature vaporizing injector. *J Chromatogr A* 1186:228–35.

Luna G, Morales MT, Aparicio R. 2006a. Characterisation of 39 varietal virgin olive oils by their volatile compositions. *Food Chem* 98:243–52.

Luna G, Morales MT, Aparicio R. 2006b. Changes induced by UV radiation during virgin olive oil storage. *J Agric Food Chem* 54:4790–4.

Mannino S, Cosio MS, Bertuccioli M. 1993. HPLC of phenolic compounds in virgin olive oils using amperometric detection. *Ital J Food Sci* 4:363–70.

Mariani C, Venturini S, Fedeli E. 1990. Sulla presenza di prodotti alogenati volatili negli oli vergini di oliva. *Riv Ital Sost Grasse* 67:239–44.

Mateos R, Espartero JL, Trujillo M, Rios JJ, Camacho ML, Alcudia F, Cert A. 2001. Determination of phenols, flavones and lignans in virgin olive oils by solid-phase extraction and high-performance, liquid chromatography with diode array ultraviolet detection. *J Agric Food Chem* 49:2185–92.

Mildner-Szkudlarz S, Jelen HH. 2008. The potential of different techniques for volatile compounds analysis coupled with PCA for the detection of the adulteration of olive oil with hazelnut oil. *Food Chem* 110:751–61.

Montedoro G. 1972. I costituenti fenolici presenti negli oli vergini di oliva. *Sci Tecnol Alimenti* 2:177–85.

Montedoro G, Cantarelli C. 1969. Indagini sulle sostanze fenoliche presenti negli oli d'oliva. *Riv Ital Sost Grasse* 46:3–12.

Montedoro GF, Servili M, Baldioli M, Miniati E. 1992. Simple and hydrolyzable phenolic compounds in olive oil. 2. Initial characterization of the hydrolyzable fraction. *J Agric Food Chem* 40:1577–80.

Morales MT, Alonso MV, Rios JJ, Aparicio R. 1995. Virgin olive oil aroma: Relationship between volatile compounds and sensory attributes by chemometrics. *J Agric Food Chem* 43:2925–31.

Morales MT, Angerosa F, Aparicio R. 1999. Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: Chemical and sensory implications. *Grasas Aceites* 50:114–21.

Morales MT, Aparicio R. 1993. Characterizing some European olive oil varieties by volatiles using statistical tools. *Grasas Aceites* 44:113–5.

Morales MT, Aparicio R. 1999. Effect of extraction conditions on sensory quality of virgin olive oil. *J Am Oil Chem Soc* 76:295–300.

Morales MT, Aparicio R, Gutierrez F. 1992. Técnicas de aislamiento y concentración de volátiles de aceites vegetales. *Grasas Aceites* 43:164–73.

Morales MT, Aparicio R, Rios JJ. 1994. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J Chromatogr A* 668:455–62.

Morales MT, Berry AJ, McIntyre PS, Aparicio R. 1998. Tentative analysis of virgin olive oil aroma by SFE-HRGC-MS. *J Chromatogr A* 819:267–75.

Morales MT, Calvente JJ, Aparicio R. 1996. Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flav Frag J* 11:171–8.

Morales MT, Luna G, Aparicio R. 2005. Comparative study of virgin olive oil sensory defects. *Food Chem* 91:293–301.

Morales MT, Przybylski R. 2000. Olive oil oxidation. In Harwood J, Aparicio R (eds.), *Handbook on Olive Oil: Analysis and Properties*. Gaithersburg, MA: Aspen, pp. 459–90.

Morales MT, Rios JJ, Aparicio R. 1997. Changes in the volatile composition of virgin olive oil during oxidation: Flavors and off-flavors. *J Agric Food Chem* 45:2666–73.

Morales MT, Tsimidou M. 2000. The role of volatile compounds and polyphenols in olive oil sensory quality. In Harwood J, Aparicio R (eds.), *Handbook on Olive Oil: Analysis and Properties*. Gaithersburg, MA: Aspen, pp. 393–458.

Olías JM, Pérez AG, Ríos JJ, Sanz LC. 1993. Aroma of virgin olive oil: Biogenesis of the "green" odor notes. *J Agric Food Chem* 41:2368–73.

Owen RW, Mier W, Giacosa A, Hule WE, Spiegelhalder B, Bartsch H. 2000. Phenolic compounds and squalene in olive oils: The concentration and antioxidant potential of total phenols, simple phenols, secoroids, lignans and squalene. *Food Chem Toxicol* 38:647–59.

Ragazzi E, Veronese G. 1973a. Indagine sul componenti fenolici degli oli di oliva. *J Chromatogr* 77:369–75.

Ragazzi E, Veronese G. 1973b. Quantitative analysis of phenolic compounds after thin layer chromatographic separation. *Riv Ital Sost Grasse* 50:443–52.

Ranalli A, Serraiocco A. 1996. Evaluation of characteristics of olive oil produced by innovative or traditional processing technologies. *Riv Ital Sost Grasse* 73:303–14.

Rios JJ, Gil MJ, Gutierrez-Rosales F. 2005. Solid-phase extraction gas chromatography-ion trap-mass spectrometry qualitative method for evaluation of phenolic compounds in virgin olive oil and structural confirmation of oleuropein and ligstroside aglycons and their oxidation products. *J Chromatogr A* 1093:167–76.

Rogers PJ. 1996. Food choice, mood and mental performance: Some examples and some mechanisms. In Meiselman HL, MacFie HJH (eds.), *Food Choice, Acceptance and Consumption*. London: Chapman & Hall, pp. 319–41.

Rovellini P, Cortesi N, Fedeli E. 1997. Analysis of flavonoids from *Olea europaea* by HPLC-UV and HPLC-electrospray-MS. *Riv Ital Sost Grasse* 74:273–9.

Salas JJ. 1998. Ruta de la lipoxigenasa en aceituna: Contribución a la biogénesis del aroma del aceite de oliva. PhD thesis, Universidad de Sevilla, Seville, Spain.

Schnürer J, Olsson J, Börjesson T. 1999. Fungal volatiles as indicators of food and feeds spoilage. *Fungal Genet Biol* 27:209–17.

Shahidi F. 1997. *Natural Antioxidants: Chemistry, Health Effects and Applications*. Champaign, IL: AOCS Press.

Siliani S, Mattei A, Innocenti LB, Zanoni B. 2006. Bitter taste and phenolic compounds in extra virgin olive oil: An empirical relationship. *J Food Qual* 29:431–41.

Solinas M. 1987. Analisi HRGC delle sostanze fenoliche di oli vergini di oliva in relazione al grado di maturazione e alla varieta delle olive. *Riv Ital Sost Grasse* 64:255–62.

Solinas M, Angerosa F, Marsilio V. 1988. Indagine su alcuni componenti dell'aroma degli oli vergini di oliva in relazione alla varietà delle olive. *Riv Ital Sost Grasse* 65:361–8.

Solinas M, Cichelli A. 1981. Sulla determinazione delle sostanze fenoliche dell'olio di oliva. *Riv Soc Ital Sci Aliment* 10:159–64.

Solinas M, Di Giovacchino L, Mascolo A. 1978. I polifenoli delle olive e dell'olio d'oliva. Nota III: Influenza della temperatura e della durata della gramolatura sul contenuto in polifenoli degli oli. *Riv Ital Sost Grasse* 55:19–23.

Springett MB. 1993. Formation of off-flavors due to microbiological and enzymic action. In Saxby MJ (ed.), *Food Taints and Off-Flavors*. London: Blackie Academic & Professional, pp. 244–55.

Swinnerton JW, Linnenbom VJ, Cheek CH. 1962. Revised sampling procedure for determination of dissolved gases in solution by gas chromatography. *Anal Chem* 34:1509.

Tena N, Lazzez A, Aparicio-Ruiz R, García-González DL. 2007. Volatile compounds characterizing Tunisian chemlali and chétoui virgin olive oils. *J Agric Food Chem* 55:7852–8.

Tsimidou MZ, Nenadis N, Zhang HY. 2006. Structure-radical scavenging activity relationships of flavonoids. In Boskou D (ed.), *Natural Antioxidant Phenols: Sources, Structure-Activity Relationship, Current Trends in Analysis and Characterization*. Kerala: Research Signpost, pp. 29–68.

Vas G, Vékey K. 2004. Solid-phase microextraction: A powerful sample preparation tool prior to mass spectrometric analysis. *J Mass Spectrom* 39:233–54.

Vázquez Roncero A, Janer del Valle ML, Janer del Valle C. 1973. Determinación de los polifenoles totales del aceite de oliva. *Grasas Aceites* 24:350–5.

Vázquez Roncero A, Janer del Valle ML, Janer del Valle C. 1976. Componentes fenólicos de la aceituna III. Polifenoles del aceite. *Grasas Aceites* 27:185–91.

Vázquez Roncero A, Janer del Valle ML. 1978. Evolución de los polifenoles durante el aderezo de aceitunas verdes. II. Cambios en los polifenoles totales. *Grasas Aceites* 29:23–7.

Vichi S, Castellote AI, Pizzale L, Conte LS, Buxaderas S, López-Tamames E. 2003a. Analysis of virgen olive oil volatile compounds by headspace solid-phase microextraction coupled

to gas chromatography with mass spectrometric and flame ionization detection. *J Chromatogr A* 983:19–33.

Vichi S, Pizzale L, Conte LS, Buxaderas S, Lopez-Tamames E. 2003b. Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: Characterization of virgin olive oils from two distinct geographical areas of Northern Italy. *J Agric Food Chem* 51:6572–7.

Vichi S, Pizzale L, Conte LS, Buxaderas S, Lopez-Tamames E. 2003c. Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: Modifications induced by oxidation and suitable markers of oxidative status. *J Agric Food Chem* 51:6564–71.

Vita JA. 2005. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. *Am J Clin Nutr* 81:292S–7S.

Yangkyo PS, Grove MJ, Takamura H, Gardner HW. 1995. Characterization of a C-5,13-cleaving enzyme of 13(S)-hydroperoxide of linolenic acid by soybean seed. *Plant Physiol* 108:1211–18.

Young DP, Kong DX, Zhang HY. 2007. Pharmacological effects of olive oil phenols. *Food Chem* 104:1269–71.

CHAPTER 44

Flavors in Onion: Characterization and Commercial Applications

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INTRODUCTION

Onion is an important bulb vegetable grown in as many as 175 countries, ranking second among all vegetables of economic importance. While onion bulbs are primarily consumed for their unique flavor or for their ability to enhance the flavor of other foods, they also contribute significantly to the nutritional value in the human diet and have medicinal and therapeutic properties (Table 44.1) (Farrell 1985; Fenwick and Hanley 1990). They are consumed fresh and after processing into flakes, powder, rings, granules, frozen, pickled, and canned in brine or vinegar. Several members of the *Allium* genus, particularly onion and garlic, are considered health-enhancing products. The water and alcoholic (1:1) extract of onion and garlic was shown to dose dependently inhibit the oxidation of fatty acids, such as linoleic acid, in the presence of soybean lipoxygenase (Shobana and Naidu 2000). Spice mixes such as ginger, onion and garlic, onion and ginger, and ginger and garlic have shown cumulative inhibition of lipid peroxidation, thereby exhibiting their synergistic antioxidant activities. Similarly, in cholesterol-fed (0.2 g/kg/d) rabbits, Bordia and others (1977) showed a significant reduction in cholesterol and blood coagulability by essential oil from onion and garlic (1 g raw bulb/kg/d) was more effective than clofibrate in the normal clinical dosage of 33 mg/kg/d. Both onion and garlic in raw rather than cooked form have greater antithrombotic effects (Bordia et al. 1996).

Onion has been used for thousands of years in the traditional medical practice of many cultures to treat cardiovascular, brain tumor, thrombosis, lipidemic, arthritic, and gycemic disorders (Kendler 1987; Rahman 2003; Tapiero et al. 2004; Thomas and Afzal 2000). Its radical scavenging activities are correlated positively with total phenolics with red onion having higher radical scavenging activities than yellow onions and garlic (Nuutila et al. 2003). The sulfur-containing compounds from onion

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TABLE 44.1. Composition of Fresh and Dehydrated Onion Bulbs

Sr. No.	Components	Units	Average Levels	
			Fresh Bulbs	Dry Bulbs
1	Moisture	%	88.6–92.8	5.0
2	Protein	%	0.9–1.6	10.1
3	Fat	%	Trace–0.2	1.1
4	Carbohydrates	%	5.2–9.0	80.7
5	Ash	%	0.6	3.2
6	Energy	kcal/100 g	0.023–0.038	347.0
7	Ca	mg/100 g FW	190.0–540.0	363.0
8	P	mg/100 g FW	200.0–430.0	340.0
9	K	mg/100 g FW	80.0–110.0	943.0
10	Na	mg/100 g FW	31.0–50.0	54.0
11	Mg	mg/100 g FW	81.0–150.0	122.0
12	Fe	mg/100 g FW	1.8–2.6	3.0
13	Zn	mg/100 g FW	1.5–2.8	2.0
14	Mn	mg/100 g FW	0.5–1.0	NQ
15	S	mg/100 g FW	50.0–51.0	NQ
16	Vitamin D	mg/100 g FW	0.3	NQ
17	Riboflavin	mg/100 g FW	0.05	NQ
18	Nicotinic acid	mg/100 g FW	0.2	NQ
19	Vitamin C	mg/100 g FW	10.0	15.0
20	Folic acid	µg/100 g FW)	16.0	NQ
21	Biotin	µg/100 g FW)	0.9	NQ
22	Pantothemic acid	mg/100 g FW)	0.14	NQ

Sources: Fenwick and Hanley (1990) and Farrell (1985).

NQ, not quoted.

were also found to inhibit platelet aggregation (Liakopoulou-Kyriakides et al. 1985; Morimitsu and Kawakishi 1990).

Onion bulbs also contain considerable quantities of fructan, a type of soluble carbohydrate positively correlated with higher pungency and longer bulb dormancy, which is associated with lower rates of colorectal cancer and antiplatelet activity (Havey et al. 2004).

BIOLOGY AND CLASSIFICATION OF ONION

Alliums belong to the genus *Allium*, which also includes several commercially important vegetables such as bulb onions and shallot (*Allium cepa* L.), leek (*Allium ampeloprasum* var. porrum), chive (*Allium schoenoprasum*), kurrat (*A. ampeloprasum* var. kurrat, mainly grown in Egypt), elephant garlic (*A. ampeloprasum* var. holmense), rakkyo (*Allium chinense*), and Chinese chives (*Allium tuberosum*) (Jones and Mann 1963). Alliums such as *Allium gigantheum*, *Allium aflatunense*, *Allium caesium*, *Allium unifolium*, *Allium mol*, and *Allium schubertii* are cultivated as ornamentals for their large umbels and uniquely colored flowers (Havey 1999). There are controversies regarding the taxonomic classification of the genus *Allium*. Earlier, it was placed in the Liliaceae family. Some British and American botanists

included it in the Amaryllidaceae family, but the more recent studies have recognized the genus *Allium* and its close relatives as monocotyledons and have grouped them in a distinct family, Alliaceae (Lawande 2005).

Intensive selection during domestication and natural hybridization has resulted in wide variations in the shape, size, color, and chemical composition of bulbs in this species. Jones and Mann (1963) provided a simple classification of *A. cepa* for use by horticulturists as follows.

Common Onion Group (*A. cepa* L. var. cepa, *A. cepa* L. spp. *cepa*, and spp. *Australe trofim*)

This group includes open pollinated varieties, land races, and commercial F_1 hybrids grown for dry bulbs that are large and are normally single.

Aggregate Group or Shallots (*Allium ascolonicum* auct. Non Strand and *A. cepa* L. spp. *Orientale kazak*)

This group is of minor importance and is almost exclusively propagated by daughter bulbs. The plants in this group produce small bulbs in clusters.

Ever-Ready Onions (*A. cepa* L. var. *perutile* Stearn)

Mainly used for salad, the bulbs in this group are narrow; the flower stalk is short and the umbel is small. This group is further divided into potato or multiplier onions and shallots. The multiplier onions have bulbs with 3–20 bulb sets that are wider than long and are covered by an outer dry skin. Shallots are short-season types suitable for high altitudes and form clusters of individual bulbs that are narrow.

Onions can also be grouped into two marketable categories: fresh market ("green") and storage ("dry bulb") onions. Fresh market or green onions are pulled while the tops are still green, usually before a large bulb has formed. They are available in yellow, red, and white. The "premium fresh" green onion is the preprocessed fresh produce that is precut and packaged for fresh toppings used at salad bars, in fast food restaurants, or for inclusion in premixed and packaged salad greens. In contrast, the storage or dry bulb onions account for about two-thirds of all onions grown. They come in various colors (red, yellow, and white), size, shape, and texture. The term "dry bulb" is used to distinguish them from fresh market onions. Besides these two categories, there are others like those used in canning, pickling, freezing, and dehydrated products made from varieties having higher solid content.

Based on the pungency strength, onions are divided into sweet, mild, strong, or extremely strong (Crowther et al. 2005). A significant linear relationship generally exists between pyruvate levels in the bulb and human perception of pungency strength. The average pyruvate level ranges in these categories were <4.0, 4.0–7.0, and above 7.0 μmol of pyruvate per gram fresh weight, respectively. However, it does not necessarily correspond to those that would be applied by an objective measure such as pyruvate levels in the market place. For example, Spanish (7.5 μmol of pyruvate per gram fresh weight) and Chilean (8.0 μmol of pyruvate per gram fresh weight) onions that have been marketed as "mild" contain higher average levels of pyruvate than the strong Rijnsberger cooking onions, which contain <7 μmol of

pyruvate per gram fresh weight (Crowther et al. 2005). Similarly, overwintered onions marketed in the United Kingdom as "strong cooking" onions consistently contain low levels of pyruvate, in the range of 3.5–4.6 μmol of pyruvate per gram fresh weight. Because onions with low levels of pungency and exhibiting a slightly sweet flavor are becoming increasingly popular with consumers in the U.S. and European markets, developing new onion cultivars with low pungency has become an important issue among onion researchers and producers (Yoo and Pike 2001).

Based on the likeability score (attractiveness of flavor obtained by dividing the sweetness score by 5 where the most and least acceptable flavor had a score of 2 and 0, respectively), onions can be grouped as most acceptable (likeable) or least likeable types (Crowther et al. 2005). The likeability score was found to have a linear inverse relation with pyruvate levels.

GROWTH AND DEVELOPMENT

Onion (*A. cepa* L.) is cultivated as a biennial crop, although some types can be treated as perennials. It is propagated through seeds, bulbs, or sets (small bulbs). The life cycle includes a number of developmental stages such as vegetative growth, bulb formation, bulb dormancy and sprouting, flowering, and seed production (Brewster 1997). The prevailing environmental conditions control the rate of growth and length of each developmental stage as well as the transition from one phase to another. It is considered as a biennial crop. A comprehensive review of the growth and development of onions has been provided by Rabinowitch and Brewster (1990) and by Brewster (1994).

PRODUCTION, IMPORTS, AND EXPORTS

Onion is a species of great economic importance, used, produced, and traded globally. World onion production has increased by at least 25% over the past 10 years. During 2004, onion was produced in 175 countries and covered an area of 3 million hectares producing 1.24 billion crate weight units of dry onions (FAO 2005). China was the world's third largest producer of onions, harvesting 19.0 million tons of dry onions during 2005 (FAO 2005). India harvested 5.5 million tons of dry onions during that year. Other important producers of dry onion were the United States, Turkey, Pakistan, Russia, Iran, and Egypt.

Nearly 90% of onions are normally consumed within the countries of production. The average per capita consumption of onions in the world is 7.0 kg/year, Libya (32 kg per capita per year) and Turkey (27 kg per capita per year) being the largest consumers (FAOSTAT 2002). The total import of dry onion is worth approximately US$1.12 billion, while the total export is US$0.911 billion (FAO 1997).

HISTORY AND BACKGROUND OF ONION FLAVOR

Semmler (1892) was the first to investigate and provide the empirical formula ($C_6H_{12}S_2$) for the major component of the essential oil of onion. Saghir et al. (1964) were the first to use gas chromatography (GC) to quantitatively analyze onion oil.

Since then, several studies have been conducted on the flavor chemistry of onion. Carson (1967) and Bernhard (1969) presented a general review of onion flavor research in the early years. In 1969, Brodnitz and coworkers listed 17 flavor components in onion oil. Brodnitz and Pollock (1970) examined the gas chromatographic method, whereas Bandopadhyay and others (1970) used a combination of chromatography and spectroscopic methods for analyses of onion oil. Brodnitz and Pascale (1970a, cited by Boelens et al. 1971) prepared synthetic onion oil by either adding propenyl-containing polysulfides to a skeleton consisting of methyl and propyl sulfides or by heating propyl-alkane thiosulfates. They also isolated the lachrymatory factor (LF) from raw onion extract by GC and identified the isolate as thiopropanal-S-oxide (Brodnitz and Pascale 1970b, cited by Boelens et al. 1971). They also recognized the methyl- and propylpropenyl disulfides as important constituents of onion oil (Brodnitz et al. 1969). Similarly, Boelens et al. (1971) identified several thiosulfonates in the extracts from freshly cut boiled and fried onions. Important among them were propyl thiosulfonates (freshly cut onions), propyl and propenyl di- and trisulfides (boiled onions), and dimethylthiophenes (fried onions).

FLAVOR VOLATILES

Onion, garlic, and other alliums are important sources of flavoring fresh and cooked food. Their importance in cooking comes from their typical taste and flavor. These characteristics are species specific, created by the chemical transformation of a series of volatile sulfur compounds generated by the cleavage of relatively stable, nonvolatile, odorless flavor precursors (S-alk(en)yl cysteine sulfoxide [ACSOs]) by enzyme alliinase (EC 4.4.1.4) and lachrymatory-factor synthase (Jones et al. 2004). When onion is cut, some of the volatile compounds affect the eyes and produce tears (lachrymatory effect) (Tewari and Bandopadhyay 1975). Some sulfur compounds cause a pungent, burning sensation in the back of the mouth and throat, whereas others produce milder, more pleasant, and typical onion flavors.

Sweetness is another factor in onion flavor. Terry and others (2005) compared the concentrations of fructose, glucose, and sucrose in cultivars SS1, Buffalo, and Shakespeare onions with taste panel scores for likeability, sweetness, and bitterness. They found a positive correlation between fructose and glucose and sweetness and likeability scores and a negative correlation between glucose levels and bitterness. However, they did not observe any correlation between sucrose concentration and sweetness score. Although sugars (glucose, fructose, and sucrose) and several nonstructural carbohydrates (fructo-oligosaccharides [FOS]) constitute up to 65% of the dry weight of onion bulbs, their presence is not felt in raw, strong-flavored onions because high pungency can mask high levels of sugars. Therefore, strong onions do not taste sweet, while sweet onions are believed to have higher amounts of sugar and lower levels of pungency, although for some varieties, the flavor classification based on pungency levels as indicated by pyruvate levels alone does not necessarily match with the sensory measure of pungency strength (Crowther et al. 2005). For example, the Rijnsberger onions sold in the United Kingdom as cooking onions contain 8–10% sugar on a dry weight basis but are not grouped as "sweet" (Crowther et al. 2005). The sweetness in such types of onions can be enjoyed after cooking, which vaporizes pungent sulfur volatiles and stops further enzyme actions.

Besides pungency compounds, sugars, and FOS, red and yellow onions contain large quantities of flavonoids, especially quercetin 4′-glucoside and quercetin 3,4′-diglucoside (Price and Rhodes 1997). The most pungent cultivars also contain high amounts of flavonols and give high Trolox equivalent antioxidant capacity (TEAC) values (Vagen and Slimestad 2008).

The nature and origin of flavor compounds, particularly in onion and garlic, have been studied since the 1940s. In onion, the enzyme alliinase is confined to the vacuole, whereas flavor precursors are present in the cytoplasm of an intact cell. Therefore, intact onions have no odor, but when cells are crushed, the enzyme alliinase is released into the cytoplasm where it reacts with S-alk(en)yl-L-cysteine sulfoxides to produce a series of volatile sulfur compounds that undergo further vapor-phase chemical transformations (Lancaster and Boland 1990). In onion, propenesulfenic acid gives thiosulfinates and/or rearranges to propanethial S-oxide (the LF) (Jones et al. 2004). Fresh onion consists of unstable volatile compounds and their derivatives. In contrast, the characteristic flavor of cooked onions contains disulfides, trisulfides, and several other sulfur-containing volatile and nonvolatile compounds, sweet aroma compounds, sugars, and amino acids (Tokimoto 2004). A lachrymator is produced enzymatically during the hydrolysis of S-propenyl cysteine sulfoxide (Moisio et al. 1962). Thus, alliums with S-propenyl cysteine sulfoxide have tear-producing effects, and those with S-allyl cysteine sulfoxide resemble a garlic taste.

Besides the bulb, flavor precursors are also present in leaf blades, base plates, and roots, but not in seeds (McCallion and Lancaster 1984). The flavor intensity and, thus, the taste of onion bulbs vary with varieties, growth stages, environmental conditions, and agronomic practices under which they were grown (Platenius 1944). Flavor precursors are formed in leaves and transported to scales where they are stored. Younger blades were found to be more efficient than the older ones in precursor production (Lancaster et al. 1986). The level of flavor precursors increases with the initiation of bulbing and gradually decreases toward maturity. During storage, the level of flavoring compounds increases until the sprouting of bulb at which time it quickly drops. The drastic reduction in the flavor of onion bulbs with the initiation of sprouting may be related to its metabolism and translocation of precursors in order to meet the enhanced nutritional needs of developing roots (Freeman and Whenham 1996). Kopsell and others (1999) reported an increase in *trans*-(+)-S-(1-propenyl)-1-cysteine sulfoxide and a decrease in S-methyl-L-cysteine sulfoxide in seven onion cultivars during sprouting.

FLAVOR PROFILES IN ONIONS

Four nonvolatile odorless cysteine sulfoxides (CSOs) serve as precursors of flavor and odors in alliums (Table 44.2). While S-methyl cysteine sulfoxide (MCSO, methiin) is present in most alliums, S-*trans*-prop-l-enyl cysteine sulfoxide (PeCSO, isoalliin) is typical of onions. Similarly, S-propyl cysteine sulfoxide (PCSO, propiin) is present in onion and related species. In contrast, S-allyl cysteine sulfoxide (ACSO, alliin) is limited to garlic only. The enzyme alliinase (EC 4.41.4) cleaves these precursors to give pyruvate, ammonia, and a thiosulfinate. The later undergoes further transformations; as a result, the flavor in alliums changes over time (Whitaker 1976).

TABLE 44.2. Flavor Precursors of Alliums

Flavor Precursors	Names	Formula	Occurrence
S-Alk(en)yl cysteine sulfoxide	Alliin	$CH_2 = CH–CH_2–S(=O)–CH_2–CH(NH_2)\,COOH$	Garlic
S-Methyl cysteine sulfoxide	Methiin	$CH_3–S(=O)–CH_2–CH(NH_2)\,COOH$	Alliums, Brassicaceae
trans-S-1-Propenyl cysteine sulfoxide	Isoalliin	$CH_3–CH = CH–S(=O)–CH_2–CH(NH_2)\,COOH$	Onion
S-Propyl cysteine sulfoxide	Propiin	$CH_3–CH_2–CH_2–S(=O)–CH_2–CH(NH_2)\,COOH$	Onion

The degradation of the most widely distributed of these flavor precursors, MCSO, gives odors that are described as "cabbagy," or fresh onion, while the easily distinguishable smell of garlic is derived from ACSO. The lachrymatory effect that is unique to onions is caused by the volatile product propanthial S-oxide (Brodnitz and Pascale 1971). Until recently, this was thought to be produced simultaneously from the thiosulfinate products of the alliinase reaction. However, it has now been shown to be generated by the action of the second enzyme, lachrymatory-factor synthase, following the action of alliinase on PeCSO, the major flavor precursor in onion (Imai et al. 2002). Some studies have suggested the occurrence of other (+)-S-alk(en)yl cysteine sulfoxides (e.g., 5-methyl-1-4-thiazan-3-carboxylic acid 1-oxide, cycloalliin) in alliums including onions (Whitaker 1976).

Of the three S-alk(en)yl sulfoxide flavor precursors in onions, S-(E)-l-propenyl cysteine sulfoxide is usually found in the greatest quantities (78% of the total sulfoxides) and is responsible for tearing response and pungency; MCSO occurs to a lesser concentration; and PCSO occurs in lowest concentrations (Edwards et al. 1994; Randle et al. 1995).

The levels of flavor precursors are related to the ontogeny of the individual leaf blade and the scale as well as the ontogeny of the entire plant (Lancaster et al. 1986). The leaf blades developing on a young or bulbing onion contain all the precursors (total amount between 55 and 70 mg per leaf); but as each attached scale developed, the leaf blades lose their flavor precursors. All three precursors increase in the developing scales, whereas they decrease in the senescent scales. Leaf blades that develop on an older ripening onion contain and then lose (+) S-l-propyl-L-cysteine sulfoxide, while the scales accumulate it; the levels of two other precursors, (+) S-l-methyl-L-cystiene sulfoxide and (+) *trans*-S-l-propoenyl-L-cysteine sulfoxide), are low. The scales that do not senesce during ripening accumulate all three precursors, with (+) *trans*-S-l-propoenyl-L-cysteine sulfoxide concentrations remaining constant at 30 mg per scale; however, the level of the other two precursors is reduced by almost 10-fold (from 20 to 2 mg per scale). The base plate contains mainly the (+) S-l-methyl-L-cystiene sulfoxide, which increases by fivefold in amount during ripening. The other two precursors are present at much lower levels. The authors hypothesized that leaf blades are the main source of precursors to the scales, and

in turn, they recycle their flavor precursor reserves to developing younger scales before they senesce.

Besides CSOs, several γ-glutamyl peptide (γ-GP) derivatives of these flavor compounds have been recognized in alliums (Granroth 1970; Whitaker 1976). γ-GPs such as γ-glutamyl-S-alk(en)yl glutathiones, γ-glutamyl-S-alk(en)yl cysteines, and γ-glutamyl-S-alk(en)yl CSOs that are proposed to be produced from glutathione (γ-glutamyl cysteinyl glycine) are viewed as intermediates in the biosynthesis of flavor precursors and may serve as reserves for nitrogen and sulfur (Jones et al. 2004).

Hanum and others (1995) studied the effects of γ-glutamyl transpeptidase in conjunction with exogenous C-S-lyase on the flavor profile of Spartan Banner, a pungent onion. They found 2.5-fold increases in pyruvate production in γ-glutamyl transpeptidase and exogenous C-S lyase-treated onions. The effects of these enzymes were shown by a shift of major components from methyl propyl disulfide, methyl propenyl trisulfide, dimethyl tetrasulfide, and propyl l-propenyl trisulfide into new major components like methyl l-propenyl disulfide, dipropyl disulfide, propyl l-propenyl disulfide, methyl l-propenyl trisulfide, and propyl l-propenyl trisulfude. This increase in l-propenyl-containing flavor compounds may affect the overall flavor of γ-glutamyl transpeptidase and exogenous C-S lyase-treated onion extracts.

BIOSYNTHESIS OF FLAVOR PRECURSORS

Flavor precursor formation in onion begins with the absorption of sulfates (SO_4^{-2}) by plants, which are subsequently reduced to sulfides and are assimilated into cysteine through a light-dependent reaction in leaf blades (Lancaster and Boland 1990). Sulfur in cysteine is further metabolized to produce other sulfur-containing compounds. Early radioisotope studies revealed that sulfur passed through glutathione and was incorporated into S-2-carboxy propyl cysteine or S-2-carboxy propyl glutathione, eventually assimilated into S-propenyl sulfoxide (Granroth 1970). Using the radioactively labeled sulfate in pulse-chase experiments, Lancaster and Shaw (1989) demonstrated that sulfur was first incorporated into γ-GPs as biosynthetic intermediates prior to terminating in the S-alk(en)yl cysteine sulfoxide precursors.

Figure 44.1 presents the general pathway for the production of various precursors in onions (Block 1992). These precursors are synthesized in the cytoplasm of the plant cell, whereas the enzyme alliinase is compartmentalized in the vacuole of the cell (Lancaster and Collin 1981). When the membrane of the vacuole is ruptured, alliinase is released and the precursors are hydrolyzed, producing a chain of events. Primary products include short-lived intermediate compounds, such as the 1-propenyl CSO-derived LF (propanethial sulfoxide) and other sulfenic acids from different precursors. Other products are pyruvate and ammonia, which are more stable. The LF is common in only l-propenyl CSO-accumulating alliums, and it produces tearing, mouth burn, and pungency sensations (Block et al. 1992a). A series of thiosulfinates are formed, which give the characteristic flavors and aromas of onion. Early reports of di- and polysulfides and thiosulfonates were later shown to be artifacts of hot injection port and gas chromatographic columns (Block 1992). Different flavor precursors produce different thiosulfinates, which impart unique flavors to the sensory experience (Table 44.3) (Fenwick and Hanley 1990).

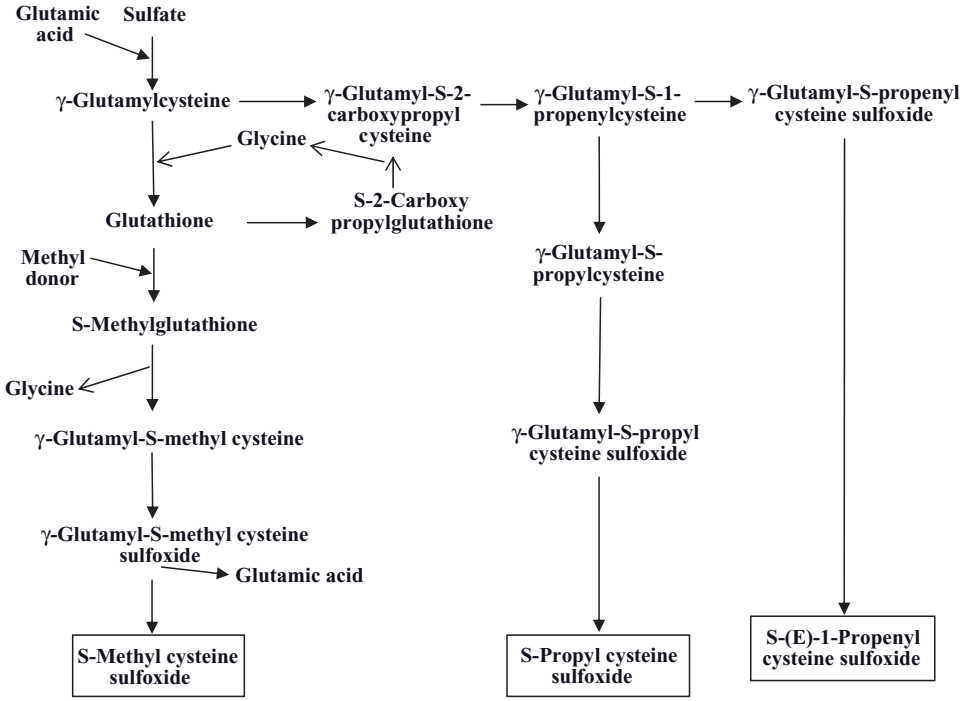

Figure 44.1. Proposed biosynthetic pathway for S-alk(en)yl cysteine sulfoxide in onions (adapted from Block 1992).

TABLE 44.3. Flavor Notes of Thiosulfinates and Lachrymatory Factor (LF)

Types of Thiosulfinates/Lachrymatory Factor	Sensory Experiences
Methyl methane thiosulfinate	Cabbage
Methyl propyl thiosulfinate	Cabbage/onion
Propyl methyl thiosulfinate	Cabbage/onion
Dipropyl thiosulfinate	Green onion, chive
Propenyl methyl thiosulfinate	Garlic, onion, metallic
Methyl propenyl thiosulfinate	Cabbage
Propenyl propyl thiosulfinate	Raw fresh onion
Lachrymatory factor (thiopropanal S-oxide)	Pungent, heat, mouth burn

Adapted from Fenwick and Hanley (1990).

Sites of Flavor Precursor Biosynthesis

Lancaster and others (1989) isolated chloroplasts, mitochondria, and the cytoplasm from the epidermal layer of leaves of sprouting onion and found glutathione in chloroplasts and in the cytoplasm, whereas identified CSOs and γ-GP within the cytoplasm only. Chloroplasts contained γ-glutamyl cysteine. One of the enzymes required for glutathione biosynthesis, γ-glutamyl cysteine synthetase, occurred in chloroplasts, whereas γ-glutamyl transpeptidase was located in the cytoplasm,

although a small portion was found in the peroxisomes. Cells in mature onion bulbs are large, thin-walled with a thin peripheral layer of cytoplasm, lining the interior of the wall (Turnbull et al. 1981). The large vacuole with small vesicles is located in the center of the cell. The cytoplasm also has vesicles. Lancaster and Collin (1981) demonstrated the presence of the alliinase in vacuoles isolated from inner bulb scales, while the flavor precursors were present in the cytoplasm. The compartmentization of alliinase and flavor precursors within the cell was further supported by their subsequent studies (Lancaster et al. 1989).

Bulbing in onions involves swelling of white nonphotosynthetic leaf blades at the bottom as concentric rings. While the inner new scales expand rapidly, the outer scales senesce equally rapidly to form papery protective layers. The entire process occurs over a 4- to 6-week period (Brewster 1997). Analysis of flavor precursors within leaves and bulbs during bulbing suggested that flavor precursors moved from the leaf blades to the base as scales developed (Lancaster et al. 1986). The precursor levels in leaf blades were very high prior to bulbing, declining by up to 90% as the bulbs developed with only the PCSO remaining in significant amounts in the leaf blades. The flavor precursors were lost from outer senescent scales and PCSO increased in the central scales as the bulbs matured, although the levels of MCSO and PeCSO were reduced. The innermost scales, which did not have leaf blades attached to them, also contained precursors. The presence of significant amounts of precursors in the basal plate suggests that the movement of precursors between scales occurs through these basal plates. After 6 months of storage of bulbs in three onion varieties (Hysam, Durco, and Grano de Oro) at 0–0.5°C, Bacon and others (1999) showed that PeCSO was the main flavor precursor in these bulbs, and its level increased in the inner fleshy scales and in the top and bottom centimeter portions of the bulbs. The levels of flavor precursors were higher in the inner fleshy layers than in the two outer fleshy layers of the bulbs.

FLAVOR ANALYSIS

Distinct flavors are produced when onion is cut and the enzyme alliinase is released into the cytoplasm, which hydrolyzes the flavor precursors to release sulfur volatiles (Lancaster and Kelley 1983). The volatiles that can be smelt and tasted are major contributors to the flavor but are difficult to measure since they undergo a rapid chemical change. The composition of ACSOs is species specific, but their content in tissue can vary according to the environmental conditions or plant parts examined (Lancaster et al. 1984; Mackenzie and Ferns 1977; Whitaker 1976). When designing an experiment to test the effects of various treatments on flavor or when selecting superior breeding lines, it is important to develop a fast, simple, and precise method to measure the inherent variability in the precursor levels in the population under investigation. For this, a reliable analytical procedure is essential. Due to the degradation of precursors and the occurrence of transient, unstable flavor compounds, it is quite difficult to define a specific type of onion odor because a tremendous number of reactions may occur depending on the processing parameters applied. Therefore, the use of appropriate analytical methods is very crucial to avoid interference from the excessive generation of "artifacts" during extraction and quantification.

The hydrolysis reaction catalyzed by alliinase is completed in 6 min (Schwimmer and Weston 1961) and produces thiopropanal S-oxide (tear-producing factor), pyruvic acid, ammonia, and many sulfur volatiles (Whitaker 1976). Therefore, onion pungency can be estimated by measuring one of the reaction products using various instruments. To date, there have been two main approaches to studying onion aroma composition: using headscape techniques to analyze the volatiles emitted from ruptured onions and preparing water slurries of onions from which volatiles are isolated by means of liquid-solvent extraction (Block et al. 1992a,b). Schwimmer and Weston (1961) developed an assay method to measure onion pungency based on the production of enzymatically derived pyruvate after the bulb tissue is crushed or juiced. Pyruvate concentration has been shown to correlate positively with perceived onion pungencies and olfactory threshold concentrations (Crowther et al. 2005; Schwimmer and Guadagni 1962; Wall and Corgan 1992); however, discrepancies in the taste panel perception of onion flavor and pre-classification based on pyruvate levels have been reported by some scientists (Bedford 1984; Crowther et al. 2005). Substantial interbulb and interclonal variations in pyruvate and sugar levels have also been reported (Hamilton et al. 1997; Randle 1992a). Even though new procedures or modifications to the Schwimmer and Weston (1961) protocol have been published in order to streamline pungency evaluation (Boyhan et al. 1999; Randle and Bussard 1993; Thomas et al. 1992; Yoo et al. 1995), the standard technique used to measure onion pungency remains to be the Schwimmer and Weston (1961) protocol. This method involves blending onions with water, filtering, centrifuging, and diluting before analysis. Pyruvic acid is then reacted with 2, 4-dinitrophenyl hydrazine to produce a colored derivative, which can be measured spectrophotometrically (Randle and Bussard 1993). Anthon and Berrett (2003) modified the Schwimmer and Weston (1961) protocol to analyze pungent sulfur compounds together with stoichiometric amounts of ammonia and pyruvic acid based on enzymatic release cascade reactions. Schwimmer and Weston (1961) have used the absorbance at 420 nm to determine pyruvate concentrations in the samples. These methods were found too slow and tedious (Randle and Bussard 1993). To shorten the time required for extracting and purifying the juice, these researchers designed and developed a press to squeeze juice from the bulb that was free of solid particles. The raw juice was then used for pyruvic acid measurements. Yoo and others (1995) extracted the juice by blending onion tissues without adding water, then reacting it with reagents without the two steps of dilution. Similarly, Sance and others (2001) modified the original Schwimmer and Weston technique by standardizing the dilution of the sample, the bench time to allow enzymatic hydrolysis of precursors, and the freezing of samples for Argentine onion cultivars.

Although pyruvic acid is relatively a stable compound in this reaction, it is also a common metabolic product in the plant and can upwardly bias enzymatic hydrolysis measurements. Schwimmer and Weston (1961) reported background levels of 2.1–4.0 μmol/mL from White Grano, Southernport Red Globe, and five other onion varieties. However, Yoo and Pike (2001) reported <0.34 μmol/mL using undiluted onion juice and suggested that the background levels may be disregarded. However, when the background levels are higher, its levels can be determined by deactivation of the alliinase enzyme by heating the onion tissue in a microwave oven for 2 min/100 g (Schwimmer and Weston 1961; Yoo et al. 1995; Yoo and Pike 2001), homogenizing with 5% trichloroacetic acid (Randle and Bussard 1993; Yoo and Pike

2001), or by the addition of 10 mM hydroxyalamine (Edwards et al. 1994) during extraction. However, 80% ethanol did not stop the alliinase activity completely (Yoo and Pike 2001). The background levels are subtracted from the total pyruvic acid to determine the enzymatically produced pyruvate.

Because of their excellent resolution and mass identification capabilities, GC and gas chromatography–mass spectroscopy (GC-MS) techniques have been used by several researchers to characterize *Allium* volatiles (Block et al. 1992b). More recent investigations suggested that better resolution can be achieved using high-performance liquid chromatography (HPLC). Yoo and Pike (1999) developed an automated system to screen a large number of onion bulbs. This method uses the same protocol as the original Schwimmer and Weston (1961) technique and involves two HPLC pumps, an autosampler, a column heater, a spectrophotometric detector, and an integrator. The new method had good repeatability (coefficient of variation [CV] = 0.99) without any human error in pipetting and showed highly positive correlation ($r^2 = 0.99$). It also estimated 7% and 17% more pyruvic acid over the HPLC and spectrophotometric methods, respectively. This method has been found very effective in mass screening of onion bulbs in a breeding program and could also be used in the commercial laboratory analysis of onion flavors where fast, efficient, and highly uniform results are required.

Jarvenpaa and others (1998) used the solid phase microextration (SPME) method for the extraction of the volatiles in the yellow onions. The SPME-GC analysis of successive samples at timed intervals provided information comparable with that obtained previously by headscape techniques; however, the SPME was more convenient and faster to perform. SPME-GC-MS also allowed easy monitoring of the fast changes in the volatile composition and produced only minor artifacts.

Saguy and others (1970) used the chemical oxygen demand method for evaluating the aroma components of onion and found it reliable and to correlate well with odor threshold values for raw onions and with taste scores for dehydrated onion products.

Boyhan and others (1999) adapted the spectroscopic assay for pyruvate in onion to a microplate reader. They found a high correlation (0.991–997 and 0.899–0.934 for sodium pyruvate standards and onion samples, respectively) between spectrophotometer and microplate readers. Onion pungency values were slightly higher with the microplate reader for both sample and background compared with the spectrophotometer when both were used in the single-wavelength mode, but there was no difference when pyruvate readings from the spectrophotometer in the single-wavelength mode were compared to those from the microplate in the dual-wavelength mode. Marcos and others (2004) developed a flow injection analysis (FIA) method for determining pyruvate in onion cultivars from Venezuela. They found no significant differences between FIA and the reference batch method (Schwimmer and Weston [1961] method) at 95% confidence level and it correlated well with taste panel tests ($r^2 = 0.8386$).

Abayomi and others (2006) constructed a disposable prototype pyruvate biosensor using pyruvate oxidase immobilized on mediated Meldolas blue electrodes to determine pungency in onions. The optimum operating potential was +150 mV (vs. Ag/AgCl), and optimum concentrations of cofactors thiamine pyrophosphate (TPP) and flavin adenine dinucleotide (FAD) and $MgSO_4$ comprising enzyme cocktail were 0.04, 0.1, and 30 mM, respectively. These authors have demonstrated a

strong correlation between biosensor response and untreated onion juice of known pyruvate concentration (2–12 μmol/g fresh weight).

Measurement of the ACSOs present in the intact tissues is an alternative approach that may be less subject to variation in the ratio of pyruvate to LF (Edwards et al. 1994). Flavor precursors can be analyzed by thin-layer chromatography (TLC) (Mackenzie and Ferns 1977), amino acid analyzer (Matikkala and Virtanen 1967), or electrophoresis and gel chromatography (Lancaster and Kelley 1983). However, the TLC method was found to be not accurate for quantifying flavor precursors in onion, and electrophoresis and gel chromatography require complicated procedures for the analysis (Yoo and Pike 1998). Thomas and Parkin (1994) analyzed the flavor precursors from onion, garlic, and leek using the HPLC method after derivatization. However, peaks of Me, Pe, and AlCSO were fused and separation of these peaks was not simple. Edwards and others (1994) were able to quantify the intact flavor precursor compounds using the HPLC method after extracting through an Amberlite IR120 anion exchange column. Even though this method has an advantage to measure the content without derivatization, it requires several steps of extraction before analysis by HPLC. Yoo and Pike (1998) modified the amino acid analysis method for screening a large number of onion breeding lines with automation. Since *Alliums* have high levels of PeCSO and AlCSO that can selectively stimulate the germination of sclerotia of *Sclerotium cepivorum* (pathogen of white rot of onion), Coley-Smith (1986) and Esler and Coley-Smith (1983) used this trait for analyzing the flavor precursor in onion.

SENSORY EVALUATION OF ONION FLAVOR

Onion flavor and taste are complex traits determined by several compounds. In taste, fresh uncooked onions vary from mild to extremely pungent. Therefore, most of the fresh onions sold all over the world are classified as sweet, mild, or strong onions, although flavor classification is assigned mainly on historical background (Crowther et al. 2005). The pungency in onion is due to the rapid production of the LF, thiopropanal S-oxide, when onion bulb is cut or crushed. The enzyme, alliinase, released from the vacuole of a damaged cell, triggers a chain of events when it comes in contact with flavor precursors present in the cytoplasm. The primary products of the hydrolysis of flavor precursors by alliinase are short-lived intermediate compounds (l-propenyl CSO-derived LF and other sulfenic acids) and are relatively more stable products (pyruvic acid, ammonia). The LF, which is common to only l-propenyl CSO-accumulating alliums, produces the tearing, mouth burn, and pungency sensations (Block et al. 1992a). A series of thiosulfinates are then formed, which characterize the unique flavor and aromas and impart a different sensory experience (Table 44.3) (Block et al. 1992a).

The thiosulfinates act as progenitor species of all sulfur compounds formed from the cut plant tissues. These compounds are unstable and undergo dissociation and rearrangement to form primary headspace volatiles (thiosulfinates formed at room temperature), secondary volatiles produced from thiosulfinates at room temperature and secondary solution components formed when thiosulfinates stand at room temperature. Therefore, the flavor of cut onions changes over time (Block 1992).

An accurate and consistent method is required to classify onions into sweet, mild, or strong for marketing purpose or to indicate to customers. The flavor or pungency strength in onion bulb is frequently determined by measuring the levels of flavor precursor compounds or one of their products, usually pyruvate (Coolong and Randle 2003a; Randle 1992a; Schwimmer and Weston 1961). Trials with small taste panels showed a high correlation between pyruvate levels and sensory ratings by taste panels (Schwimmer and Guadagni 1962; Wall and Corgan 1992). Therefore, pyruvate analysis or variations of the method to estimate pyruvate have been evolved to suit the needs of the market or breeding programs (Yoo et al. 1995). However, significant inconsistencies in repeated sensory ratings of same samples by taste panels have been reported (Bedford 1984). Also, the marketing classification of onion bulbs does not always contain corresponding pyruvate levels. For example, Spanish and Chilean onions, which are marketed in the United Kingdom as "mild," were found to have 7.21 and 8.48 μmol/g fresh weight, respectively, which is representative of strongly pungent onions. In contrast, overwintered onions sold as "strong onions" contained low levels (3.5–4.46 μmol/g fresh weight) of pyruvate.

Only trained and experienced taste panelists can accurately record their perception of onion flavor. Also, the sequence in which the onion sample is presented to the panelists influences the accuracy and consistency of sensory ratings (Crowther et al. 2005). The panelists were successful in classifying when they tasted milder onions before a strong onion. Based on the results of taste panels at classifying onions, Crowther and others (2005) suggested that the relationship between pyruvate levels to onion classification is complex, and factors other than pyruvate levels such as appearance (size, color, and shape), texture, moisture content, crispiness, and packing influence consumer acceptance of onions. Therefore, pyruvate levels cannot be a sole indicator of flavor and consumer acceptance. Hence, taste panels have an important place in establishing consumer acceptability of new onion cultivars.

FACTORS INFLUENCING FLAVOR INTENSITY

The composition and concentration of flavor precursors is influenced by cultivar (Kopsell and Randle 1997; Thomas and Parkin 1994; Yoo and Pike 1998), environment (Coolong and Randle 2003c; Lancaster et al. 1988; Randle et al. 1995; Yoo et al. 2006), soil type (Hamilton et al. 1998; Randle et al. 1998), sulfur and nitrogen nutrition (Chang and Randle 2005; Coolong and Randle 2003a,b; Durenkamp and De Kok 2004; Freeman and Mossadeghi 1970; Hamilton et al. 1997; McCallum et al. 2002, 2005; Platenius 1944; Platenius and Knott 1934; Randle 1992b; Randle and Bussard 1993; Randle et al. 1999, 2002; Randle and Lancaster 2002), irrigation (Enciso et al. 2007; Freeman and Mossadeghi 1973; Platenius 1944; Randle 1997), bulb weight, and bulb storage environment (Bacon et al. 1999; Blanchard et al. 1996; Debaene et al. 1999). Yoo and others (2006) reported that genetic differences were the major determining factor of onion pungency, accounting for 81.3% of total variation, followed by location including all environmental factors (11.35%) and clone X location (7.3%). Red and yellow onions could be mild or highly pungent, whereas white onions are only pungent to highly pungent (Rodrigues et al. 2003). It was suggested that cultivars differ in their efficiency in assimilating sulfur through the flavor biosynthesis pathway.

FLAVOR APPLICATION IN PROCESSING

Currently, onion-based products are marketed in a variety of forms—dehydrated onion pieces, toasted dehydrated onion, onion powder, onion flavoring, encapsulated flavor, onion juice, oleoresins, essential oil, onion salt, pickled onion, and canned, frozen, and packaged onions. Many patents have been granted for the production of an onion-like flavor that is being used commercially for manufacturing flavoring products. The primary function of these products is to impart a characteristic pungent flavor, disease prevention, health promotion, and therapeutic properties (Block et al. 1992a; Farrell 1985). Onion capsules and odorless products have therapeutic properties. Processed products have advantages in the food industry (reduced transportation and storage costs, reproducible organoleptic quality, freedom from seasonal fluctuations, and greater dispersion in foods).

Thiosulfinates and related sulfonic acid-derived compounds are formed from a series of reactions between the enzyme alliinase and flavor precursors when onion bulb cells are ruptured (Whitaker 1976). Dissociation of thiosulfinates such as allicin (diallyl thiosulfinates) proceeds by several pathways and produces various sulfides containing methyl, propyl and propenyl groups, thiophene derivatives, and other sulfur-containing heterocycles (Carson 1987). The allyl thiosulfonates (methyl methane-, propyl methane-, and propyl propanethiosulfonates) have been associated with fresh onion-like flavors, while propyl- and propenyl-containing di- and trisulfides have been associated with cooked onions or steam-distilled onion oils (Boelens et al. 1971). Onion and onion flavors (onion oil) are important seasonings widely used in food processing. These products, which are steam distilled, lack fresh onion flavors, and their quality varies depending on the origin of production.

In another pathway, three molecules of allicin were made to combine to form two molecules of ajoene, which is at least as potent as aspirin in preventing the aggregation of blood platelets and thus preventing blood clotting (Block 1985). Through nonenzymatic degradation pathways, thiosulfinates are converted into sulfur-containing compounds such as thiosulfinates, cepaenes, mono-, di-, tri-, and tetrasulfides, thiols, thiophenes, and sulfur dioxide. The type and concentrations of sulfur compounds extracted from onion are affected by bulb maturity, production practices, cultivar, location in the plant, and processing conditions. Therefore, it is important to take these factors into consideration when selecting for pungency, flavor, and bioactive compounds.

Processing of onion into essence extracts and dehydrated, canned, or frozen foods leads to formation of products with significantly different physicochemical properties and biological characteristics. For example, supercritical CO_2 (SC CO_2) extraction of onion flavors produced onion-like flavor components from onions (Sinha et al. 1992). The GC-MS analysis of this extract showed the presence of 28 sulfur-containing compounds, including diallyl thiosulfinates (or its isomer, di-l-propenyl thiosulfinate), propyl methanethiosulfonate, dithiin derivatives, diallyl sulfide, diallyl trisulfide, and six other tentatively identified compounds. In contrast, the commercial steam distillation produced a dark-yellow water-insoluble liquid (onion oil). This oil, analyzed under similar conditions, did not contain detectable amounts of the above compounds but had 13 compounds in common with the SC CO_2 onion extract and methyl propyl trisulfide, dipropyl trisulfide, and dipropyl tetrasulfide in high concentrations. Similarly, extraction with a mixture of freon–water at 0°C

yielded an LF, whereas extraction with ethyl alcohol at subzero temperature had an LF and 28 other compounds. This shows that the biological activity of the extract depends on the method of extraction.

Onion juice normally contains undesirable bitter components. These components can be eliminated by initially acidifying to pH 3.9 and then raising it to 5.5–6.8 (Schwimmer and Guadagni 1967). Determination of headspace volatiles and thiosulfinate in fresh, dehydrated, freeze-dried, pickled, canned, fried, and boiled onion showed that freeze-drying retained most characteristic flavor components.

TRANSGENIC AND GENETIC ENGINEERING FOR FLAVOR IN ONION

Alliums have large genomes with 2C DNA amounts per genome ranging from 16.93 to 63.57 pg (Ohri and Pastrik 2001). Onion is a diploid (2n = 16) with a nuclear genome estimated to be 15.290 Mbp per 1C, of which 6% is a single-copy DNA. The GC content of onion DNA is 32%, one of the lowest for any angiosperm, but within coding regions is approximately equal to that of *Arabidopsis thaliana* (Kuhl et al. 2004). A low-intensity genetic map of onion with 116 indicated that duplicate loci were present more frequently than would be expected for a diploid (King et al. 1998). They mapped duplicate genes for alliinase and glutathione-S-transferase. McCallum and others (2002) cloned onion homologues of key genes in the sulfur assimilation pathway.

A detailed analysis of the genomic region around the gene is necessary to elucidate the genomic organization of onion. Genes encoding alliinase have been isolated from *A. cepa*, *Allium asculonicum*, and *Allium sativum* (Clark 1993; Gilpin et al. 1995; Van Damme et al. 1992). These genes, referred to as "bulb alliinase genes" by Do and others (2004), are expressed in both bulbs and leaves. The alliinase genes are thought to form a multigene family because several alliinase-like genes were detected in genomic DNA gel blot analysis (Van Damme et al. 1992). Recently, Lancaster and others (2000) isolated two isoforms with alliinase activity (I and II) in onion roots and cloned the gene encoding for isoform II. They demonstrated the existence of a novel alliinase gene (root alliinase gene) expressed in onion roots. Suzuki and others (2001) constructed a partial bacterial artificial chromosome (BAC) library of onion for molecular cytogenetic studies. They have also isolated and characterized onion 86-kb BAC clones containing a novel alliinase gene, ALLI, which most likely encodes the root alliinase isoform I. These results provided the first evidence of the onion genomic sequence around the genes that are different from the central genome.

Several researchers have reported estimates of general combining ability (GCA) and specific combining ability (SCA) for onion inbreds and populations. Hosfield and others (1976, 1977a,b) observed large GCAs for yield, bulb quality, and maturity, but SCAs were low to moderate. The enzymatically produced pyruvic acid (EPY) and soluble solids were found to be determined by additive gene action with sporadic dominance effects (Lin et al. 1995; Simon 1995). Heterosis estimates were most often significant for yield and soluble solids, but less often for pungency (Havey and Randle 1996). A large GCA estimate suggests that superior onion inbreds and populations can be developed using recurrent selection strategies that increase the frequency of desirable alleles with additive effects. Ohsumi and others (1993) examined the volatile flavor compounds in the interspecific hybrid between onion

(*A. cepa*) and garlic (*Allium sativa*) by GC and GC-MS analysis of headspace gas and found flavor compounds that are specific to each species and some new ones. The hybrid contained thiopropanal S-oxide, which is the major specific flavor and LF in onion, and diallyl sulfide and allyl methyl disulfide, which are the characteristic flavor compounds in garlic. The hybrid was recognized as a new plant.

McCallum and others (2007) used expressed sequence tag (EST) sequencing and homology-based cloning to develop markers for candidate gene mapping and to measure gene expression at mRNA and protein levels. Their results have confirmed significant differences in gene expression and enzyme activities among varieties and on transition to bulbing. Using quantitative trait loci (QTL) analysis, they further observed a significant association of pungency and bulb soluble solids with marker intervals on chromosomes 3 and 5 (McCallum et al. 2007). They also noted significant associations between ATPs and SiR loci and bulb pungency, but not for bulb soluble solids.

Eady and others (2005) successfully produced transgenic onions with an antisense version of the bulb alliinase gene under CaMV35s promotional control. Biochemical pyruvate analysis and multiplex reverse transcription–polymerase chain reaction (RT-PCR) showed a significant reduction in the alliinase transcript. They are currently developing molecular fingerprinting techniques.

Enormous breeding opportunities exist for manipulating onion flavor based on differential SO_4^{-2} absorption due to changes in root morphology or S-permease concentration, total bulb S-accumulation, ratios between SO_4^{-2} to organic sulfur due to cysteine synthase or other enzymes in the ASCO biosynthetic pathway, differential accumulation of various ACSOs, and manipulation of alliinase action (Randle 2001). These opportunities offer a vast scope for the application of modern molecular and conventional breeding tools for manipulating sulfur metabolism in onion.

CONCLUSIONS

Onions make an important contribution to the human diet because of their unique flavor compounds and their potential benefits to human health. They are the second most important horticultural crop after tomatoes and are produced in 175 countries. There is a growing interest in optimizing breeding and production techniques to produce fresh or processed products with specific flavor profiles and health characteristics.

Onion flavors are dominated by primary and secondary products of the enzymatic breakdown of a common flavor precursor compound, S-alk(en)yl cysteine sulfoxide (ACSO). When onion bulb is cut or crushed, the enzyme alliinase is released from the vacuole and acts upon the odorless flavor precursor (ACSOs) present in the cytoplasm forming volatile compounds responsible for the characteristic flavor and aroma. The synthesis of ACSOs occurs when sulfur is absorbed by the plant and metabolized through the ACSO biosynthetic pathway. The metabolism of sulfur within the ACSO biosynthetic pathway and the breakdown of ACSOs are highly variable both among and within onion varieties. The biosynthetic pathway proposed by Lancaster and coworkers provided an important breakthrough in the understanding of sulfur metabolism leading to the synthesis of the ACSOs, although the relationship between CSOs and γ-GPs remains to be fully elucidated.

Investigations on the effects of sulfur nutrition and environmental control on flavor composition have provided additional information on the behavior of various flavor precursors. The existence of wide variations in the levels of flavor compounds is well documented but poorly understood at the molecular level. Therefore, more studies are necessary to identify and to characterize enzymes and genes that regulate sulfur absorption by the roots, accumulation of sulfur in the bulb and production and accumulation of ACSOs in various tissues, and regulation of alliinase action. These are both exciting and challenging goals for future research in this crop. This can be addressed by using a combination of genomic, agronomic, and biochemical methods. Understanding of variations in flavor biosynthesis among and within cultivars will ultimately help in developing strategies to produce onions with tailor-made flavor profiles and health benefits.

REFERENCES

Abayomi LA, Terry LA, White SF, Warner PJ. 2006. Development of a disposable pyruvate biosensor to determine pungency in onions (*Allium cepa* L.). *Biosens Bioelectron* 21:2176–9.

Anthon GE, Berrett DM. 2003. Modified method for the determination of pyruvic acid with dinitrophenylhydrazine in the assessment of onion pungency. *J Sci Food Agric* 63: 1210–13.

Bacon JRG, Moates K, Ng A, Rhodes MJC, Waldron KW. 1999. Quantitative analysis of flavor precursors and pyruvate levels in different tissues and cultivars of onion (*Allium cepa*). *Food Chem* 64:257–61.

Bandopadhyay CA, Srirangarajan N, Sreenivasn A. 1970. Studies on flavor components of onion (*Allium cepa* L.) Thin layer chromatographic investigation of onion oil. *J Chromatogr* 47:400–7.

Bedford LV. 1984. Dry matter and pungency tests on British grown onions. *J Natl Inst Agric Bot* 16:581–91.

Bernhard RA. 1969. Comparative distribution of volatile aliphatic disulfides derived from fresh and dehydrated onions. *J Food Sci* 33:298–304.

Blanchard M, Castaigne F, Willemot C, Makhlouf J. 1996. Modified atmosphere preservation of freshly prepared diced yellow onion. *Postharvest Biol Technol* 9:173–85.

Block E. 1985. The chemistry of garlic and onion. *Sci Am* 252:114–20.

Block E. 1992. The organosulfur chemistry of the genus *Allium*—Implications for the organic chemistry of sulfur. *Angew Chem* 31:1135–78.

Block E, Naganathan S, Putman D, Zhao SH. 1992a. *Allium* chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (Ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive and Chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. *J Agric Food Chem* 40:2418–30.

Block E, Putnam D, Zhao SH. 1992b. *Allium* chemistry. GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive and Chinese chive. *J Agric Food Chem* 40:2431–8.

Boelens M, de Valois PJ, Wobben HJ, Gen Van der A. 1971. Volatile flavor compounds from onion. *J Agric Food Chem* 19:984–5.

Bordia A, Verma SK, Vyas AK, Khabya BL, Rathore AS, Bhu N, Bedi HK. 1977. Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. *Atherosclerosis* 26:379–86.

Bordia T, Mohammed N, Thomson M, Ali M. 1996. An evaluation of garlic and onion's antithrombotic agents. *Prostaglandins Leukot Essent Fatty Acids* 54:183–6.

Boyhan GE, Schmidt NE, Woods FM, Himelrick DG, Randle WM. 1999. Adaptation of a spectrophotometric assay for pungency in onion to a microplate reader. *J Food Qual* 22:225–33.

Brewster JL (ed.). 1994. *Onion and Other Vegetable Alliums*. Wallingford: CAL Intl.

Brewster JL. 1997. Onion and garlic. In Wien HC (ed.). *The Physiology of Vegetable Crops*. Wallingford, UK: CAB International, pp. 581–619.

Brodnitz CL, Pollock DL, Vallon PP. 1969. Flavor components of onion oil. *J Agric Food Chem* 17:760–3.

Brodnitz MH, Pascale JV. 1971. Thiopropanal S-oxide: A lachrymatory factor in onions. *J Agric Food Chem* 19:269–72.

Brodnitz MH, Pollock DL. 1970. Gas chromatographic analysis of distilled onion. *Food Technol* 24:78.

Carson JF. 1967. *Chemistry and Physiology of Flavors*. Westport, CT: AVI.

Carson JF. 1987. Chemical and biological properties of onions and garlic. *Food Rev Int* 3:71–103.

Chang T-T, Randle WM. 2005. Sodium timing and length of exposure affect onion growth and flavor. *J Plant Nutr* 28:1755–66.

Clark SA. 1993. Molecular cloning of a cDNA encoding alliinase from onion (*Allium cepa* L.). PhD dissertation, University of Canterbury, Christchurch, New Zealand.

Coley-Smith JR. 1986. Interaction between *Sclerotium cepivorum* and cultivars of onion, leek, garlic and *Allium fistulosum*. *Plant Pathol* 35:362–9.

Coolong TW, Randle WM. 2003a. Sulfur and nitrogen availability interact to affect the flavor biosynthetic pathway in onion. *J Am Soc Hortic Sci* 128:776–83.

Coolong TW, Randle WM. 2003b. Ammonium nitrate fertility levels influence flavour development in hydroponically grown "Granex 33" onion. *J Sci Food Agric* 83:477–82.

Coolong TW, Randle WM. 2003c. Temperature influences flower intensity and quality in "Granex 33" onion. *J Am Soc Hortic Sci* 128:176–81.

Crowther T, Collin HA, Smith B, Tonmsett AB, O'Connor D, Jones MG. 2005. Assessment of flavour of fresh uncooked onions by taste-panels and analysis of flavour precursors, pyruvate and sugars. *J Sci Food Agric* 85:112–20.

Debaene JEP, Goldman IL, Yandell BS. 1999. Postharvest flux and genotype X environment effects for onion-induced antiplatelet activity, pungency and soluble solids in long-day onion during postharvest cold storage. *J Am Soc Hortic Sci* 124:366–72.

Do GS, Suzuki G, Mukai Y. 2004. Genomic organization of a novel root alliinase gene, ALL1, in onion. *Gene* 325:17–24.

Durenkamp M, De Kok LJ. 2004. Impact of pedospheric and atmospheric sulphur nutrition on sulphur metabolism of *Allium cepa* L., a species with a potential sink capacity for secondary sulphur compounds. *J Exp Bot* 55:1821–30.

Eady CC, Trueman L, McCallum J, Shaw M, Pither-Joyce M, Davis S, Reader J. 2005. Transgenic onions with reduced alliinase activity: Biochemical and molecular assessment. *Acta Hortic* 688:181–90.

Edwards SJ, Britton G, Collin HA. 1994. The biosynthetic pathway of the S-alk(en)yl-L-cysteine sulfoxide (flavour precursors) in species of *Allium*. *Plant Cell Tissue Organ Cult* 38:181–8.

Enciso J, Jifon J, Wiedenfeld B. 2007. Subsurface drip irrigation of onions: Effects of drip tape emitter spacing on yield and quality. *Agric Water Manage* 92:126–30.

Esler G, Coley-Smith JR. 1983. Flavor and odor characteristics of species of *Allium* in relation to their capacity to stimulate germination of sclerotia of *Sclerotium cepivorum*. *Plant Pathol* 32:13–32.

FAOSTAT. 2002. Agriculture data. Food balance sheets. http://apps.fao.org.

Farrell KY. 1985. *Spices and Condiments Onion*. New Delhi, India: National Book Trust, pp. 173–5.

Fenwick GR, Hanley AB. 1990. Chemical composition. In Brewster JJ, Rabinovitch HD (eds.), *Onions and Allied Crops*. Boca Raton, FL: CRC Press, pp. 17–31.

Food and Agriculture Organization (FAO). 1997. *Trade Yearbook*, Vol. 51. Rome: Food and Agriculture Organization, pp. 130–1.

Food and Agriculture Organization (FAO). 2005. *Trade Yearbook*. Rome: Food and Agriculture Organization. http://www.fao.org/.

Freeman GG, Mossadeghi N. 1970. Effects of sulfate nutrition on flavor components of onion (*Allium cepa*). *J Sci Food Agric* 21:610–5.

Freeman GG, Mossadeghi N. 1973. Studies on relationship between water regime and flavour strength in watercress (*Rorippa nassturtium-aquaticum* (L) Hayek), cabbage (*Brassica oleracea capitata*) and onion (*Allium cepa*). *J Hort Sci* 48:365–78.

Freeman GG, Whenham RJ. 1996. Effect of overwintering storage at three temperatures on the flavor intensity of fry bulb onions. *J Sci Food Afric* 27:37.

Gilpin BJ, Leung DW, Lancaster JE. 1995. Nucleotide sequence of a nuclear clone of alliinase (accession no L48614) from onion (PGR 95-125). *Plant Physiol* 110:336.

Granroth B. 1970. Biosynthesis and decomposition of cysteine derivatives in onion and other allium species. *Ann Acad Sci Fenn A* 154:1–71.

Hamilton BK, Pike LM, Yoo KS. 1997. Clonal variation of pungency, sugar content, and bulb weight of onions due to sulphur nutrition. *Sci Hortic* 71:131–6.

Hamilton BK, Yoo KS, Pike LM. 1998. Changes in pungency of onions by soil type, sulphur nutrition and bulb maturity. *Sci Hortic* 74:249–56.

Hanum T, Sinha NK, Guyer DE, Cash JN. 1995. Pyruvate and flavor development in macerated onion (*Allium cepa* L.) by γ-glutamyl transpeptidase and exogenous C-S lyase. *Food Chem* 54:183–8.

Havey M, Randle WM. 1996. Combining abilities for yield and bulb quality among long- and intermediate-day open-pollinated onion populations. *J Am Soc Hortic Sci* 121:604–8.

Havey MJ. 1999. Advances in new Alliums. In Janick J (ed.), *Perspectives on New Crops and New Uses*. Alexandria, VA: American Society for Horticultural Science Press, pp. 374–8.

Havey MJ, Galmarini CR, Gokce AF, Henson C. 2004. QTL affecting soluble carbohydrate concentrations in stored onion bulbs and their association with flavor and health-enhancing attributes. *Genome* 47:463–8.

Hosfield G, West G, Peterson C. 1976. A ten-parent diallel cross to evaluate inbred line performance and combining ability in onions. *J Am Soc Hortic Sci* 101:324–9.

Hosfield G, West G, Peterson C. 1977a. A seven-parent diallel cross in onions to evaluate general and specific combining ability and their interaction with years and locations. *J Am Soc Hortic Sci* 102:56–61.

Hosfield G, West G, Peterson C. 1977b. Heterosis and combining ability in a diallel cross of onions. *J Am Soc Hortic Sci* 102:355–60.

Imai S, Tsuge N, Tomotake M, Nagatome Y, Sawada H, Nagata T, Kumagai H. 2002. An onion enzyme that makes the eyes water. *Nature* 419:685.

Jarvenpaa EP, Zang Z, Huopalahti R, King JW. 1998. Determination of fresh onion (*Allium cepa* L.) volatiles by solid phase microextraction combined with gas chromatography-mass spectrometry. *Z Lebensm Unters Forsch A* 207:39–43.

Jones HA, Mann LK. 1963. *Onions and Their Allies—Botany, Collection and Utilization*. New York: Interscience.

Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA. 2004. Biosynthesis of the flavor precursors of onion and garlic. *J Exp Bot* 55:1903–18.

Kendler BS. 1987. Garlic (*Allium sativa*) and onion (*Allium cepa*): A review of their relationship to cardiovascular disease. *Prev Med* 16:670–85.

King JJ, Bradeen JM, Bark O, McCollum JA, Havey MJ. 1998. A low-density genetic map of onion reveals a role for tandem duplication in the evolution of an extremely large diploid genome. *Theor Appl Genet* 96:52–62.

Kopsell DE, Randle WM. 1997. Onion cultivars differ in pungency and bulb quality changes during storage. *HortScience* 32:1260–3.

Kopsell DE, Randle WM, Eitemen MA. 1999. Changes in the S-alk(en)yl-cysteine sulfoxides and their biosynthetic intermediates during onion storage. *J Nat Prod* 65:960–4.

Kuhl JC, Cheung F, Yuan Q. 2004. A unique set 11 008 onion expressed sequence tags reveals expressed sequence and genomic differences between the monocot orders asparagus and poals. *Plant Cell* 16:114–25.

Lancaster JE, Boland MJ. 1990. Flavor chemistry. In Rabinowitch H, Brewster J (eds.), *Alliums and Allied Crops*, Vol. 3. Boca Raton, FL: CRC Press, pp. 33–72.

Lancaster JE, Collin HA. 1981. Presence of alliinase in isolated vacuoles and of alkyl cysteine sulphoxides in the cytoplasm of bulbs of onions (*Allium cepa*). *Plant Sci Lett* 22:169–76.

Lancaster JE, Kelley KE. 1983. Quantitative analysis of the S-alk(en)yl-L-cysteine sulphoxides in onion (*Allium cepa* L.). *J Sci Food Agric* 34:1229–35.

Lancaster JE, McCallion BJ, Shaw ML. 1984. The levels of S-alk(en)yl-L-cysteine sulfoxides during the growth on onion (*Allium cepa* L.). *J Sci Food Agric* 35:415–20.

Lancaster JE, McCallion BJ, Shaw ML. 1986. The dynamics of the flavour precursors, the S-alk(en)yl-L-cysteine sulphoxides, during leaf blade and scale development in the onion (*Allium cepa*). *Physiol Plant* 66:293–7.

Lancaster JE, Reay PF, Mann JD, Bennett WD, Sedcole JR. 1988. Quality in New Zealand-grown onion bulbs—A survey of chemical and physical characteristics. *N Z J Exp Agric* 16:279–85.

Lancaster JE, Reynolds PHS, Shaw ML, Dommise EM, Munro J. 1989. Intercellular localization of the biosynthetic pathway to flavour precursors in onion. *Phytochemisty* 28:461–4.

Lancaster JE, Shaw ML. 1989. γ–Glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in *Allium*. *Phytochemistry* 28:455–60.

Lancaster JE, Shaw ML, Pither-Joyce MD, McCallum JA, McManus MT. 2000. A novel alliinase from onion roots: Biochemical characterization and cDNA cloning. *Plant Physiol* 122:1269–79.

Lawande KE. 2005.Onion. In Peter KV (ed.), *Handbook of Herbs and Spices*. Cambridge, England: Woodhead Publishing, pp. 249–60.

Liakopoulou-Kyriakides M, Sinakos Z, Kyriakids DA. 1985. Identification of alliin, a constituent of *Allium cepa* with an inhibitory effect on platelet aggregation. *Phytochemistry* 24:600–1.

Lin M, Watson J, Baggett J. 1995. Inheritance of soluble solids and pyruvic acid contents in bulb onions. *J Am Soc Hortic Sci* 120:119–22.

Mackenzie IA, Ferns DA. 1977. The composition of volatiles from different parts of *Allium tuberosum* plants. *Phytochemistry* 16:763–4.

Marcos P, Lue-Meru MP, Ricardo R, Maximo G, Maribel V, Jose Luis B, Marcela B. 2004. Pungency evaluation of onion cultivars from the Venezuelan West-Center region by

flow injection analysis—UV- visible spectroscopy pyruvate determination. *Talanta* 64:1299–303.

Matikkala EJ, Virtanen AI. 1967. On the quantitative determination of the amino acids and γ-glutamyl peptide of onion. *Acta Chem Scand* 21:2891–3.

McCallion BJ, Lancaster JE. 1984. Changes in the content and distribution in different organs, of the flavor precursors, the S-alk(en)yl-L-cysteine sulfoxides, during seedling development of onion (*Allium cepa*) grown under light and dark regimes. *Physiol Plant* 62: 370–4.

McCallum JA, Pither-Joyce M, Shaw M. 2002. Sulfur deprivation and genotype affect gene expression and metabolism of onion roots. *J Am Soc Hortic Sci* 127:583–9.

McCallum JA, Porter N, Searle B, Shaw M, Bettjeman B, McManus M. 2005. Sulfur and nitrogen fertility affects flavor of field-grown onions. *Plant Soil* 269:151–8.

McCallum J, Pither-Joyce M, Shaw M, Kenel F, Davis S, Buttler R, Scheffer J, Jakse J, Havey M. 2007. Genetic mapping of sulfur assimilating genes reveals a QTL for onion bulb pungency. *Theor Appl Genet* 114:815–22.

Moisio T, Space CG, Vitenan AJ. 1962. Mass spectral studies of chemical nature of the lachrymatory factors formed enzymatically from S-(l-propenyl)-cysteine sulfoxide isolated from onion (*Allium cepa*). *Suom Kemistil B* 35:29.

Morimitsu Y, Kawakishi S. 1990. Inhibitors of platelet aggregation from onion. *Phytochemistry* 29:3435–9.

Nuutila AM, Puupponen-Pima R, Aami M, Oksman-Caldentey KM. 2003. Comparison of antioxidant activity of onion and garlic extracts by inhibition of lipid peroxidation and radial scavenging activity. *Food Chem* 81:485–93.

Ohri D, Pastrick K. 2001. Phenology and genome size variation in *Allium cepa* L.—A tight correlation. *Plant Biol* 3:654–60.

Ohsumi C, Hayashi T, Kubota K, Kobayashi A. 1993. Volatile flavour compounds formed in an interspecific hybrid between onion and garlic. *J Agric Food Chem* 41:1808–10.

Platenius H. 1944. Factors affecting onion pungency. *J Agric Res* 62:371.

Platenius M, Knott JE. 1934. Pungency and sugars of short-day onions as affected by sulfur nutrition. *J Am Soc Hortic Sci* 118:755–70.

Price KR, Rhodes MJC. 1997. Analysis of major flavonol glycosides present in four varieties of onion (*Allium cepa*) and changes in composition resulting from autolysis. *J Sci Food Agric* 74:331–9.

Rabinowitch HD, Brewster JL (eds.). 1990. *Onions and Allied Crops, Vol. 3, Biochemistry, Food Science and Minor Crops*. Boca Raton, FL: CRC Press.

Rahman K. 2003. Garlic and ageing: New insights into an old remedy. *Ageing Res Rev* 2:39–56.

Randle WM. 1992a. Sampling procedures to estimate flavor potential in onion. *HortScience* 27:1116–7.

Randle WM. 1992b. Onion germplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency. *Euphytica* 59:151–6.

Randle WM. 1997. Onion flavor chemistry and factors influencing flavor intensity. In Risch SJ, Chi-Tang H (eds.), *Spice: Flavor Chemistry and Antioxidant Properties*. ACS Symposium Series. American Chemical Society, pp. 41–52.

Randle WM. 2001. Opportunities and strategies for manipulating onion flavour. *Acta Hortic* 555:51–61.

Randle WM, Bussard ML. 1993. Pungency and sugars of short-day onions as affected by sulfur nutrition. *J Am Soc Hortic Sci* 118:770–6.

Randle WM, Kopsell DA, Kopsell DE, Snyder RL, Torrance R. 1998. Field sampling short-day onion for bulb pungency. *Horttechnology* 8:329–32.

Randle WM, Kopsell DE, Kopsell DA, Snyder RL. 1999. Total sulfur and sulfate accumulation in onion is affected by sulfur fertility. *J Plant Nutr* 22:45–51.

Randle WM, Lancaster JE. 2002. Sulfur compounds in alliums in relation to flavor quality. In Rabionpwitch HD, Currah L (eds.), *Allium Crop Science: Recent Advances*. Wallingford, UK: CAB International, pp. 329–56.

Randle WM, Lancaster JE, Shaw ML, Suton KH, Hay RL, Bussard ML. 1995. Quantifying onion flavor compounds responding to sulfur fertility—Sulfur increases levels of alk(en)yl cysteine sulphoxides and biosynthetic intermediates. *J Am Soc Hortic Sci* 120:1075–81.

Rodrigues AA, Fogliano V, Graziani G, Mendes S, Vale AP, Goncalves C. 2003. Nutritional value of onion regional varieties in northwest Portugal. *J Environ Agric Food Chem* 2:519–24.

Saghir AR, Mann LK, Bernard RA, Jacobson JV. 1964. Determination of aliphatic mono- and disulfides in *Allium* by gas chromatography and their distribution in the common food species. *J Am Soc Hortic Sci* 84:386–98.

Saguy M, Mannheim CH, Peleg Y. 1970. Estimation of volatile onion aroma and flavour compounds. *Int J Food Sci Technol* 5:165–71.

Sance M, Galmanini CR, Bauza MM. 2001. Pungency determination of Argentine onion cultivars. *Acta Hortic* 688:133–8.

Schwimmer S, Guadagni DG. 1962. Relation between olfactory threshold concentration and pyruvic acid content of onion juice. *J Food Sci* 27:94–7.

Schwimmer S, Guadagni DG. 1967. Cysteine induced odor intensification in onions and other foods. *J Food Sci* 32:405–8.

Schwimmer S, Weston WK. 1961. Enzymatic development of pyruvic acid in onion as measure of pungency. *J Agric Food Chem* 9:301–4.

Semmler FW. 1892. *Arch Pharm* 230:434–43.

Shobana S, Naidu KA. 2000. Antioxidant activity of selected Indian spices. *Prostaglandins Leukot Essent Fatty Acids* 62:107–10.

Simon P. 1995. Genetic analysis of pungency and soluble solids in long-storage onions. *Euphytica* 82:1–8.

Sinha NK, Guyer DE, Gage DA, Lira CT. 1992. Super carbon dioxide extraction of onion flavors and their analysis by gas chromatography-mass spectrometry. *J Food Agric Chem* 40:842–5.

Suzuki G, Ura A, Saito N, Do GS, Seo BB, Yamamoto M, Mukai Y. 2001. BAC FISH analysis in *Allium cepa*. *Genes Genet Syst* 76:251–5.

Tapiero H, Townsend DM, Tew KD. 2004. Organosulfur compounds from Alliaceae in the prevention of human pathologies. *Biomed Pharmacother* 58:183–93.

Terry LA, Law KA, Hipwood KJ, Bellamy PH. 2005. Non-structural carbohydrate profiles in onion bulbs influence taste preference. *Frutic* 5:12–16.

Tewari GM, Bandopadhyay C. 1975. Quantitative evaluation of lachrymatory factor in onion thin-layer chromatography. *J Agric Food Chem* 23:645–7.

Thomas DJ, Parkin KI. 1994. Quantification of alk(en)yl-L-cysteine sulfoxides and related amino acids in alliums by high-performance liquid chromatography. *J Agric Food Chem* 42:1632–8.

Thomas DJ, Parkin KL, Simon PW. 1992. Development of a simple pungency indicator test for onion. *J Sci Food Agric* 60:499–504.

Thomas MAM, Afzal M. 2000. Garlic and onions: Their effect on eicosanoid metabolism and its chemical relevance. *Prostaglandins Leukot Essent Fatty Acids* 62:55–73.

Tokimoto Y. 2004. Flavour components of onion and their changes on cooking. *Koryo* 223:97–107.

Turnbull A, Galpin IJ, Smith JL, Collin HA. 1981. Comparison of the onion plant (*Allium cepa*) and onion tissue culture. IV. Effects of shoot and root morphogenesis on flavour precursor synthesis in onion tissue culture. *New Phytol* 87:257–68.

Vagen IM, Slimestad R. 2008. Amount of characteristic compounds in 15 cultivars of onion (*Allium cepa* L.) in controlled field trials. *J Sci Food Agric* 88:404–11.

Van Damme EJ, Smeets MK, Torrekens S, van Leuven F, Peumans WJ. 1992. Isolation and characterization of alliinase cDNA clones from garlic and related species. *Eur J Biochem* 209:751–7.

Wall MM, Corgan JN. 1992. Relationship between pyruvate analysis and flavor perception for onion pungency determination. *HortScience* 27:1029–30.

Whitaker JR. 1976. Development of flavor, odor and pungency in onion and garlic. *Adv Food Res* 22:73–133.

Yoo KS, Pike L, Crosby K, Jones R, Leskovar D. 2006. Differences in onion pungency due to cultivars, growth environment and bulb size. *Sci Hortic* 110:144–9.

Yoo KS, Pike LM. 1998. Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by a method and their distribution in *Allium* species. *Sci Hortic* 75:1–10.

Yoo KS, Pike LM. 1999. Development of an automated system for pyruvic acid analysis in onion breeding. *Sci Hortic* 82:193–201.

Yoo KS, Pike LM. 2001. Determination of background pyruvic acid concentration in onions, *Allium* species and other vegetables. *Sci Hortic* 89:249–56.

Yoo KS, Pike LM, Hamilton A. 1995. A simplified pyruvic acid analysis suitable for onion breeding programs. *HortScience* 20:1306.

CHAPTER 45

Onion: A Food, Spice, and Remedy in the Middle Eastern Gastronomy

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A Middle Eastern story tells us that once upon a time there was a very poor man, and people asked him what he would do if he ever becomes a rich man, he answered that "he would eat the heart of the onion."

BIOLOGICAL CLASSIFICATION OF ONION

Whether white, yellow, or red, onion (*Allium cepa* L.) is among the oldest of all cultivated plants; its history can be traced back 3500 years with Egyptian cultivation along the Nile River. In ancient Egypt, onion rings were pronounced holy, symbolizing eternal life, and supplemented the diet of slaves to promote their health during the construction of pyramids (Rosengarten 1969).

Allium is botanically included in the Alliaceae family, which comprises nearly 450 species and is the versatile, largest representative genus of the Liliaceae family (Lanzotti 2006). The three colors of onion—red, white, and yellow—represent the most commonly cultivated subspecies of *A. cepa*. Nearly 88% of the crop production is committed to yellow onion, followed by about 7% of red onions and 5% of white onions (http://www.fao.org/). Growers also make a distinction between bulb onions (*A. cepa* L. group common onion) and freshly consumed sprouts (*A. cepa* L. group Aggregatum). *A. cepa* is propagated by seeds, bulbs, or small bulbs as biennials, but there are some types that are treated as perennials.

Onions are universally consumed for their unique flavor to enhance the flavor of foods or to mask off-flavors in certain dishes (Gonzalvez et al. 2008; Kopsell and Randle 1997). Besides being used as a food ingredient, onion has been for over 3500 years and is still being used in traditional folk medicinal applications for the treatment of a variety of ailments including fever, headache, stomachache, cholera, dysentery, heart problems, skin diseases and infections, worms, and tumors (Chang et al. 2004; Corzo-Martinez et al. 2007).

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Onion is used as an important food commodity and healing agent by most people of the Mediterranean area. In recent years, extensive research is devoted to determine the pharmacological properties of onion scientifically proven that onion possesses antifungal, antibacterial, antiviral, antiparasitic, anti-infective, antithrombotic, antimutagenic, antitumor, anticarcinogenic (gastrointestinal and prostate), antispasmodic, anti-inflammatory, antiasthmatic, antihypertensive, antidiabetic, antihyperhomocysteinemia, antioxidant, antiallergic, antiarthritic, hypoglycemic, hypocholesterolemic, hypolipidemic, antiobesity, and antihelminthic activities (Ceylan and Fung 2004; Kris-Etherton, et al. 2002; Shon et al. 2004; Srinivasan 2005). These already identified prevention and treatment properties of onion are further attracting considerable interest to determine biochemically active ingredients that exhibit physiological health effects.

ONION PRODUCTION AND TRADE IN THE MIDDLE EASTERN COUNTRIES

Onion is the second most important horticultural vegetable crop after tomatoes on a global basis (Griffiths et al. 2002). According to the United Nations FAO report, over the last decade, the total surface area dedicated to the onion crop in the world has doubled, reaching 2.74 million hectares per year, which produces nearly 64.1 million tons of onion each year (FAO 2002). It is cultivated in a wide range of latitudes and altitudes worldwide, and leading onion-producing countries are China, India, the United States, Turkey, and Pakistan (Table 45.1). Onion also constitutes a major economic exchange agricultural crop; approximately 8% of global onion production is traded internationally (FAO 2004).

Onion is a typical spice of Middle Eastern cuisine and substantial quantities of onion are produced and consumed within the Middle Eastern countries, which makes the crop a remarkable commercial trade commodity. The average annual consumption in the world is 7 kg per capita per year, whereas Middle Eastern countries are among the major consumers; for instance, Libya and Turkey consume 32 and 37 kg, respectively (per capita per year) (FAOSTAT 2002). Among the Middle Eastern countries, Turkey, with 2.1 million tons of bulb, is the fourth leading global onion producer and shares 3.2% of world production (FAO 2004; Kadayifci et al. 2005); Iran, with 1.45 million tons and a prospective 2.6% of the world production,

TABLE 45.1. Major Producers of Onions in the World (2004)

Ranking	Country	Production (Million Tons)	Production Share (%)
1	China	20,000	33.0
2	India	5570	8.7
3	United States of America	3700	5.8
4	Turkey	2100	3.3
5	Pakistan	1780	2.8
6	Russia	1700	2.6
7	Iran	1450	2.2

Source: Food and Agriculture Organization of the United Nations (http://www.fao.org/).

TABLE 45.2. Proximate Chemical Composition of Onions

Component	Amount
Water	>80 g/100 g
Carbohydrates	7.9 g/100 g
Starch	Not detected
Sugar	5.2 g/100 g
Protein	1.2 g/100 g
Fat	Not detected
Essential minerals (K, Na, Ca, Mg, Fe, Se, folic acid, etc.)	Trace
Vitamins (B and C)	Trace
Low-molecular-weight flavor components	Trace

is the seventh leading producer globally. In Egypt, onion ranks fourth after cotton, rice, and citrus as an export crop, and the total production reaches 305,200 tons (Bahnasawy et al. 2004). On the other hand, Saudi Arabia is a major onion importer with annual consumption of 239,738 tons (FAO 2004).

VOLATILE AND NONVOLATILE FLAVOR COMPOUNDS OF ONION

Onions contain a wide variety of low-molecular-weight flavor components such as volatile sulfur compounds (thiosulfinates, hydrolysis compounds of thiosulfinates, or sulfenic acids), nonvolatile flavonoids, saponins, and sapogenins (Lanzotti 2006) (Table 45.2).

Organosulfurs and Thiosulfinate Compounds

Sulfur volatiles are the typical flavor and odor components of allium plants. Sulfide-containing aroma components were first reported in the early 19th century (Semmler 1982). Only in late 1950s were structures of aroma precursors elucidated by Virtaned and his colleagues (Whitaker 1976). To date, thiosulfinates are the most studied aroma precursor, with various forms, structures, biological functions, and stability (Block 1992).

Three organosulfurs in the form of (+) *S*-methyl-L-cysteine sulfoxide, *S*-propyl-L-cysteine sulfoxide, and *trans*-(+)-*S*-(1-propenyl)-L-cysteine sulfoxide are the dominating flavor precursors located in the cytoplasm of intact onion cells (Auger et al. 2004; Block 1992; Hrazdina 2006). The precursor *S*-methyl-L-cysteine sulfoxide is found in the highest concentrations in the cytoplasm of plant cells and dominates the onion flavor profile (Auger et al. 2004). When the tissue is mechanically damaged (cut, crushed, macerated, or chewed), allinase enzyme (EC 4.4.1.4) is released from the vacuoles and transforms organosulfur into S-alk(en)yl-L-cysteine sulfoxides, which are pungent aroma and taste components (Block 1992). This hydrolysis is completed within 6 min and also results in the production of by-products such as thiopropanol S-oxide, pyruvate, ammonia, sulfenic acids, and other sulfur volatiles (Whitaker 1976). These primary compounds of S-alk(en)yl-L-cysteine sulfoxides are

unstable and, during analysis, further decompose to ajoenes, dithiins, and sulfides, depending on the solvents used and the extraction conditions (Auger et al. 2004). Although pyruvate itself does not contribute to the aroma, its concentration (mole per gram fresh weight) is used as an indicator of pungency quality due to its correlation with aroma compounds and its ease of quantitation (Crowther et al. 2005; Randle and Bussard 1993). Pungency varies with genotype and growing conditions (Randle et al. 1998). The genetic system of onion controls the sulfur uptake and assimilation of the sulfur. Fertilization with sulfate, higher growing temperatures, and dry growing conditions increase flavor intensity (Ketter and Randle 1998; Randle 1997). Onion has the ability to uptake selenium from the soil and to incorporate it into amino acids that normally implicated the sulfur (S) pathways (Hong et al. 2000). The volatile sulfur compounds are unstable and give rise to transformation products shortly after tissue damage, and more recent attention has been focused on biologically active polar compounds such as phenolics, flavonoids, sapogenins, and saponins (Lanzotti 2006).

Phenolics and Flavonoids

Flavonoids, a family of polyphenolic secondary plant metabolites, are minor constituents in onions (Hertog et al. 1993). Prakash and others (2007) analyzed the total phenolic content of various onions (red, violet, white, and green sprout) and observed that its content ranges from 4.6 to 74.1 mg/g depending on the variety. Yellow- and red-pigmented onions have higher flavonoid content than the white varieties (Herrmann 1976). Flavonoids possess a unique diphenylpropane structure (C6-C3-C6) with phenolic OH groups; the structure contains two phenolic benzene rings linked by a hererocyclic pyrone or a pyran ring containing an oxygen atom. Flavonoids are usually found as sugar conjugates in which hydrogens are substituted to a sugar, which is hydrolyzed by the microflora found in the digestive system (Terao 1999). The most common sugar found in the composition of flavonoids is glucose, whereas galactose and rhamnose are also frequently detected (Wach et al. 2007). To date, over 4000 different flavonoids have been identified, and they are categorized as quercetin, flavonols, flavones, flavanones, isoflavones, catechins, anthocyanins, dihydroflavonols, kaempferol, gallic acid, myricetin, and chalcones (Cook and Samman 1996; Shon et al. 2004; Terao 1999; Wach et al. 2007). Lin and Tang (2007) analyzed the total phenolic content of nine different vegetables and, among these, onion was leading, with 319 mg gallic acid mg/100 g fresh material.

Two flavonoid subgroups responsible for color in onion are flavonols such as quercetin and kaempferol, which provide the yellow and brown color in the skins of many varieties, and the anthocyanins, which provide the red/purple color in some varieties (de Vries et al. 1998; Griffiths et al. 2002).

Onions are rich sources of quercetin so as that among 28 vegetables tested, onion has been identified as the highest quercetin source (Hertog et al. 1993) Quercetin is the major flavonol in onion; different onion varieties accumulate between 300 and 900 mg/kg fresh weight of quercetin (Hertog et al. 1992; Rhodes and Price 1996). These two glucosides account for more than 80% of the total flavonoids found in onion. Quercetin contains phenolic OH groups at the 5′ and 7′ positions in the A ring and at the 3′ and 4′ positions in the B ring (Shimoda et al. 2007). Although quercetin glycosides contain a sugar group at the 3′ position, the site for sugar binding is not

restricted to the 3′ position (Shimoda et al. 2007). For example, in quercetin 3,4-O-diglucoside and quercetin 4-O-glucoside (Hertog et al. 1992; Rhodes and Price 1996), it is predominantly present as quercetin 3,7,4′-O-β-triglucopyranoside together with quercetin 4′-O-β-glucopyranoside, quercetin 3,4′-O-β-diglucopyranoside, and isorhamnetin-4-glucoside (Caridi et al. 2007). Small amounts of taxifolin 4′-O-β-glucopyranoiside were isolated from red onion (Fossen et al. 1998). Quercetin monoglucoside and quercetin diglucoside accounted for 80% of the total flavonoids in onions (Rhodes and Price 1996), and more recently, Lombard and others (2005) demonstrated that quercetin content increases during baking and sautéing, whereas boiling reduced the content in the range of 20.6–75.0% for cooking times ranging from 3 to 60 min (Crozier et al. 1997; Ewald et al. 1999; Hirota et al. 1998; Makris and Rossiter 2002; Price et al. 1997). Flavonoids in the form of aglycosides are not absorbable through the gut wall and the free forms are absorbed only after hydrolysis (Hollman and Katan 1997). The intestinal mucosa and the liver are believed to be the location of major quercetin metabolism in humans (Wittig et al. 2001). The quercetin glucosides in onion appear to be highly bioavailable since they are absorbed more rapidly and accumulate at levels of up to threefold higher in the human bloodstream compared with rutin (Hollman et al. 1995; Hollman and Katan 1997). Neverthless, quercetin in various forms available in blood circulation is still a matter of dispute.

Anthocyanins are only minor components of the flavonoid spectrum in the edible portion of red varieties (Rhodes and Price 1996). The color of red onions is due primarily to anthocyanins present in the epidermal cells (Donner et al. 1997). Total antocyanin was 233 mg/kg, while nearly three times more 943 mg/kg (fresh weight) was detected (Ferreres et al. 1996). The main anthocyanin in red onions was first reported as cyanidin 3-glucoside (Robinson and Robinson 1932). The major identified anthocyanins were cyanidin 3-glucoside, cyanidin 3-laminaribioside, cyanidin 3-(6″-malonylglucoside), and cyanidin 3-(6″-malonyllaminaribioside), while cyanidin 3-(3″-malonylglucoside), peonidin 3-glucoside, peonidin 3-malonylglucoside, and cyanidin 3-dimalonyllaminaribioside were present in minor quantities (Donner et al. 1997; Ferreres et al. 1996). Later, two anthocyanins with 4-substituted aglycone have been isolated from the edible and dry outer layers of red onion, and the structures were elucidated by 2-D nuclear magnetic resonance (NMR) and liquid chromatography–mass spectrometry (LC-MS) (Fossen and Andersen 2003). In addition to six known anthocyanins, recently, six new anthocyanins (cyanidin 3-O-(3″-O-β-glucopyranosyl-6″-O-malonyl-β-glucopyranoside)-4′-O-β-glucopyranoside, cyanidin 7-O-(3″-O-β-glucopyranosyl-6″-O-malonyl-β-glucopyranoside)-4′-O-β-glucopyranoside, cyanidin 3,4′-di-O-β-glucopyranoside, cyanidin 4′-O-β-glucoside, peonidin 3-O-(6″-O-malonyl-β-glucopyranoside)-5-O-β-glucopyranoside, and peonidin 3-O-(6″-O-malonyl-β-glucopyranoside) in minor amounts were identified by the use of 2-D NMR and electospray LC-MS (Fossen et al. 2003). The anthocyanins show great variation in stability; for instance, the malonated anthocyanins are much more stable than the corresponding non-acylated pigments.

Sapogenins and Saponins

Sapogenins are steroidal compounds; their aglycone forms are referred to as the sapogenins (Dini et al. 2005). They typically occur in small quantities in the *Allium*

genus (Dini et al. 2005). One of the earliest reports on onion dated back in 1982, where Smoczkiewicz and others determined saponin glycosides in onion. Several different methods of identification were applied, which included gas chromatography–mass spectrometry (GC-MS, aided by NMR, infrared (IR), and UV. To date, those identified saponins compounds are sitosterol, gitogenin, oleanolic acid, amyrin, diosgenin, β-chlorogenin, cepagenin, alliospirosides A–D, tropeosides A1/A2, and tropeosides B1/B2 (Corea et al. 2005; Dini et al. 2005; Kravets et al. 1987).

Flavor Analytical Methodology

The elemental composition of onion has been reviewed for paying special attention to methods used in the literature, specifically to determine possible components or the products that contribute to flavor. The chemical characterization of onion cultivars is important as it is an important quality factor (Alvarez et al. 2003). Even small differences in flavor components play an important role in the characterization and differentiation of onion varieties. Losses or increases of phytochemicals can also vary with the processing method. The degree of change in the chemical composition of onion during processing depends on the sensitivity of the phytochemical and length of processing (Breene 1994). Discrepancies between reports might be related to cultivar differences or sample preparation prior to analysis.

The selection of the appropiate analytical method is crucial to determine unstable transient flavor compounds.

The identification and quantification of flavonoids are usually performed with high-performance liquid chromatography (HPLC) combined with UV detection and fluorescence detection (Ewald et al. 1999). Although both UV absorbance and fluorescence detection methods are applied, fluorescence detection has been reported to be more sensitive (Huck et al. 2002). For the quantitation of flavonol aglycones, an initial hydrolysis procedure is required to break the glycosidic bonds, and the aglycones are quantified. The method of choice compromises the efficient release and degradation of aglycones (Nuutila et al. 2002). Mondy and others (2002) analyzed quantitatively and qualitatively the aroma of fresh and frozen, freeze-dried, and sterilized onion products by solid phase microextraction (SPME), liquid extraction, and GC-MS. The hydrolysis methods and related conditions were compared, and hydrolysis in 1.2 M HCl in methnol and 2 ppm antioxidant ascorbic acid was the optimum onion flanoids and phenolic acids (Nuutila et al. 2002). The extraction recovery is dependent on the molarity of HCl, hydrolysis time and temperature, and the composition of the extraction solvent (Wach et al. 2007). Due to their large number, quantification of individual flavonoid glycosides is difficult (Nuutila et al. 2002). Yoo and Pike (1998) applied dansyl-Cl derivatization prior to the HPLC analysis of pungency precursor compounds S-methyl (Me), S-2-propenyl (allyl, Al), and S-propenyl (Pe)-cysteine sulfoxides (CSOs) and observed PeCSO as the main flavor precursor. Free and conjugated flavonoids are also analyzed quantitavely by reversed-phase HPLC (Crozier et al. 1997). Andlauer and others (1999) utilized reversed-phase HPLC combined with UV and MS detection in order to determine phytochemicals. Free and conjugated flavonoids are analyzed quantitavely by reversed-phase HPLC (Crozier et al. 1997). When reversed-phase HPLC was used for free and conjugated flavonoids, the limit of detection for endogenous quercetin and other aglycones was 3 μg/g fresh weight.

Some studies demonstrate that HPLC and gas chromatography (GC) connected with mass spectrometry (MS) are superior as compared to UV or electrochemical detection and have been used for the separation and identification of thiosulfinate compounds and their decomposition products. Phenolic composition performed through HPLC and LC-MS/MS showed the presence of gallic acid, ferulic acid, protocatechuic acid, quercetin, and kaempferol (Prakash et al. 2007). The sensitivity of fluorescence is enhanced by MS detection. HPLC-MS techniques have been applied in the separation and identification of flavonols in a number of studies. A hydrophilic poly (carboxylic acid)-coated silica was investigated for the HPLC determination of flavonoids using UV absorbance and MS detection (Huck et al. 2002). In the most recent report by Bonaccorsi and others (2008), onion bulbs were subjected to chemical analysis by means of HPLC–diode array detection (DAD) together with electron spray mass spectrometry (LC-ESI-MS-MS). Among the seven flavonols identified, two of which quercetin 3,4′-diglucoside and quercetin 4′-glucoside constituted 90%. Great differences from 7 to 700 mg/kg in flavonol contents were reported in white, red, and gold varieties, respectively. Donner et al. (1997) determined the anthocyanin content by HPLC and expressed total anthocyanin as cyanidin 3-glucoside. Subtantial differences in the quantity of the anthocyanins in various cultivars were further observed.

Trenerry (1996) applied capillary electrophoresis for the determination of the sulfite content in onion. Prior to loading, sulfite was eventually converted to sulfate by distillation and was loaded onto the electrophoresis only when the detection limit was 5 mg/kg. Caridi and others (2007) applied capillary zone electrophoresis for profiling and quantification of the levels of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside in HPLC.

Biosensor technology is also tested in flavor analysis. The main advantage of the biosensoric techniques over HPLC is that there is no need for chromatographic separation. Keusgen and others (2003) developed a biosensor-based detection method for S-alk(en)yl cysteine sulfoxides. Alliinase enzyme combined with an ammonia gas electrode is immobilized in direct contact with an electrode surface. The method was sensitive enough to detect 10^{-5} to 10^{-3} mM. Later, Abayomi and others (2006) tested a disposable amperometric biosensor to determine the pyruvate content, a quality indicator of pungency in onion, and were able to detect 1–2 μmol/g fresh weight.

The major difficulty of identification compounds responsible for the flavor of onion released after cut is due to their sensitivity to thermal heating with traditional GC. Ferary and Auger (1996) have utilized GC-MS with particle beam and atmospheric pressure ionization interfaces. Asbury and others (1999) analyzed lipid components in onion seeds by using matrix-assisted laser desorption/ionization (MALDI) MS. Arnault and others (2000) improved the GC-MS analysis of onion volatiles by studying solvent partition with SPME followed by cryo-trapping isolation. Thiosulfinates were better identified using GC-MS with routine splitless injection.

In recent years, heteronuclear NMR spectroscopy has also found application in the structural analysis of chemical compounds in onion. The structures of quercetins from red onions were determined with using homo- and heteronuclear NMR spectroscopy (Fossen et al. 1998). On the bases of 1-D and 2-D NMR and mass spectometry data, the structure of eight furostanol saponins was elucidated (Dini et al. 2005).

Organoleptic Evaluation of Onion

Whether consumed cooked or raw, onions always have had a principal role in the taste and aroma of typical traditional Middle Eastern dishes. Depending on the variety, onion can be sharp, spicy, tangy and pungent, mild, or sweet. Low-pungency onions typically have a pyruvate concentration of ca. <5 mol/g (fresh weight). Onion flavors and pungency are affected primarily from genetic factors, tissue type, and bulb size; neverthless, many environmental factors such as growth sulfate availability, nitrogen (Randle 2000) and selenium (Kopsell and Randle 1997) concentrations, irrigation water, growing temperature, storage conditions, and later food processing methods and conditions (Coolong and Randle 2003) also have great influence on onion pungency (Yoo et al. 2006). The contents of soluble carbohydrates like sugars and organic acids also contribute to to the distinctive flavor and aroma (Dhumal et al. 2007).

The average volatile oil content of onion is 1.5 ppm, depending on the variety and the extraction and/or processing conditions (Shaath and Flores 1998). A sulfur-containing imino compound is present at lower levels in fresh onions but increases during cooking and accounts for about 50% of all sulfur-containing compounds (Shaath and Flores 1998). Depending on the processing conditions, a great number of complex reactions occur in the degradation of flavor compounds (Granvogl and Schieberle 2006). On heating, S-propenyl-cysteine sulfoxide, the main sulfur amino acid in onion, changes to a stable cyclic compound, cycloalliin (Yanagita et al. 2003). Among all volatile S-compounds, cycloalliin, at 83.43%, is also the predominant compound in Welsh onion (Gyawali et al. 2006). Bacon and others (1999) quantatively analyzed the flavor precursors and pyruvate levels of different tissues of onions. In all cultivars tested, the top and bottom onion sections have the highest levels of alk(en)yl cysteine sulfoxides; the levels of pyruvate and flavor precursors increased in the inner tissue during storage.

Onion Flavor in Semi-Processed and Processed Foods

Onions can be dried and simply preserved for several months with small or no quality change. Due to simple preservation, long storage time, ease of transportation, and strong, desirable aroma, onion is one of the most common food ingredients used globally in gastronomy (FAO 2004).

Onion-rich ethnic and traditional foods are popular in the Middle Eastern countries. Consumption of raw onion in garnishing prevents flavonoid breakdown, which occurs during cooking (Crozier et al. 1997). Onion is not eaten alone but usually acts as a garnishing in raw or processed dishes. In ethnic and traditional products, fresh and raw onion is used in sautéing, baking, and boiling of almost all meat and vegetable products. The typical flavors of meat dishes result with the use of fresh bulb onion.

There is an increasing interest in industrial research in alternatively providing onion aroma components in a ready-to-use format. Although in recent years a substantial proportion of onion is processed as a ready-to-use ingredient in food products as peeled, dehydrated, powder, chops and flakes (packed and unpacked), frozen, pickled aromatic oil in the world market, onion is traditionally consumed as fresh in the Middle Eastern countries. Onion powders are produced freeze-dried, flow-dried, or vacuum packed. In order to protect their characteristic pungent flavor

and aroma, dehydrated onions are produced without blanching or without the addition of sulfites. Dried, powdered, pickled, lactic acid fermented, or other processing in comparison to conventionally prepared is commercially not so popular in the Middle Eastern diet because onion is easier to preserve than other vegetables.

Minimally processed or shredded onions are produced fresh and "ready-to-eat." The main quality factor in the case of fresh, ready-to-eat shredded onions is preservation of color components and resulting discoloration during storage. Oxidation is generally the major cause of the chemical deterioration of the nutritional and organoleptic (color, flavor, and texture) quality of processed onion products (Antolovich et al. 2002). Polymeric film packaging has been applied in the preparation of ready-to-eat salads, including minimally processed or fresh, ready-to-eat shredded onion (Howard et al. 1994). In the 1980s, the industry began to adopt controlled atmosphere (CA) storage. CA uses a low-oxygen, high-carbon dioxide refrigerated environment to store onions. The influence of CA storage conditions on the microbiological and sensory quality of diced, "ready-to-use" onion was investigated, and increasing the length of storage caused undesirable browning after cooking (Blanchard et al. 1996). Studies have been conducted for an alternative novel processing of onion. The effect of gamma irradiation on the volatile compounds of dried Welsh onions has been investigated for microbial decontamination, but the treatment resulted in decreased S-containing compounds (Gyawali et al. 2006). The efficiency of IR convective drying has been tested (Pathare and Sharma 2006).

TRANSGENICITY AND GENETIC STUDIES FOR ONION

There are several prerequests for the production of transgenic plant lines. First, a reliable transformation system for foreign genetic materials and a reliable plant regeneration method either from callus or suspension cultures must be established. Second, a construct carrying specific target gene sequences should be prepared. To distinguish transgenic lines from nontrangenic lines, a moleculer characterization technique based on either protein or DNA sequences should be identified. Only following these, an effective transformation system could be applied (Zheng et al. 2001). In this line, the poor *in vitro* plant regeneration rate shown by onion is reported as the main limiting factor in obtaining transgenic onion plants (Eady et al. 2000). Only just in 2000, Eady and others reported an onion transformation protocol based on immature zygotic embryos as target tissue. After that, in 2001, Zheng et al. reported an *Agrobacterium tumefaciens*-mediated transformation procedure for onion using immature embryos as the explant source. In recent years, studies to determine the genomic mapping of *A. cepa* (onion) have inceased; for example, Masuzaki et al. (2008) performed chromosome-specific random amplified fragment length polymorphism (RAPD) markers for establishing linkage maps. These studies, in return, are expected to contribute to develop transgenic onion varieties.

To date, three different alliinase genes, a bulb alliinase gene and two root alliinase genes, whose protein products operate in the biochemical pathways that are responsible for the characteristic onion flavor, have been isolated and characterized (Clark 1993; Do et al. 2004; Gilpin et al. 1995). The genomic DNA gel blotting has also further confirmed three different locations of alliinase genes that form the alliinase

multigene family in the onion genome (Do et al. 2004). The deduced amino acid sequence homology among the three genes is around 65% (Do et al. 2004). Eady et al. (2000), by using gene silencing method, were able to produce an antisense version of a bulb alliinase gene under the CaMV35s promoter; transgenic lines carrying this antisense gene had significantly reduced levels of allinase activity. Tearless onion, by silencing the lachrymatory factor synthase gene, has been reported. The elimination of the gene resulted in the conversion of sulfur compounds to flavor instead of tearing agents (Eady et al. 2000).

In molecular studies, onion epidermis cells present a great tool for the molecular expression of novel fusion proteins from various sources. A transient expression system using onion epidermal cells to investigate the expression of foreing proteins and cellular localization has been a very popular technique (dos Reis Figueira et al. 2002). Che et al. (2002) have performed a comparative analysis of gene expression during *Pseudomonas avenae* infection of rice; in order to achieve this, they have introduced recombitant fusion into onion cells, which confirmed the expression location and the possible function of the specific cDNA region with its involvement in the repair mechanism of cell walls damaged by bacteria (Che et al. 2002). Ha and others (2007) tested a ginseng-specific abundant protein (GSAP) involved in abiotic stress tolerance by using onion. Fores and others (2006) localized proteins that were involved in the regulation of cellular lipid homeostasis exclusively onto the endoplasmic reticulum by expressing these proteins first in onion epidermal cells. Later, Liu and others (2006) expressed a rice transcription factor reponsible for defense response in onion epidermis cells, and the protein was localized in the nucleus. More recently, the wheat Myb transcription factor crucial in regulating secondary metabolism was tested *in vivo* subcellular targeting in onion epidermal cells and was localized in the nucleus (Chen et al. 2005).

There are several published studies where genetic studies in onion have found applications in plant pathology. Eady and others (2003) produced trangenic onion containing a bar gene construct encoding the visual green fluorescent reporter protein and resistance to the herbicide phosphinothricin. Recently, field trials of herbicides tolerant to glyphosate genetically transformed onion seedlings have been conducted (Eady et al. 2005). Pectins in plants are capable of providing a self-defense against sap-sucking insects by adversely affecting the survivability of the insects (Hossain et al. 2006). Hossain and others (2006) found that a mannose-binding lectin gene from onion was expressed in a bacterial system, and the purified recombinant lectin peptides were utilized against the sap-sucking mustard aphid *Lipaphis erysimi*.

It is proposed that by genetic engineering, isolated uridine diphosphate glucosyltransferase genes from onion could be transformed into other crops in order to increase the quercetin content. Alternatively, quercetin-like flavonoid glucosides could be manufactured through biofermentation. To date, two flavonoid glucosyltransferases isolated from yellow onion were functionally expressed in *Eschricha coli* (Kramer et al. 2003).

CONCLUSIONS

Increasing scientific attention is focused on functional foods and in ethnomedicines. Although tremendous amounts of effort are directed, the functional secrets of the

biologically active properties of onion are far from being entirely exposed. The increasing interest in the potential usability of onion as a functional food in ethnomedicine will challenge scientists and researchers in the field to concentrate on the identification of chemical constitutes and on using novel molecules in ethnomedicine. As today's consumers ask for more natural nutraceuticals, new challenges are presented to researchers to develop methods to identify, to isolate, and perhaps to preserve biologically active components from onion. Cell and tissue culture methods are expected to find applications in investigating the commercial production of flavors and biologically active chemical components in large quantities (Hughes et al. 2005).

Currently, onion is cultivated under both irrigated and non-irrigated conditions. With regard to production, global climate change and decrease in water resources available for agricultural production have incerased the concerns, because an excessive amount of irrigation water is generally needed as onions are shallow-rooted (Al-jamal et al. 2000; Bahnasawy et al. 2004). Molecular biology and the selection of cultivars will find applications in developing varieties that will require less water.

DEDICATION

We would like to dedicate this chapter to Mr. Hasan Tokgoz, a major supporter and guide of this chapter and of similar studies, who passed away in January 2006.

REFERENCES

Abayomi LA, Terry LA, White SF, Warner PJ. 2006. Development of a disposable pyruvate biosensor to determine pungency in onions (*Allium cepa* L.). *Biosens Bioelectron* 21:2176–9.

Al-Jamal MS, Sammis TW, Ball S, Smeal D. 2000. Computing the crop water production function for onion. *Agricult Water Manag* 46(1):29–41.

Alvarez J, Marco LM, Arroyo J, Greaves ED, Rivas R. 2003. Determination of calcium, potassium, manganese, iron, copper and zinc levels in representative samples of two onion cultivars using total reflection X-ray fluorescence and ultrasound extraction procedure. *Spectrochim Acta Part B At Spectrosc* 58:2183–9.

Andlauer W, Martena MJ, Fürst P. 1999. Determination of selected phytochemicals by reversed-phase high-performance liquid chromatography combined with ultraviolet and mass spectrometric detection. *J Chromatogr A* 849:341–8.

Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. 2002. Methods for testing antioxidant activity. *Analyst* 127:183–98.

Arnault I, Mondy N, Cadoux F, Auger J. 2000. Possible interest of various sample transfer techniques for fast gas chromatography-mass spectrometric analysis of true onion volatiles. *J Chromatogr A* 896(1–2):117–24.

Asbury GR, Al-Saad K, Siems WF, Hannan RM, Hill HH. 1999. Analysis of triacylglycerols and whole oils by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *J Am Soc Mass Spectrom* 10(10):983–91.

Auger J, Yang W, Arnault I, Pannier F, Potin-Gautier M. 2004. High-performance liquid chromatographic–inductively coupled plasma mass spectrometric evidence for Se-"alliins" in garlic and onion grown in Se-rich soil. *J Chromatogr* 1032(1–2):103–7.

Bacon JR, Moates GK, Ng A, Rhodes MJC, Smith AC, Waldron KW. 1999. Quantitative analysis of flavour precursors and pyruvate levels in different tissues and cultivars of onion (*Allium cepa*). *Food Chem* 64(2):257–61.

Bahnasawy AH, El-Haddad ZA, El-Ansary MY, Sorour HM. 2004. Physical and mechanical properties of some Egyptian onion cultivars. *J Food Process Eng* 62(3):255–61.

Blanchard M, Castaigne F, Willemot C, Makhlouf J. 1996. Modified atmosphere preservation of freshly prepared diced yellow onion. *Postharvest Biol Technol* 9(2):173–85.

Block E. 1992. The organosulfur chemistry of the genus *Allium*—Implications for the organic *Allium* chemistry of sulfur. *Angew Chem Int Ed Engl* 31:1135–78.

Bonaccorsi P, Caristi C, Gargiulli C, Leuzzi U. 2008. Flavonol glucosides in species: A comparative study by means of HPLC-DAD-ESI-MS-MS. *Food Chem* 107(4):1668–73.

Breene WM. 1994. Healthfulness and nutritional quality of fresh versus processed fruits and vegetables: A review. *J Foodserv Syst* 8:1–45.

Caridi D, Trenerry VC, Rochfort S, Duong S, Laugher D, Jones R. 2007. Profiling and quantifying quercetin glucosides in onion (*Allium cepa* L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. *Food Chem* 105(2):691–9.

Ceylan E, Fung DYC. 2004. Antimicrobial activities of spices. *J Rapid Methods Autom Microbiol* 12:1–55

Chang HS, Yamato O, Sakai Y, Yamasaki M, Maede Y. (2004) Acceleration of superoxide generation in polymorphonuclear leukocytes and inhibition of platelet aggregation by alk(en)yl thiosulfates derived from onion and garlic in dogs and humans. *Prostaglandins Leukot Essent Fatty Acids* 70:77–83.

Che F-S, Entani T, Marumoto T, Taniguchi M, Takayama S, Isogai A. 2002. Identification of novel genes differentially expressed in compatible and incompatible interactions between rice and *Pseudomonas avenae*. *Plant Sci* 162(3):449–58.

Chen R, Ni Z, Nie X, Qin Y, Dong G, Sun Q. (2005). Isolation and characterization of genes encoding Myb transcription factor in wheat (*Triticum aestivem* L.). *Plant Sci* 169(6):1146–54.

Clark SA. 1993. Molecular cloning of a cDNA encoding alliinase from onion (*Allium cepa* L.). PhD dissertation, University of Canterbury, Christchurch, New Zealand.

Cook NC, Samman S. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem* 7(2):66–76.

Coolong TM, Randle WM. 2003. Sulfur and nitrogen availability interact to affect the flavor biosynthetic pathway in onion. *J Am Soc Hortic Sci* 128(5):776–83.

Corea G, Fattorusso E, Lanzotti V, Capasso R, Izzo AA. 2005. Antispasmodic saponins from bulbs of red onion *Allium cepa* L. Tropea. *J Agric Food Chem* 53:935–40.

Corzo-Martinez M, Corzo N, Villamiel M. 2007. Biological properties of onions and garlic. *Trends Food Sci Technol* 18(12):609–25.

Crowther T, Collin HA, Smith B, Tomsett AB, O'Connor D, Jones MG. 2005. Assessment of the flavour of fresh uncooked onions by taste panels and analysis of flavour precursors, pyruvate and sugars, *J Sci Food Agric* 85:112–20.

Crozier A, Jensen E, Lean MEJ, McDonald MS. 1997a. Quantitative analysis of flavonoids by reversed-phase high-performance liquid chromatography. *J Chromatogr A* 761(1–2): 315–21.

Crozier A, Lean MEJ, McDonald SM, Blak C. 1997b. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J Agric Food Chem* 45:590–5.

de Vries JHM, Hollman PCH, Meyboom S, Buysman MN, Zock PL, Staveren WAS, Katan MB. 1998. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. *Am J Clin Nutr* 68:60–5.

Dhumal K, Datir S, Pandey R. 2007. Assessment of bulb pungency level in different Indian cultivars of onion (*Allium cepa* L.). *Food Chem* 100:1328–30.

Dini I, Tenore GC, Trimarco E, Dini, A. 2005. Furostanol saponins in *Allium cepa* L. Var. tropeana seeds. *Food Chem* 93:205–14.

Do GS, Suzuki G, Mukai Y. 2004. Genomic organization of a novel root alliinase gene, *ALL1*, in onion. *Gene* 325:17–24.

Donner H, Gao TL, Mazzab G. 1997. Separation and characterization of simple and malonylated anthocyanins in red onions, *Allium cepa L. Food Res Int* 30(8):637–43.

dos Reis Figueira A, Golem S, Goregaoker SP, Culver JN. 2002. A nuclear localization signal and a membrane association domain contribute to the cellular localization of the tobacco mosaic virus 126-kDa replicase protein. *Virology* 301(1):81–9.

Eady C, Davis S, Catanach A, Kenel F, Hunger S. 2005. *Agrobacterium tumefaciens*-mediated transformation of leek (*Allium porrum*) and garlic (*Allium sativum*). *Plant Cell Rep* 24:209–15.

Eady CC, Reader J, Davis S, Dale T. 2003. Inheritance and expression of introduced DNA in transgenic onion plants (*Allium cepa*). *Ann Appl Biol* 142:219–24.

Eady CC, Weld RJ, Lister CE. 2000. *Agrobacterium tumefaciens*-mediated transformation and transgenic-plant regeneration of onion (*Allium cepa* L.). *Plant Cell Rep* 19:376–81.

Ewald C, Fjelkner-Modig S, Johansson K, Sjöholm I, Åkesson B. 1999. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chem* 64(2):231–5.

FAO. 2002. FAO Statistical Database (online). Consultation 26 March 2006. http://www.fao.org/page/collections?Suset=agriculture&language=ES.

FAOSTAT. 2002. Agriculture Data. Food Balance Sheets. URL: http://apps.fao.org. Consulted in 03 October 2002. Last actualization: 28 May 2007.

Ferary S, Auger J. 1996. What is the true odour of cut Allium? Complementarity of various hyphenated methods: Gas chromatography-mass spectrometry and high-performance liquid chromatography-mass spectrometry with particle beam and atmospheric pressure ionization interfaces in sulphenic acids rearrangement components discrimination. *J Chromatogr A* 750(1–2):63–74.

Ferreres F, Gil MI, Tomás-Barberán FA. 1996. Anthocyanins and flavonoids from shredded red onion and changes during storage in perforated films. *Food Res Int* 29(3–4):389–95.

Fores O, Arró M, Pahissa A, Ferrero S, Germann M, Stukey J, McDonough V, Nickels JT Jr., Campos N, Ferrer A. 2006. *Arabidopsis thaliana* expresses two functional isoforms of Arvp, a protein involved in the regulation of cellular lipid homeostasis. *Biochimi Biophys Acta, Molecular and Cell Biology of Lipids* 1761:725–35.

Fossen T, Andersen OM. 2003. Anthocyanins from red onion, *Allium cepa*, with novel aglycone. *Phytochem* 62(8):1217–20.

Fossen T, Pedersen AT, Andersen OM. 1998. Flavonoids from red onion (*Allium cepa*). *Phytochem* 47(2):281–5.

Fossen T, Slimestad R, Anderson OM. 2003. Anthocyanins with 4′-glucosidation from red onion, *Allium cepa*. *Phytochem* 64(8):1367–74.

Gilpin BJ, Leung DW, Lancaster JE. 1995. Nucleotide sequence of a nuclear clone of alliinase (accession no L48614) from onion (PGR 95-125). *Plant Physiol* 110:336.

Gonzalvez A, Armenta S, Cervera ML, de la Guardia M. 2008. Elemental composition of seasoning products. *Talanta* 74:1085–95.

Granvogl M, Schieberle P. 2006. Facts and "artefacts" in the flavour chemistry of onions. *Dev Food Sci* 43:359–62.

Griffiths G, Trueman L, Crowther T, Thomas B, Smith B. 2002. Onions- A global benefit to health. *Phytother Res* 16(7):603–15.

Gyawali R, Seo H-Y, Lee H-J, Song H-P, Kim D-H, Byun M-W, Kim K-S. 2006. Effect of [gamma]-irradiation on volatile compounds of dried Welsh onion (*Allium fistulosum* L.). *Radiat Phys Chem* 75(2):322–8.

Ha YI, Lim JM, Ko S-M, Liu JR, Choi D-W. 2007. A ginseng-specific abundant protein (GSAP) located on the cell wall is involved in abiotic stress tolerance. *Gene* 386:115–22.

Herrmann J. (1976). Flavonols and flavones in food plants: A review. *J Food Technol* 11:433–7.

Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. 1993. Dietary antioxidant flavonoids and the risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* 342:1007–11.

Hertog MGL, Hollman CH, Venema DP. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J Agric Food Chem* 40:1591–8.

Hirota S, Shimoda T, Takahama U. 1998. Tissue and spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *J Agric Food Chem* 46:3497–502.

Hollman PCH, de Vries JHM, van Leeuwen SD, Mengeler SMJB, Katan MB. 1995. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *Am J Clin Nutr* 62:1276–82.

Hollman PCH, Katan MB. 1997. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed Pharmacother* 51(8):305–10.

Hong G, Peiser G, Cantwell MI. 2000. Use of controlled atmospheres and heat treatment to maintain quality of intact and minimally processed green onions. *Postharvest Biol Technol* 20:53–61.

Hossain MA, Maiti MK, Basu A, Sen S, Ghosh AK, Sen SK. 2006. Transgenic expression of onion leaf lectin gene in Indian mustard offers protection against aphid colonization. *Genomics Mol Genet Biotechnol* 46:2022–32.

Howard LR, Yoo KS, Pike LM, Miller GH Jr. 1994. Quality changes in diced onions stored in film packages. *J Food Sci* 59:110–5.

Huck CW, Buchmeiser MR, Bonn GK. 2002. Fast analysis of flavonoids in plant extracts by liquid chromatography-ultraviolet absorbance detection on poly (carboxylic acid)-coated silica and electrospray ionization tandem mass spectrometric detection. *J Chromatogr A* 943(1):33–8.

Hughes J, Tregova A, Tomsett, AB, Jones MG, Cosstick R, Collin HA. 2005. Synthesis of the flavour precursor, alliin, in garlic tissue cultures. *Phytochemistry* 66(2):187–94.

Hrazdina G. 2006. Aroma production by tissue cultures. *J Agric Food Chem* 54:1116–23.

Kadayifci A, Tuylu GI, Ucar Y, Cakmak B. 2005 Crop water use of onion (*Allium cepa* L.) in Turkey. *Agricult Water Manag* 72:59–68.

Ketter CAT, Randle WM. 1998. Pungency assessment in onions. In Karcher SJ (ed.), Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education, vol. 19.

Keusgen M, Junger M, Krest I, Schöning MJ. 2003. Development of a biosensor specific for cysteine sulfoxides. *Biosens Bioelectron* 18(5–6):805–12.

Kopsell DE, Randle WM. 1997. Onion cultivars differ in pungency and bulb quality changes during storage. *HortScience* 32(7):1260–3.

Kramer CM, Prata RTN, Willits MG, De Luc V, Steffens JC, Graser G. 2003. Cloning and regiospecificity studies of two flavonoid glucosyltransferases from *Allium cepa*. *Phytochemistry* 64(6):1069–76.

Kravets SD, Vollerner YS, Shashkov AS, Gorovits MB, Abubakirov NK. 1987. Steroids of the spirostan and furostan series from plants of the genus *Allium*. XXIII—Structure of cepagenin and alliospiraniside C and D. from *Allium cepa*. *Him Prir Soedin* 6:843–9.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med* 113:71–88.

Lanzotti V. 2006. The analysis of onion and garlic. *J Chromatogr A* 1112(2006):3–22.

Lin JY, Tang CY. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem* 101:140–7.

Liu X, Bai X, Wang X, Chu C. 2006. OsWRKY71, a rice transcription factor, is involved in rice defense response. *J. Plant Physiol* 17:1691–9.

Lombard K, Peffley E, Geoffriau B, Thompson L, Herring A. 2005. Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. *J Food Compost Anal* 18(6):571–81.

Makris DP, Rossiter JT. 2002. An investigation on structural aspects influencing product formation in enzymic and chemical oxidation of quercetin and related flavonols. *Food Chem* 77:177–85.

Masuzaki S, Miyazaki T, McCallum JA, Heusden SV, Kik C, Yamashita K-I, Tashiro Y, Yamauchi N, Shigyo M. 2008. Conversion of chromosome-specific RAPDs into SCAR-based anchor markers for onion linkage maps and its application to genetic analyses in other *Allium* species. *Sci Hortic* 115:323–8.

Mondy N, Duplat D, Christides JP, Arnault I, Auger J. 2002. Aroma analysis of fresh and preserved onions and leek by dual solid-phase microextraction–liquid extraction and gas chromatography–mass spectrometry. *J Chrom A* 963:89–93.

Nuutila AM, Kammiovirta K, Oksman-Caldentey K-M. 2002. Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. *Food Chem* 76(4):519–25.

Pathare PB, Sharma GP. 2006. Effective moisture diffusivity of onion slices undergoing infrared convective drying. *Biosyst Eng* 93(3):285–91.

Prakash D, Singh BN, Upadhyay G. 2007. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem* 102(4):1389–93.

Price CL, Shuler ML, Yamada Y. 1997. Altering flavor profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis. *Biotechnol Prog* 13:506–10.

Randle WM. 1997. Onion flavor chemistry and factors influencing flavor intensity. In Risch SJ, Ho C (eds.), *Spices: Flavor Chemistry and Antioxidant Properties*. Washington, DC: American Chemical Society, pp. 41–52.

Randle WM. 2000. Increasing nitrogen concentration in hydroponic solutions affects onion flavor and bulb quality. *J Am Soc Hortic Sci* 125(2):254–9.

Randle WM, Bussard ML. 1993. Streamlining onion pungency analyses. *HortScience* 28:60.

Randle WM, Kopsell DA, Kopsell DE, Snyder RL, Torrance R. 1998. Field sampling short-day onions for bulb pungency. *Horttechnology* 8:329–32.

Rhodes MJC, Price KR. 1996. Analytical problems in the study of flavonoid compounds in onions. *Food Chem* 57(1):113–17.

Robinson GM, Robinson R. 1932. A survey of anthocyanins II. *Biochem J* 26:1647–64.

Rosengarten F. 1969. *The Book of Spices*. Wynnewood, PA: Livingston Publishing, pp. 297–305.

Semmler FW. 1982. The essential oil of onion (*Allium cepa* L.). *Arch Pharm* 434–43.

Shaath NA, Flores FB. 1998. Egyptian onion oil. *Dev Food Sci* 40:443–53.

Shimoda K, Otsuka T, Morimoto Y, Hamada H, Hamada H. 2007 Glycosylation and malonylation of quercetin, epicatechin, and catechin by cultured plant cells. *Chem Lett* 36:1292–3.

Shon MY, Choi SD, Kahng GG, Nam SH, Sung NJ. 2004. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem Toxicol* 42:659–66.

Smoczkiewicz MA, Nitschke D, Wieladek H. 1982. Microdetermination of steroid and triterpene saponin glycosides in various plant materials I. *Allium* species. *Pol Mikrochim Acta* 2:43–53.

Srinivasan K. 2005. Spices as influencers of body metabolism: An overview of three decades of research. *Food Res Int* 38:77–86.

Terao J. 1999. Dietary flavonoids as plasma antioxidants on lipid peroxidation: Significance of metabolic conversion. In *Antioxidant Foods: Supplements in Human Health*, Packer L, Hiramatsu M, Yoshikawa T (eds.). New York: Academic, pp. 255–68.

Trenerry VC. 1996. The determination of foods and beverages the sulphite content of some by capillary electrophoresis. *Food Chem* 55(3):299–303.

Wach A, Pyrzynska K, Biesaga M. 2007. Quercetin content in some food and herbal samples. *Food Chem* 100:699–704.

Whitaker JR. 1976. Development of flavor, odor and pungency in onion and garlic. *Adv Food Res* 22:73–133.

Wittig J, Herderich M, Graefe, EU, Veit M. 2001. Identification of quercetin glucuronides in human plasma by highperformance liquid chromatography–tandem mass spectrometry. *J Chromatogr B* 753:237–43.

Yanagita T, Han S-Y, Wang Y-M, Tsuruta Y, Anno T. 2003. Cycloalliin, a cyclic sulfur imino acid, reduces serum triacylglycerol in rats. *Nutrition* 19:140–3.

Yoo KS, Pike LM. 1998. Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci Hortic* 75:1–10.

Yoo KS, Pike L, Crosby K, Jones R, Leskovar D. 2006. Differences in onion pungency due to cultivars, growth environment, and bulb sizes. *Sci Hortic* 110(2):144–9.

Zheng S, Khrustaleva L, Henken B, Sofiari E, Jacobsen E, Kik C, Krens FA. 2001. *Agrobacterium tumefaciens*-mediated transformation of *Allium cepa* L.: The production of transgenic onions and shallots. *Mol Breed* 7:101–15.

CHAPTER 46

Mushrooms in the Middle Eastern Diet

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> Without leaves, without bud, without flowers: yet they form fruits. As a food, as a toxic, as a medicine: the entire creation is precious. (Chang 1990)

INTRODUCTION

Mushrooms are fungi and do not contain chlorophyll, therefore they are not considered as plants. They are used a source of nutritionally rich food, and many species of mushrooms have also been reported as folk medicines for thousands of years. Ancient Egyptians believed that mushrooms were the plants of immortality according to the hieroglyphics of 4600 years ago. The Egyptians considered mushrooms as a delicacy; mushrooms were food for royalty and no commoner could ever touch them. The Greeks believed that mushrooms provided strength for warriors in battle. The Romans regarded mushrooms as a gift from God and served them only during festivals, while the Chinese treasured them as a health food.

Mushrooms have been used around the world as human food for centuries, not as a source of nutrients but as a delicacy. Though mushrooms may not seem to offer much nutritional value, many species are in fact rich in fiber, vitamins (such as thiamine, riboflavin, niacin, cobalamins, and ascorbic acid), and minerals (such as iron, selenium, potassium, and phosphorus).

Mushrooms can also have a positive effect on the environment. Those available in the supermarket have been commercially grown using agricultural wastes such as wheat and paddy straw (Pant et al. 2006). Button mushroom (*Agaricus bisporus*), one of the most widely cultivated mushrooms in the world, has an excellent capacity for recycling these farm waste materials (Tewari 2003). Among the commonly grown mushrooms, *A. bisporus* is one of the safest mushrooms for most people to eat because it is grown under controlled, sterilized environmental conditions.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Not all is good about mushrooms. There are a number of mushroom species that are known to be poisonous and could prove fatal if eaten. Some resemble edible mushrooms, and identification of toxic species becomes difficult for a normal consumer as there is no single trait that can be used for the identification purposes. This toxicity plays a role in protecting the function of mushroom caps to achieve efficient distribution of its spores against consumption and premature destruction.

BIOLOGICAL CLASSIFICATION OF MUSHROOMS

Out of 38,000 known mushroom varieties, about 3000 varieties are edible and fewer than 1% are recognized as poisonous. Only about two dozen edible mushrooms have so far been successfully cultivated and worldwide, and only 5–6% have been commercialized. They differ in their organoleptic properties of shape, size, color, and biochemical composition. White button mushroom (*A. bisporus* and *A. bitorquis*), oyster mushroom (*Pleurotus* spp.), and paddy straw mushroom (*Volvariella* spp.) are the main ones being cultivated commercially. The French started cultivating the button mushroom (*A. bisporus*) in the 17th century. Mushroom cultivation has a long history; cultivation of *A. bisporus* originated in France, when agriculturist Olivier de Serres noted that transplanting mushroom mycelia would lead to more mushrooms. In 1893, a pure culturing method was discovered by the Pasteur Institute in France. Some species are difficult to cultivate; others (particularly mycorrhizal species) have not yet been successfully cultivated.

A. bisporus, named as champignon, common mushroom, or table mushroom, is cultivated in many countries around the world. It is mild in flavor, smooth and round in appearance, creamy white to beige in color, and comes in various sizes. *A. bisporus* is also known by many names, several of which refer to different stages: "white button mushroom" when sold, collected, or eaten in young, unopened form; "crimini or brown mushrooms," with a more earthy flavor and firmer texture; and "portobello or portabella mushrooms," with a large, brown, umbrella-shaped cap, mature, and with a meaty flavor. *A. bisporus* alone comprises more than 90% of total mushroom production. Other cultivated species now available at many grocers include shiitake, maitake or hen-of-the-woods, oyster, and enoki. Shiitakes are the second most-consumed mushrooms in the world, after button mushrooms, and are the preferred variety in Asia.

Table 46.1 summarizes the common cultivated mushroom composition. The nutritional value of mushrooms varies according to species. More recently, Tsai and others (2007) reported a detailed analysis of the nonvolatile taste components in *A. bisporus*, which is harvested at different stages of maturity. The moisture contents were in the range of 89.3–92.3% on a fresh weight basis. Based on dry weight, these values were carbohydrate (38.3–48.9%), crude protein (21.3–27.0%), crude fiber (17.7–23.3%), crude ash (7.77–11.0%), and crude fat (2.53–3.92%).

Wild mushrooms are rare in the Middle East. Wild varieties are found only in regions with sufficient quantities of seasonal rain, like the mountain areas, so recipes with mushrooms are not usually found in traditional cookbooks. With changes in dietary habits during recent years, the cultivation and consumption of *A. bisporus* has become common. The most popular mushroom in the Middle Eastern countries is the button mushroom. The activities of the Food

TABLE 46.1. Nutritional Composition of Mushroom (*Agaricus bisporus*)

Constituent	Content
Carbohydrate	3.3%
Fat	0.3%
Protein	3.1%
Vitamin B_1	0.08 mg/100 g
Vitamin B_2	0.4 mg/100 g
Niacin	3.6 mg/100 g
Vitamin B_5	1.5 mg/100 g
Vitamin C	2 mg/100 g
Iron	0.5 mg/100 g

Source: USDA nutrient database.

and Agriculture Organization (FAO) Forestry and Food Security Project in the Mediterranean and Near East Region (Turkey, Syria, and Jordan) include cultivation of *A. bisporus*.

The next best-known mushroom, the commonly collected wild mushroom known as truffle (Heine 2004), is found throughout much of the Middle East. In fact, wild mushrooms are among the most widely used wild food plants in Turkey; although the fungal flora of Turkey has not been fully documented, a recent estimate puts the number of mushroom species at 5000 with at least 2000 being edible (Baytop 1994; Oder 1990). Some of the other wild consumed varieties in Turkey are *Morchella* spp., *Boletus edulis*, *Cantherellus cibarus*, *Lactarlus songuifluis*, and *Amenita coesdnia*.

A variety of truffles can be found on the common market. The so-called desert truffles found in Kuwait and Iraq are said to have nearly no smell or taste, and it is nearly impossible to clean them of sand. The white desert truffles of Saudi Arabia are more flavorsome and are consumed as a meat substitute (Heine 2004). There are also reports of truffles in Morocco and Tunisia that taste like potatoes.

Traditionally, truffles are better known throughout the Middle East and Europe, where they are collected wild from the environment. Only since the 1990s have they been successfully cultivated in the United States, and they are now being commercially grown there. Truffles grow underground, feeding on nutrients supplied by the roots of trees (Blech 2002). Their underground fruiting bodies, which resemble potatoes, do not completely emerge from the ground, and only grow in conjunction with roots of higher plants, which must have reached a certain physiological maturity. Their size ranges from a pea to a potato and usually gives off a distinctive odor when cooked. Their composition is: 75.5% moisture, 4.4% protein, 1.4% fat, 1.2% ash, 1.2% fiber, 15.1% nitrogen-free extract (NFE), (Pellet and Shadarevian 1970).

Shiitake mushrooms (Japanese shi = oak and take = mushroom) (*Lentinus edodes*), also named Chinese or black forest mushrooms, were originally grown on oak logs but today are grown on oak sawdust. The flavor depends on the type of wood on which they grow (Blech 2002). Shiitake has been known for quite a long time as a cultivated mushroom (Chang and Miles 2004).

MUSHROOM PRODUCTION AND TRADE IN THE MIDDLE EASTERN COUNTRIES

There is little or no information on the production and trade of mushrooms in the Middle Eastern countries. *A. bisporus* is the only commercially cultivated mushroom available in Middle Eastern countries. In Iran in 2005, production was reported to be 22,000 tons, an increase of 20% as compared with the previous year (*Tehran Times* 2005). Although the first notations of primitive cultivation originated in the Middle East ca. 800 years ago, the commercial cultivation of mushrooms is very recent. Other varieties of mushroom, such as shiitake, portabella, and morels, are becoming more available in local markets, as they are relatively easy to cultivate.

HISTORY OF MUSHROOM FLAVOR STUDIES

Mushrooms have long been used as a food or food-flavoring material due to their unique and subtle flavor. Mushroom aroma is one of the most desirable aromas in foods (Zawirska-Wojtasiak 2006). The flavor qualities are greatly dependent on the numerous volatile (Maga 1981a) and nonvolatile compounds (Litchfield 1967; Tsai et al. 2007) contained within the mushroom complex. Most recent reports on mushroom flavor have been concerned with the importance of volatile fraction, and this has been investigated by many authors (Chen and Wu 1984; Chen et al. 1984; Fischer and Grosch 1987; Maga 1981a,b; Mau et al. 1992, 1997; Wasowicz 1974; Venkateshwarlu et al. 1999). Nonvolatile compounds have also been investigated in detail by a number of researchers (Chiang et al. 2006a,b; Tsai et al. 2007, 2008).

About 150 different volatile compounds have been identified in various mushroom species, and the volatile constituents from *A. bisporus* have been well documented (Card and Avisse 1977; Cronin and Ward 1971; Dijkstra and Wiken 1976; Picardi and Issenberg 1973; Pyysalo 1976; Pyysalo and Suihko 1976; Wasowicz 1974). Tressl and others (1982) have shown that in *Agaricus compestris*, some of the C_8 and C_{10} compounds can be normally present in the mushroom. Some of the more important flavor volatiles are believed to be a series of 8-carbon (C_8), and less volatile 10-carbon (C_{10}) compounds. The most important compounds among these eight-carbon are 1-octene-3-ol, 3-octanol, 3-oktanone, 1-octene-3-on, 2-octene-1-ol, and 3-methyl-butanol (Fischer and Grosch 1987; Macleod and Panchasara 1983; Maga 1981a; Mau et al. 1992; Venkateshwarlu et al. 1999; Zawirska-Wojtasiak 2006).

1-Octene-3-ol is the most important of these components in *A. bisporus* and in most other species of wild mushrooms, and is known as "mushroom alcohol" (Flegg et al. 1985). This product of the enzymatic breakdown of linoleic acid (LA) in mushrooms by a lipoxygenase is the most important among all (Cruz et al. 1997; Mau et al. 1992). The amount of 1-octene-3-ol may vary in the total volatile compounds depending on the edible mushroom and its origin; for instance, it can be more than 78% in *A. bisporus* (Wasowicz 1974), 60–74% in oyster mushroom, *Pleurotus florida* (Venkateshwarlu et al. 1999), and more than 80% in edible boletus, *B. edulis* (Maga 1981a). The amount of the mushroom alcohol also has an important effect on the flavor of mushrooms. In small caps, higher concentrations of 1-octene-3-ol were observed in comparison to those bigger in size, but the differences were not

statistically significant in every case (Tsai et al. 2007). Dijkstra (1976) found that *A. bitorquis* had a stronger mushroom flavor than *A. bisporus* due to its higher amount of 1-octene-3-ol content. It is also found in smaller amounts in truffles, *Tuber aestivum*, not as an intrinsic flavor in mushrooms (Diaz et al. 2002) or the shiitake, *L. edodes* (Chen et al. 1984). It occurs as two optically active isomers, the natural (–) form having a stronger flavor than the (+) form (Flegg et al. 1985; Mosandl et al. 1986). The natural form (–), 1-octene-3-ol, is the compound that is responsible for the flavor in fresh mushrooms.

Mushrooms have been found to contain numerous middle-chain aliphatic alcohols, aldehydes, and ketones, which are believed to be the degradation products of fatty acids. The compounds 3-methyl-butyraldehyde and 2,6 bis (1,1-dimethyl ethyl)-phenol were identified in each of the mushrooms with the exception of the giant puffball, *Calvatia*. These compounds are thought to contribute to the characteristic aroma of the mushrooms. Phenylacetaldehyde, which appears to be an important flavor compound, was present in each of the mushrooms except in *A. bisporus*. The flavor compound benzaldehyde was also detected in portabella as well as in *A. bisporus*. *Lactarius* sp. contained a high concentration of the aliphatic compound 1-octene-3-ol. 3-Octanone was also found in the shiitake, portabella, and *A. bisporus* mushrooms (Flegg et al. 1985).

The formation of C_8 compounds in *L. edodes*, the shiitake mushroom, such as 1-octene-3-ol, and sulfurous compounds, such as dimethyl disulfide and dimethyl trisulfide, is affected by the pH value, while the characteristic aroma of shiitake is related to a cyclic sulfurous compound, lenthionine (Chen et al. 1984). Shibuya and others (2005) also studied the flavor compound (Lenthionine) in Japanese shiitake mushroom. Lenthionine, a cyclic sulfur compound, has already been identified to be a key flavor compound in shiitake mushroom and is produced from lentinic acid by the action of γ-glutamyl transferase and C-S lyase. They also suggested that lenthionine is a promising bioactive compound to be used as a new remedy for thrombosis.

VOLATILE AND NONVOLATILE FLAVOR COMPOUND PROFILES

The mushroom odor is located mainly in the central parts of the cap and in the stem in *A. compestris* (Flegg et al. 1985). To a lesser extent, aroma occurs in the hymenium, in the outer levels of the cap, and in the gills. It is not yet certain which chemical fraction is responsible for the flavor properties of mushrooms. Some investigators consider that certain nonvolatile substances may contribute the characteristic flavor, and among these is glutamic acid (Flegg et al. 1985; Guha and Banerjee 1970). Short-chain fatty acids (Stäuble and Rast 1971) and carbohydrates (Litchfield 1967) have also been suggested as flavor contributors. Proteins may contribute to the overall flavor, as a well as nonprotein nitrogenous substances such as nucleotides, which are well-known flavor components in other foods. Altamura and others (1967) identified some unusual amino acids, which they suggested could be of importance in mushroom flavor, especially upon heating.

Any form of processing of fresh mushrooms can be expected to alter the overall composition of the volatile components, many of which are chemically reactive. New compounds are formed during cooking of mushrooms. Picardi and Issenberg (1973)

found that the main difference in the volatiles of *A. bisporus* observed after cooking was an increase in the content of 1-octene-3-ol. Apart from an increase in 1-octene-3-one after cooking, Card and Avisse (1977) found that benzaldehyde and 3-octanone concentrations increased, while furfural and methyl furfural were newly formed. Various methods of drying mushrooms have been found to result in major losses (as much as 90%) of 1-octene-3-ol (Dijkstra 1976).

The taste of edible mushrooms is primarily due to the presence of nonvolatile compounds such as several small water-soluble substances, including 5′-nucleotides, free amino acids, soluble sugars, and polyols (Litchfield 1967). The taste components of mushrooms, including fruit bodies and mycelia, have been extensively studied, and their equivalent umami concentrations (EUCs) were calculated (Mau 2005). The profile of taste components in canned mushrooms has also been investigated by Chiang and others (2006a).

Tsai and others (2007) reported that mannitol was the major soluble sugar alcohol in *A. bisporus*, and its content increased dramatically with maturation from 157 to 260 mg/g. The content of total free amino acids was in the range of 48.8–64.2 mg/g; it peaked and then decreased significantly with maturation. The content of monosodium glutamate (MSG)-like components was in the range of 10.6–13.5 mg/g and was similar to that of other sweet components (11.4–14.3 mg/g) but was lower than those of bitter components (19.7–26.9 mg/g). Contents of total 5′-nucleotides fluctuated in the range of 6.59–8.14 mg/g. The contents of flavor 5′-nucleotides were higher in mushrooms harvested when they are really small and decreased with maturation. EUC values were higher in matured mushrooms. *Agaricus* mushrooms possessed a highly intense umami taste (Tsai et al. 2007).

Interestingly, carbohydrate and fat contents increased with maturation while protein content decreased. Crude fiber content peaked at stage 3 and greatly decreased when fully mature. However, the content of reducing sugar was in the range of 13.9–15.9%. The difference between carbohydrate and reducing sugar contents is the content of soluble polysaccharides, which were thought to be biologically active substances in mushrooms (Wasser and Weis 1999), and the amount of the soluble polysaccharides increased by maturation from 23.4% to 33.6% (Tsai et al. 2007).

Nonvolatile Compounds

The taste of edible mushrooms is primarily due to the presence of several small water-soluble substances, including 5′-nucleotides, free amino acids, and soluble sugars and sugar alcohols (polyols) (Litchfield 1967). The good taste provided by mushrooms is the umami taste, also called palatable taste or the perception of satisfaction, which is an overall food flavor sensation induced or enhanced by MSG (Yamaguchi 1979). Because the second functionality of foods is their taste properties, Mau (2005) calculated the EUCs of mushrooms based on their contents of nonvolatile components. The EUC value is the concentration of MSG equivalent to the umami intensity given by a mixture of MSG and 5′-nucleotide (Yamaguchi 1979). Among the fruit bodies studied, common mushrooms and paddy straw mushrooms were the two mushrooms with the most umami taste (Mau 2005).

Common mushrooms (*A. bisporus*) are usually harvested at a veil intact stage all around the world. However, in Europe, mushrooms are harvested at veil-opened and gills-exposed stages. In addition to cultural diversity, the divergence might be

due to the difference in flavor preference. In fact, no remarkable difference in the volatile components, especially 1-octene-3-ol, was found in mushrooms harvested at different stages of maturity (Mau et al. 1993). However, the profiles of nonvolatile components of mushrooms harvested at different stages of maturity are unknown.

Soluble Sugars

The high content of sugars and polyols in mushroom contributed a sweet taste (Litchfield 1967) and gave rise to a moderately sweet taste perception. Chen (1986) found that mannitol was the taste-active component in mushroom sugar alcohols. However, mannitol was not found in the mycelia of common mushrooms (Chang et al. 2001; Hwang and Mau 1997). Soluble sugars and arabitol, glucose, and trehalose in three mushroom mycelia exceeded 10% with glucose > arabitol > trehalose (Chang et al. 2001). Mannitol and trehalose are found in the fruit bodies of common mushrooms (Bano and Rajarathnam 1988; Hammond and Nichols 1976; Mau et al. 1997). Later on, Tsai and others (2007) investigated the nonvolatile compounds in fresh mushroom (*A. bisporus*) at different stages of maturity. They found that mannitol was the major soluble sugar alcohol in fresh mushrooms, and its content increased dramatically with maturation from 157 to 260 mg/g. Glucose was the second highest, and its content was in the range of 17.6–28.1 mg/g, but was almost absent in the least mature and the most mature stages. Generally, the amount of the total sugars and polyols increased with maturation. Tseng and Mau (1999) found that the total sugar and polyol content of *A. bisporus* was lower than that of *Volvariella volvacea* (Mau et al. 1997) but was higher than *Ganoderma* spp. (Mau et al. 2001a), *Agrocybe cylindracea* (Mau and Tseng 1998), *Auricularia* spp., and *Tremella fuciformis* (Mau et al. 1998b), and *Pleurotus eryngii* (Mau et al. 1998a).

Tsai and others (2008) reported that arabitol, myo-inositol, mannitol, and trehalose were detected in *A. cylindracea*, *Agaricus blazei*, and *B. edulis* mushrooms, whereas glucose was not found in *B. edulis*. The contents of total soluble sugars and polyols in these three mushrooms ranged from 150.33 to 225.08 mg/g, and in the descending order of mannitol and trehalose, which were the major mushroom polyol and sugar, respectively (Bano and Rajarathnam 1988; Hammond and Nichols 1976; Mau et al. 1997). As compared with other mushrooms, the contents of total soluble sugars or polyols were found to be 349–457 mg/g in *V. volvacea* (Mau et al. 1997), 205–320 mg/g in *A. bisporus* (Tseng and Mau 1999), 98.7–316.4 mg/g in *Auricularia* spp. and *T. fuciformis* (Mau and Tseng 1998), and 169 mg/g in *Pleurotus citrinopileatus* (Huang 2003).

Free Amino Acids

Chen (1986) conducted a series of sensory evaluations on synthetic mushroom extracts prepared by omitting and adding soluble components and found that alanine, glycine and threonine (sweet), and aspartic and glutamic acids (MSG-like) were taste-active amino acids in common mushrooms, whereas none of the bitter components was found to be taste active. The bitterness from the bitter components in the three mushrooms could probably be masked by the sweetness from sweet components and mainly the high amount of soluble sugars and polyols. Therefore, MSG-like and sweet components would be responsible for the natural taste of these

three mushrooms (Chang et al. 2001). Aspartic acid and glutamic acid are MSG-like components, which give the most typical mushroom taste, that is, the umami taste (or palatable taste) (Komata 1969).

The MSG-like taste comes from MSG and from aspartic and glutamic acid; the sweetness comes from alanine, glycine, serine, and threonine; the bitterness comes from arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine; and the tastelessness comes from lysine and tyrosine. With regard to the contents of MSG-like components in other mushrooms, including common mushrooms, their contents were: 22.67–47.12 mg/g (Tseng and Mau 1999); in *L. edodes*, 3.75–9.06 mg/g (Lin 1999); and in *P. citrinopileatus*, 16.12 mg/g (Huang 2003). Evidently, these three mushrooms possessed a low content of MSG-like components. Yang and others (2001) found that contents of MSG-like components in several commercial mushrooms, including *L. edodes*, *Flammulina velutipes* strain white, *Pleurotus cystidiosus*, and *Pleurotus ostreatus*, ranged from 0.84 to 1.93 mg/g. Yang and others (2001) also found that that in the yellow strain of *F. velutipes*, the content was 7.06 mg/g. However, Mau and others (2001) found that contents of MSG-like components in medicinal mushrooms, including *Ganoderma lucidum*, *G. tsugae*, and *Coriolus versicolor*, were in the range of 0.17–0.50 mg/g. Mau and Tseng (1998) reported that the contents of MSG-like components in three strains of *A. cylindracea* ranged from 10.85 to 11.89 mg/g, and it was found to be higher to that of *A. cylindracea*.

Flavor 5′-Nucleotides

The flavor 5′-nucleotides are 5′-adenosine monophosphate (5′-AMP), 5′-cytosine monophosphate (5′-CMP), 5′-guanosine monophosphate (5′-GMP), 5′-inosine monophosphate (5′-IMP), 5′-uridine monophosphate (5′-UMP), and 5′-xanthosine monophosphate (5′-XMP). 5′-GMP is responsible for the meaty flavor, and is a flavor enhancer, which is much stronger than MSG (Litchfield 1967). MSG-like compounds together with flavor 5′ nucleotides might give the synergistic effect and might increase the umami taste of mushrooms (Yamaguchi et al. 1971). Obviously, the amount of these contents changes the strength of the umami or the palatable taste of mushrooms (Chen 1986).

The flavor 5′-nucleotides, which also gave the umami or palatable taste, were found to be 5′-GMP, 5′-IMP, and 5′-XMP (Chen 1986). The contents of the flavor 5′-nucleotides were found to be 4.19–6.13 mg/g in *A. bisporus* (Tseng and Mau 1999), 1.63–4.89 mg/g in *P. eryngii* (Mau et al. 1998), 1.51 mg/g in *P. citrinopileatus* (Huang 2003), and 1.47 mg/g in *Hypsizygus marmoreus* (Lee 2003). Yang and others (2001) have also reported that contents of flavor 5′-nucleotides in several commercial mushrooms, including *F. velutipes* (white strain), *P. cystidiosus*, and *P. ostreatus*, ranged from 5.52 to 8.60 mg/g. Mau and others (2001) found that contents of flavor 5′-nucleotides in *Dictyophora indusiata*, *Grifola frondosa*, *Hericum erinaceus*, and *Tricholoma giganteum* were 9.04, 0.64, 0.62, and 13.6 mg/g, respectively. They divided the contents of MSG-like components into three ranges: low (<5 mg/g), middle (5–20 mg/g), and high (>20 mg/g).

A few other studies have reported the contents of MSG-like components to be 22.67–47.12 mg/g dry weight in common mushrooms (*A. bisporus*) (Tseng and Mau 1999), 11.20–26.21 mg/g in paddy straw mushrooms (*V. volvacea*) (Mau et al. 1997), 10.85–11.89 mg/g in black poplar mushrooms (*A. cylindracea*) (Mau and Tseng

1998), 3.75–9.06 mg/g in shiitake *(L. edodes)* (Lin 1988), 1.01–1.77 mg/g in king oyster mushrooms (*P. eryngii*) (Mau et al. 1998a), and 0.05–0.34 mg/g in ear mushrooms (*Auricularia* spp. and *T. fuciformis*) (Mau et al. 1998b).

Fatty Acids

The constituents of lipids in the cultivated mushroom *A. bisporus* have been investigated quite extensively (Weete 1980). These include C_{12}–C_{20} even-numbered fatty acids (Holtz and Schisler 1971; Prostenik et al. 1978; Weete et al. 1985) and C_{16}–C_{24} hydroxy fatty acids (Prostenik et al. 1978), but with oleic, linoleic, and palmitic acids predominating. These fatty acids may exist in their free form or as conjugated to other lipid constituents. Byrne and Brennan (1975) have reported on levels of palmitic, stearic, and oleic acids in the free form, and Stancher and others (1992) expanded the observed range of free and bound fatty acids to include C_8 and C_{13}–C_{17} odd-numbered acids. A preliminary study was reported by Hugues (1962), who identified 10 fatty acids, among which LA (18:2) varied from 63% to 74%. The chief unsaturated fatty acid of mushroom lipids, LA, is the precursor of the mushroom alcohol (1-octene-3-ol) (Grosch and Wurzenberger 1984; Mau et al. 1992; Tressl et al. 1982; Wurzenberger and Grosch 1982). This alcohol, together with the two associated C_8 ketones (1-octene-3-one and 3-octanone), constitutes the main group of volatiles that are considered to be the major contributors of the characteristic mushroom flavor (Cronin and Ward 1971; Maga 1981a,b; Pyysalo 1976).

MUSHROOM FLAVOR ANALYTICAL METHODOLOGY

Several analytical techniques are applied to identify and quantitatively estimate the flavor components present in mushrooms, which are responsible for flavor and off-flavors, as well as to identify compounds, which may be toxic or unique to the species of mushroom. Santford and others (1999) studied the volatile organic compounds to compare and to quantify the volatile organics contained within different kinds of edible (cultivated or wild) mushrooms (shiitake, *A. bisporus*, *A. compestris*, *Lactarius*, and puffball *Calvatia*). The compounds were analyzed by using a purge-and-trap technique (P&T), followed by trapping on to an adsorbent resin and subsequent analysis by thermal desorption–gas chromatography–mass spectrometry (TD-GC-MS). These mushrooms were found to contain numerous middle-chain aliphatic alcohols, aldehydes, and ketones, which are believed to be the degradation products of fatty acids (Kawabe and Morita 1993). The compounds 3-methyl-butyraldehyde and 2,6-bis (1,1-dimethyl ethyl)-phenol were identified in each of the mushrooms with the exception of *Calvatia*. Phenylacetaldehyde, which appears to be an important flavor compound, was present in each of the mushrooms except in *A. bisporus*. The flavor compound benzaldehyde was also detected in portabella as well as in *A. bisporus*. *Lactarius* sp. contained a high concentration of the aliphatic compound 1-octene-3-ol. 3-Octanone was also found in the shiitake, portabella, and *A. bisporus* mushrooms. The presence of 1-octene-3-ol and 3-octanone suggests that the activity of lipoxygenase and hydroperoxide lyase-producing C_8 compounds from LA was stronger in these mushrooms (Combet et al. 2006; Kawabe and Morita 1993).

Zawirska-Wojtasiak (2004) investigated the characteristic enantiomeric ratio of 1-octene-3-ol in various species of edible mushrooms, including *A. bisporus*, *P. ostreatus*, *Hericium erinaceum*, *Pholiota ameco*, and *L. edodes*; and in wild-growing mushrooms, including *B. edulis*, *Xerocomus badius*, and *Macrolepiota procera* by using micro-distillation–extraction and GC capillary column. The optical purity of (R)-O-1-octene-3-ol in all the species was very high, being the highest in *A. bisporus* (over 98.5%) and the lowest in *X. badius* (over 82.1%).

Sunesson and others (1995) studied the volatile compounds produced by five fungal species, *Aspergillus versicolor*, *Penicillium commune*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, and *Phialophora fastigiata*, which were cultivated in two media, malt extract agar and dichloran glycerol agar. Air samples from the cultures were analyzed by TD-GC connected with an MS. For the isolation of the volatiles from mushrooms and mushroom-like products, a micro-distillation–extraction procedure followed by GC was also applied (Sunesson et al. 1995). Solid phase microextraction has been successfully used for the separation of volatile organic compounds in mushrooms and truffles (Diaz et al. 2002; Pelusio et al. 1995).

Although the number of the reports is very limited, nonvolatiles compounds (soluble sugars, free amino acids, and fatty acids) have also been studied. A number of researchers have analyzed soluble sugars by the method of Ajlouni and others (1995) by using high-performance liquid chromatography (HPLC) with a selective column separation (Chiang et al. 2006a,b; Mau et al. 2001a,b; Tsai et al. 2007). Mondal and others (2004) analyzed soluble sugars from *Termitomyces eurhizus* by a gas-liquid chromatography (GLC) MS analysis followed by nuclear magnetic resonance (NMR) (1H, 13C, 2D-COSY, TOCSY, and NOESY) spectroscopy. A heteropolysaccharide composed of D-glucose, D-mannose, D-galactose, and D-galacturonic acid was detected.

Free amino acids in mushrooms were analyzed by the HPLC with the same system as used for sugars and polyols but equipped with a fluorescence detector (Mau et al. 2001a,b; Tsai et al. 2007, 2008). Trans-esterification of fatty acids in mushrooms was studied by Kavishree and others (2008). Fatty acid methyl esters were prepared and analyzed by GC equipped with hydrogen flame ionization detector (FID). Samples were separated by using a coated silica capillary column (Zawirska-Wojtasiak 2004). Flavor 5′ nucleotides were analyzed by the HPLC with the same system used for sugars and polyols except that it was equipped with a UV detector and a Prodigy 5 ODS-2 column (Mau et al. 2001b; Tsai et al. 2007, 2008).

SENSORY EVALUATION OF MUSHROOMS

Mushrooms have a wide scope of sensorial variation; the majority are not cultivated and are generally collected from the native environment. With hundreds of locally known varieties, nearly every species of mushroom has its own unique flavor. Mushroom colors have been reported to range from white to very dark brown (Antmann et al. 2008; Ares et al. 2006). Generally, the flavor gets intense as the color gets darker. In local markets, shiitake, portabella, and morels are becoming more available and easy to cultivate. The button of the shiitake mushroom has a smooth and elastic texture and solid flesh and is high in dry matter content compared with other varieties. The flavor is woody but moderate resembling that of typical forest

mushrooms while being stronger in taste. Portabella mushrooms are rounded, with black, visible gills under the cap. As they age, their color darkens and their meaty flavor gets more intense. Morels have short stems and a closed chimney-like cap. They are varied in color (tan, yellow, or black) and have a nutty or sweet flavor; the flavor gets more intense with color. Their texture is noticeable during consumption. Chanterelle-type mushrooms are light brown and capped similar to flowers and have a fresh, fruity flavor. Funnel-shaped chanterelles (golden or white) are among the most well-known edible mushrooms. They have a meaty texture and a pepper-like aroma. Black trumpet, one of the well-known chanterelles, is black and comparably very small. Its taste is mild and does not have distinctive odor. Maitake-type mushrooms come in various brown colors and taste woody. Beech mushroom are white or cream colored and leave a mild sugary aroma in the mouth. Button mushrooms and portabella mushrooms are light tan colored. Button mushrooms have a rounded cap with no obvious colored gills. Hedgehog mushrooms are distinguished for their toothlike gills, usually tan colored, and they have a crunchy texture and nutty flavor. Although rarely found, saffron milk mushrooms have an orange sticky cap; their flesh exudes a milky liquid when cut. Shiny, orange-red capped amanitas are rare but are very popular. Rhizopogon mushrooms are white or yellow, and they have a sponge-like but firm structure (Aguirre et al. 2008).

MUSHROOM FLAVOR IN PROCESSED FOODS

Among different types of wild mushrooms that are regularly harvested and commercialized, only a fraction of a large variety of fungi are currently cultivated and sold commercially. Mushrooms comprise one of the favorite dishes in Middle Eastern countries. Some varieties are still not commercially cultivated because cultivation is a challenge. Local varieties are only known to people who live in that rural area. These mushrooms are collected in season and are generally consumed as fresh. Fresh mushrooms are rarely consumed raw because some are locally recognized for their allergic reactions. Fresh mushrooms are grilled, roasted, stewed, or lightly fried with tomato juice in a pan or sliced and sauced in baked goods and meat products (Cliffe-Byrnes and O-Beirne 2008).

Mushrooms are highly perishable in nature and last only for a few days in cold storage. Obviously, the shelf life of mushrooms in the fresh form is very short. Due to a short shelf life, only about 45% of mushrooms produced are consumed fresh; the rest are processed as canned (50%) or dehydrated (5%). Hence, mushrooms are traded in the world market mostly in the processed form (Kar and Gupta 2003; Wang and Sheng 2004).

Mushrooms have different kinds of enzymes with high activity; therefore, a fresh mushroom must be processed immediately after picking; otherwise it will quickly lose flavor and quality (Liu et al. 2004). One way of adding commercial value to fresh mushrooms is by drying mushrooms for sale in the off-season. Drying whole or sliced mushrooms is the oldest and most commonly used preservation method in the Middle Eastern countries. Dehydrated mushrooms are easily available in the local markets. Fresh mushrooms shrink shortly after collection and change color, and in order to preserve for consumption later in the year, a number of traditional techniques (such as drying, pickling, and salting) are applied. Drying can be achieved

by placing the mushrooms in sunlight, which further decreases their vitamin content, and the dried mushroom yield is as low as 14%. The dried mushrooms must be rehydrated by soaking in water or sautéed in tomato juice before consumption. The soaking liquid can add flavor as well, but should be strained before using to remove any sand. Often, dried mushrooms are used in small quantities to add flavor to stewed meat.

Mushrooms can also be found in the market as flash-blanched and frozen, a process in which they retain most of their taste, aroma, texture, and nutrients, or as marinated; the two styles have become very popular among consumers. In recent years, more innovative applications in commercial processing technologies, such as freezing, freeze-drying, canning, pickling, flash-blanched freezing, and vacuum-drying have been applied, but due to cost, these products are not so popular in the Middle Eastern countries. These are used on the basis of their value in market demand and end use. Although canning in vinegar or brine constitutes the major preservation technique in international trade, due to the changes in aroma, canned mushrooms are not very popular among Middle Eastern consumers. The common processing method for mushrooms, at present, is canning, but the flavor and quality are seriously impaired during this process, leading to poor acceptability by the consumers (Zhimin et al. 2001).

In steeping preservation, mushrooms are preserved by steeping in a solution having 10–12% salt, whereas the radiation preservation of mushrooms is done by radiating with gamma rays at the dose of 3 kGy to stop postharvest growth and discoloration/deterioration (Lescano 1994).

NUTRITIVE VALUE OF MUSHROOMS

Edible mushrooms compare favorably well with most vegtables. Mushrooms are rich in many B-complex vitamins and contain reasonable amounts of vitamin A, D, K, and C. They also include many other nutrients such as potassium, copper, iron, LA, folate, and a few trace elements. Mushrooms are mainly water (91%) but also supply proteins (3.15%), carbohydrates (2.4%), fiber (1%), and fat (0.4%) apart from vitamins and minerals (Hayes and Haddad 1976). Mushroom proteins contain all the essential amino acids required by humans (Chang 1980). The protein content of certain species of mushrooms (especially *Agaricus*) may range from 19% to 38% on a dry basis (Braaksma and Schaap 1996). The protein content measurement gets complicated by the presence of nonprotein nitrogen-containing compounds such as N-acetyl glucosamine. The protein quality of four types of edible mushrooms, *Terfezia claveryi*, *P. ostreatus*, *Tricholoma terreum*, and *Agaricus macrosporus*, in terms of protein efficiency ratio and net protein utilization, has been reported to be lower than casein controls (Dabbour and Takruri 2002).

As mushrooms do not contain starch, they form an excellent food for diabetic persons (FAO 1972). Mushrooms, being low in fat (and with no cholesterol), high in fiber, and rich in minerals (with an especially high potassium:sodium ratio) and B-complex vitamins, are the ideal food of choice for persons suffering from obesity and heart disease.

Mushrooms contain various polyphenolic compounds recognized as excellent antioxidants due to their ability to scavenge free radicals by single-electron transfer

(Hirano et al. 2001). Some common edible mushrooms, which are widely consumed in the Asian culture, have currently been found to possess antioxidant activity, which is well correlated with their total phenolic content (Barros et al. 2007; Cheung and Cheung 2005; Cheung et al. 2003; Lo and Cheung 2005; Mau et al. 2002, 2004; Yang et al. 2002; Yen and Hung 2000). Abulude and others (2001) have analyzed 10 varieties of mushrooms found in Nigeria for their phytate, trace minerals, and phosphorus contents. They found iron to be the most abundant mineral in all the 10 varieties that they analyzed. In another study (Akindahuns and Oyetayo 2005), potassium was reported to be the most abundant element in *Pleurotus tuber-regium* (3.3 mg/g, dry basis). They found crude protein content to be highest (13.8%, dry basis) in the cap than in any other parts of the mushrooms.

There are many species of mushrooms that are used in folk medicine for thousands of years and now have become a topic of intense study by medical researchers. In folk medicine, some believed that mushrooms had properties that could produce superhuman strength and could lead the soul to the realm of the gods. Many types of mushrooms such as maitake, shiitake, chaga, and reishi are being studied for their anticancer, antiviral, or immune-boosting properties (Chang 1996; Chang and Miles 2004).

TRANSGENICITY AND GENETIC ENGINEERING FOR MUSHROOM PRODUCTION

As consumers increasingly show their interest in alternative flavors, there is a great amount of laboratory and industrial research in isolating newer flavor compounds to produce varieties of mushrooms with thousands of flavor components. Initially, the lack of an efficient gene transformation system for manipulation constrained transgenic studies in mushrooms. Recently, *Agrobacterium*-mediated transformation and particle bombardment techniques have also been applied (Mikosch et al. 2001). The developments of gene transfer techniques in recent years enable the exploration of genetic solutions to problems confronting mushroom production.

By using molecular methodologies, molecular, physiological, and biochemical processes essential in mushroom production will be better understood (Stoop and Mooibroek 1998). Understanding the role of key genes involved in pathogenesis, crop agronomy, pre- and postharvest quality, and substrate utilization will probably be given priority in mushroom research. Genetic manipulations are mostly expected to concentrate on the improvement of agronomic and pre- and postharvest quality properties such as yield, size, enhanced shelf life, consumer quality aspects (color, flavor, and texture), prevention of brown discoloration, resistance against heat and water stress, carbon and nitrogen substrate utilization, and fruit body development (Stoop and Mooibroek 1998). In order to prevent mushroom browning, a quality problem, tyrosinase gene was isolated and introduced in antisense orientation, a method called gene silencing.

Furthermore, developments in transformation systems for mushrooms based on synthetic genes provided further ground for research to use the culturing advantage of mushrooms as bioreactors for the mass biomanufacturing of commercially valuable heterologous proteins or useful human drugs. High yield containment, facile reproduction, low cost, easy scaling up, and a generally recognized as safe (GRAS)

status make mushrooms favorable. It is possible to completely sterilize rooms after harvest to prevent environmental contamination, and there is no limit to the number of crops obtained in a year. The transformed mushroom chromosomes facilitate the production of human pharmaceutical products including vaccines and antibodies. For example, cytokine hormone and human milk proteins have been expressed in mushrooms. Recent studies are aimed at the production of genetically modified mushrooms to deal with human nutritional deficiencies such as omega-3 fatty acids by inserting a desaturase gene, producing human interleukin, and a human milk protein, beta-casein.

CONCLUSIONS

Mushroom, being a low calorie, no-cholesterol, low-fat, low-carbohydrate food, is an ideal choice for all persons who wish to shed extra pounds. Although mushrooms are valued for their typical meat-like texture and delicate flavor, they also supply a host of nutrients such as proteins, vitamins, minerals, and fiber. Other than these usual nutrients, mushrooms need to be investigated for their other minor but important constituents such as polyphenols and N-acetyl glucosamine for their health-promoting properties, if any.

Many kinds of flavors are used in the food industry, and there is a demand for new and improved natural flavors. Introduction of locally consumed, wild edible mushroom varieties for commercial production may extend the horizon of variability of mushroom aroma in processed and semi-processed food products. Some commercially harvested wild edible species are difficult to cultivate, and there are some mushroom species that have not yet been successfully cultivated so far. In order to conserve biodiversity, more research and development efforts should be directed for cultivation of such species on a commercial scale.

REFERENCES

Abulude FO, Akajagbor C, Dafiewhare BH. 2001. Distribution of trace minerals, phosphorus and phytate in some varieties of mushrooms found in Nigeria. *Adv Food Sci* 23(3): 113–16.

Aguirre L, Frias JM, Barry-Ryan C, Grogan H. 2008. Assessing the effect of product variability on the management of the quality of mushrooms (*Agaricus bisporus*). *Postharvest Biol Technol* 49(2):247–54.

Ajlouni SO, Beelman RB, Thompson DB, Mau JL. 1995. Changes in soluble sugars in various tissues of cultivated mushrooms, *Agaricus bisporus*, during postharvest storage. In Charalambous G (ed.), *Food Flavors*. Amsterdam: Elsevier Science, pp. 1865–80.

Akindahuns AA, Oyetayo FL. 2005. Nutrient and antinutrient distribution of edible mushroom, *Pleurotus tuber-regium* (fries) singer. *LWT-Food Sci Technol* 39(5):548–53.

Altamura MR, Robbins FM, Andreotti RE, Long L Jr., Hasselstrom T. 1967. Mushroom ninhydrin-positive compounds. Amino acids, related compounds, and other nitrogenous substances found in cultivated mushroom, *Agaricus campestris*. *J Agric Food Chem* 15:1040–3.

Antmann G, Ares G, Lema P, Lareo C. 2008. Influence of modified atmosphere packaging on sensory quality of shiitake mushrooms. *Postharvest Biol Technol* 41(2):164–70.

Ares G, Parentelli C, Gambaro A, Lareo C, Lema P. 2006. Sensory shelf life of shiitake mushrooms stored under passive modified atmosphere. *Postharvest Biol Technol* 41(2):191–7.

Bano Z, Rajarathnam S. 1988. *Pleurotus* mushrooms part II. Chemical composition, nutritional value, post-harvest physiology, preservation and role as human foods. *CRC Crit Rev Food Sci Nutr* 27(2):87–158.

Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem* 103(2):413–9.

Baytop T. 1994. *Dictionary of Vernacular Names of Wild Plants of Turkey*. Turkish Language Association, Publ. No. 578, Ankara.

Blech RZ. 2002. A fungible feast, mushrooms in Halacha. *MK Vaad News and Views*, 3(4):2.

Braaksma A, Schaap DJ. 1996. Protein analysis of the common mushroom *Agaricus bisporus*. *Postharvest Biol Technol* 7(1–2):119–27.

Byrne PFS, Brennan PJ. 1975. Lipids of *Agaricus bisporus*. *J Gen Microbiol* 89:245–55.

Card A, Avisse C. 1977. Comparative study of the aroma of raw and cooked mushrooms (*Agaricus bisporus* L.). *Ann Technol Agric* 27:287–93.

Chang HL, Chao GR, Chen CC, Mau JL. 2001. Non-volatile taste components of *Agaricus blazei*, *Antrodia comphorata* and *Cordyceps militaris mycelia*. *Food Chem* 74:203–7.

Chang R. 1996. Functional properties of edible mushrooms. *Nutr Rev* 54:S91–3.

Chang S, Miles PG (eds.). 2004. *Mushrooms, Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*, 2nd Ed. Boca Raton, FL: CRC Press.

Chang ST. 1980. Mushrooms as human food. *Bioscience* 30:399–401.

Chang ST. 1990. Mushrooms as food. *Food Lab News* 21:7–8.

Chen CC, Chen SD, Chen JJ, Wu CM. 1984. Effects of pH value on the formation of volatiles of shiitake (*Lentinus edodes*) and edible mushroom. *J Agric Food Chem* 32:999–1001.

Chen CC, Wu CM. 1984. Studies on enzyme reduction of 1-octen-3-ol in mushrooms (*Agaricus bisporus*). *J Agric Food Chem* 49:1208–9.

Chen HK. 1986. Studies on the characteristics of taste-active components in mushroom concentrate and its powderization. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.

Cheung LM, Cheung PCK. 2005. Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chem* 89:403–9.

Cheung LM, Cheung PCK, Ooi VEC. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem* 81:249–55.

Chiang PD, Yen CT, Mau JL. 2006a. Non-volatile taste components of canned mushrooms. *Food Chem* 97(3):431–7.

Chiang PD, Yen CT, Mau JL. 2006b. Non-volatile taste components of various broth cubes. *Food Chem* 101(3):932–7.

Cliffe-Byrnes V, O-Beirne D. 2008. Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushrooms (*Agaricus bisporus*). *Postharvest Biol Technol* 48(2):283–94.

Combet E, Henderson J, Eastwood DC, Burton KS. 2006. Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. *Mycoscience* 47(6):1618–2545.

Cronin DA, Ward MK. 1971. The characterization of some mushroom volatiles. *J Sci Food Agric* 22:477–9.

Cruz C, Noel-Subrville C, Montury M. 1997. Fatty acids content and some flavor compounds release in two strains of *Agaricus bisporus*, according to three stages of development. *J Agric Food Chem* 45:64–7.

Dabbour IR, Takruri HR. 2002. Protein quality of four edible mushrooms found in Jordan. *Plant Food Hum Nutr* 57(1):1–11.

Diaz P, Senorans FJ, Reglero G, Ibanez E. 2002. Truffle aroma analysis by headspace solid phase microextraction. *J Agric Food Chem* 50:6468–72.

Dijkstra FY. 1976. Studies on mushroom flavours 3. Some flavour compounds in fresh, canned and dried edible mushrooms. *Eur Food Res Technol* 160(4):401–5.

Dijkstra FY, Wiken TO. 1976. Studies on mushroom flavors, part 1. Organoleptic significance of constituents of the cultivated mushroom *Agaricus bisporus*. *Z Lebensm Unters Forsch* 160:255–62.

FAO. 1972. *Food Composition Table for Use in East Asia (Part-I)*. Rome: Food Policy and Nutrition Division, FAO.

Fischer KH, Grosch W. 1987. Volatile compounds of importance in the aroma of mushrooms (*Psalliota bispora*). *Lebenson Wiss Technol* 20:233–6.

Flegg PB, Spencer DM, Wood DA (eds.). 1985. Food value and chemical composition. In *The Biology and Technology of the Cultivated Mushroom*. Chichester, NY: John Wiley and Sons, pp. 225–7.

Grosch W, Wurzenberger M. 1984. Enzymic formation of 1-octen-3-ol in mushroom. *Dev Food Sci* 10:253–9.

Guha AK, Banerjee AB. 1970. Effect of different nitrogenous compounds on the submerged production of *Agaricus campestris* mycelium. *J Food Sci Technol* 7:23–5.

Hammond JBW, Nichols R. 1976. Carbohydrate metabolism in *Agaricus bisporus* (Lange) Imbach: Change on soluble carbohydrate during growth of mycelium and sporephore. *J Gen Microbiol* 93:309–20.

Hayes WA, Haddad N. 1976. The food value of cultivated mushrooms and its importance to the mushroom industry. *Mushroom J* 40:104–10.

Heine P. 2004. *Food Culture in the Near East, Middle East, and North Africa*. Westport, CN: Greenwood Press.

Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. 2001. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *J Nutr Sci Vitaminol (Tokyo)* 47:357–62.

Holtz RB, Schisler LC. 1971. Lipid metabolism of *Agaricus bisporus* (Lange) sing. I. Analysis of sporophore and mycelial lipids. *Lipids* 7:176–80.

Huang GW. 2003. Taste quality and antioxidant and antimutagenic properties of *Pleurotus citrinopileatus*. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.

Hugues DH. 1962. Preliminary characterization of the cultivated mushroom, *Agaricus compestris*. *J Mushroom Sci* 5:540–6.

Hwang SJ, Mau JL. 1997. Quality evaluation of *Agaricus bisporus* mycelia. *Food Sci* 24:44–55.

Kar A, Gupta DK. 2003. Air drying of osmosed button mushrooms. *J Food Sci Technol* 40(1):23–7.

Kavishree S, Hemavathy J, Lokesh BR, Shashirekha MN, Rajarathnam S. 2008. Fat and fatty acids of Indian edible mushrooms. *Food Chem* 106(2):597–602.

Kawabe T, Morita H. 1993. Volatile components in culture fluid of *Polyporus tuberaster*. *J Agric Food Chem* 41(4):637–40.

Komata Y. 1969. The taste and constituents of foods. *Nippon Shokuhin Kogyo Gakkaishi* 3:2–6.

Lee YL. 2003. Taste quality, antioxidant and antimutagenic properties of *Hypsizigus marmoreus* fruit bodies. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.

Lescano G. 1994. Extension of mushroom (*Agaricus bisporus*) shelf life by gamma radiation. *Postharvest Biol Technol* 4(3):255–60.

Lin SY. 1988. Studies on the characteristics of taste-active components in shiitake mushroom and the powderization of the concentrate. Master's thesis, National Chung-Hsing University, Taichung, Taiwan.

Lin SY. 1999. Evaluation of taste quality and antioxidant properties of edible and medicinal mushrooms. Master's Thesis, National Chung-Hsing University, Taichung, Taiwan.

Litchfield JH. 1967. Morel mushroom mycelium as a food flavoring material. *Biotechnol Bioeng* 9:289–304.

Liu SM, Zhou JH, Zeng YL, Ouyang XL. 2004. The enhancement and encapsulation of *Agaricus bisporus* flavor. *J Food Eng* 65(3):391–6.

Lo KM, Cheung PCK. 2005. Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. *Food Chem* 89:533–9.

Macleod AJ, Panchasara SD. 1983. Volatile aroma components, particularly glucosinolate products of cooked edible mushroom *Agaricus bisporus* and cooked dried mushrooms. *Phytochem* 22(3):705–9.

Maga JA. 1981a. Mushroom flavor. *J Agric Food Chem* 29:1–4.

Maga JA. 1981b. Mushroom flavor. *J Food Process Preserv* 5:95–101.

Mau JL. 2005. The umami taste of edible and medicinal mushrooms. *Int J Med Mushrooms* 7:113–19.

Mau JL, Beelman RB, Ziegler GR. 1992. 1-Octen-3-ol in the cultivated mushroom *Agaricus bisporus*. *J Food Sci* 57(3):704–6.

Mau JL, Beelman RB, Ziegler GR. 1993. Factors affecting 1-octen-3-ol in mushrooms at harvest postharvest storage. *J Food Sci* 57:704–6.

Mau JL, Chang CN, Huang SJ, Chen CC. 2004. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem* 87:111–18.

Mau JL, Chyau CC, Li JY, Tseng YH. 1997. Flavor compounds in straw mushrooms *Volvariella volvacea* harvested at different stages of maturity. *J Agric Food Chem* 45:4726–9.

Mau JL, Lin YP, Chen P-T, Wu YH, Peng JT. 1998a. Flavor compounds in king oyster mushrooms *Pleurotus eryngii*. *J Agric Food Chem* 46:4587–91.

Mau JL, Wu KT, Wu YH, Lin YP. 1998b. Nonvolatile taste components of ear mushrooms. *J Agric Food Chem* 46:4583–6.

Mau JL, Lin HC, Chen CC. 2001a. Non-volatile components of several medicinal mushrooms. *Food Res Int* 34:521–6.

Mau JL, Lin HC, Ma JT, Song SF. 2001b. Non-volatile taste components of several speciality mushrooms. *Food Chem* 73:461–6.

Mau JL, Lin HC, Song SF. 2002. Antioxidant properties of several speciality mushrooms. *Food Res Int* 35:519–26.

Mau JL, Tseng YH. 1998. Nonvolatile taste components of three strains of *Agrocybe cylindracea*. *J Agric Food Chem* 45:2071–4.

Mikosch TS, Lawrijssen B, Sonnenberg AS, van Griensven LJ. 2001. Transformation of the cultivated mushroom *Agaricus bisporus* (Lange) using T-DNA from *Agrobacterium tumefaciens*. *Curr Genet* 39:35–9.

Mondal S, Chakraborty I, Pramanik M, Rout D, Islam SS. 2004. Structural studies of water-soluble polysaccharides of an edible mushroom, *Termitomyces eurhizus*: A reinvestigation. *Carbohydr Res* 339:1135–40.

Mosandl A, Heusinger G, Gessner M. 1986. Analytical and sensory differentiation of 1-octen-3-ol enantiomers. *J Agric Food Chem* 34:119–122.

Oder N. 1990. Important edible and poisonous mushrooms of Turkey. Panel on Biological Diversity in Turkey, October 26, Ankara, Turkey.

Pant D, Reddy UG, Adholeya A. 2006. Cultivation of oyster mushrooms on wheat straw and bagasse substrate amended with distillery effluent. *World J Microbiol Biotechnol* 22(3):267–75.

Pellet PL, Shadarevian S. 1970. *Food Composition Tables for Use in the Middle East*, 2nd Ed. Beirut: American University of Beirut.

Pelusio F, Nilsson T, Montanarella L, Tilio R, Larsen B, Facchetti S, Madsen JO. 1995. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black truffle aroma. *J Agric Food Chem* 43:2138–43.

Picardi SM, Issenberg PJ. 1973. Investigation of some volatile constituent of mushroom (*Agaricus bisporus*): Changes which occur during heating. *J Agric Food Chem* 21:959–62.

Prostenik M, Burcar I, Castek A, Coscovic C, Golem J, Jandric Z. 1978. Lipids of higher fungi. III. The fatty acids and 2-hydroxy acids in same species of basidiomycetes. *Chem Phys Lipids* 22:97–103.

Pyysalo H. 1976. Identification of volatile compounds in seven edible fresh mushrooms. *Acta Chem Scand* B30:235–44.

Pyysalo H, Suihko M. 1976. Odour characterization and threshold values of some volatile compounds in fresh mushrooms. *Lebenson Wiss Technol* 9:3713.

Santford V, Overton J, Manura J. 1999. Determination of volatile organic compounds in mushrooms. http://www.sisweb.com/referenc/applnote/app-18-a.htm (accessed on February 14, 2008).

Shibuya T, Shimada S, Sakurai H, Kumagai H. 2005. Mechanism of inhibition of platelet aggregation by lenthionine, a flavor component from shiitake mushroom. IFT Annual Meeting, Presentation 54G-9, New Orleans, LA, July 15–20..

Stancher B, Procida G, Calabrese M. 1992. Characteristics of the most common mushrooms cultivated in Italy. IV. Lipids: Determination of the content of free and bound fatty acids. *Ind Aliment Italy* 31:744–50.

Stäuble EJ, Rast D. 1971. Leichtflüchtige Stoffe in *Agaricus bisporus*. *Experientia* 27:886–8.

Stoop JMH, Mooibroek H. 1998. Cloning and characterization of NADPH-dependent mannitol dehydrogenase from the button mushroom *Agaricus bisporus* and its expression in response to NaCl stress. *Appl Environ Microbiol* 64:4689–96.

Stoop JMH, Mooibroek H. 1999. Advances in genetic analysis and biotechnology of the cultivated button mushroom, *Agaricus bisporus*. *Appl Microbiol Biotechnol* 52:474–83.

Sunesson AL, Wouter HJ, Nilsson CA, Blomquist G, Andersson B, Carlson R. 1995. Identification of volatile metabolites from five fungal species cultivated on two media. *Appl Environ Microbiol* 61(8):2911–18.

Tehran Times. 2005. Iran's mushroom production increases by over 20%. In FAO Agriculture News, Sept. 8. http://agriculturenews.net/index.aspx?Type=Briefnews&ID=1699 (accessed on March 3, 2008).

Tewari RP. 2003. Cultivation of edible mushrooms. *Extension Bulletin No. 1, NRCM*. Chambaghat, Solan, India: Indian Council of Agricultural Research.

Tressl R, Bahri D, Engel KH. 1982. Formation of eight carbon and ten carbon components in mushrooms (*Agaricus compestris*). *J Agric Food Chem* 30:89–93.

Tsai SY, Tsai HL, Mau JL. 2008. Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. *Food Chem* 107:977–83.

Tsai SY, Wu TP, Huang SJ, Mau JL. 2007. Nonvolatile taste components of *Agaricus bisporus* harvested at different stages of maturity. *Food Chem* 103(4):1457–64.

Tseng YH, Mau JL. 1999. Contents of sugars, free amino acids and free 5′-nucleotides in mushrooms, *Agaricus bisporus*, during post-harvest storage. *J Sci Food Agric* 79:1519–23.

Venkateshwarlu G, Chandravadana MV, Tewari RP. 1999. Volatile flavour components of some edible mushrooms (Basidiomycetes). *Flavour Frag J* 14:191–4.

Wang J, Sheng KC. 2004. Modeling of multilayer far-infrared dryer. *Dry Technol* 22(4):809–20.

Wasowicz E. 1974. Identification of the volatile flavor compounds in mushrooms *Agaricus bisporus*. *Bull Acad Pol Sci* 22(3):143–51.

Wasser SP, Weis AL. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspective (review). *Int J Med Mushrooms* 1:31–62.

Weete JD. 1980. *Lipid Biochemistry of Fungi and Other Organisms*. New York: Plenum Press.

Weete JD, Furthe R, Haenseler E, Rast DM. 1985. Cellular and chitosomal lipids of *Agaricus bisporus* and *Mucor rousii*. *Canadian J Microbiol* 31:1120–6.

Wurzenberger M, Grosch W. 1982. The enzymic oxidative breakdown of linoleic acid in mushroom (*Psalliota bisporus*). *Z Lebensm Unters Forsch* 175:186–90.

Yamaguchi, S. 1979. The umami taste. In Boudreau JC (ed.), *Food Taste Chemistry ACS Symposium Series 115*. Washington, DC: American Chemical Society, pp. 33–51.

Yamaguchi S, Yoshikawa T, Ikeda S, Ninomiya T. 1971. Measurement of the relative taste intensity of some a-amino acid and 5′-nucleotides. *J Food Sci* 36:846–9.

Yang JH, Lin HC, Mau JL. 2001. Non-volatile taste components of several commercial mushrooms. *Food Chem* 72:465–71.

Yang JH, Lin HC, Mau JL. 2002. Antioxidant properties of several commercial mushrooms. *Food Chem* 77:229–35.

Yen GC, Hung CY. 2000. Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsiantsao (*Mesona procumbens* Hemsl.). *Food Res Int* 33:487–92.

Zawirska-Wojtasiak R. 2004. Optical purity of (R)-(–)-1-octene-3-ol in the aroma of various species of edible mushrooms. *Food Chem* 86(1):113–18.

Zawirska-Wojtasiak R. 2006. Chirality and the nature of food authenticity of aroma. *Acta Sci Polit Technol Alimenentarus* 5(1):21–36.

Zhimin L, Hong JC, Fang L. 2001. Influence of pretreatments and blanching treatments on the yield and color of canned mushrooms. *J Food Process Preserv* 25(6):381–8.

CHAPTER 47

Flavoring Compounds in Red Pepper Fruits (*Capsicum* genus) and Processed Products

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INTRODUCTION

The genus *Capsicum* includes several vegetal species widely cultivated in America, Asia, Africa, and in the Mediterranean countries. Pepper plants are native to America where the fruits represent a culinary tradition and a cultural symbol in some countries, being economically important because of the widespread consumption of diverse varieties.

The pepper plant (*Capsicum* genus) belongs to the Solanaceae, a large tropical family that includes potato, tomato, tobacco, and petunia. The *Capsicum* genus comprises 22 wild species and 5 domesticated species, *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens*. Although several hundred pepper pod types and cultivars are grown worldwide, the most economically important species in the world are *C. frutescens* and *C. annuum*. The fruits of these plants are used in the manufacture of selected commercial products known for their pungency and color, respectively. The application of those products improves the color, taste, and aroma of foodstuffs to which they are added, so that pepper products have found application in sauces, soups, processed meats, snacks, candies, soft drink, and alcoholic beverages.

Consequently, the overall quality of *capsicum* fruits arises from contribution of the different sensory properties color, taste, and aroma. Harmonization of such properties, especially the aroma, to achieve a quality product seems to be a complicated subject, considering the high number and variability of compounds responsible of those properties. Therefore, the parameters selected to determine the quality and economical value of *capsicum* fruits and processed products have been components responsible for color, carotenoids, and components responsible for pungency, capsaicinoids (Govindarajan 1986; Govindarajan et al. 1987), although vast research

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

has been focused on aroma to use this property as a parameter to discriminate quality fruits (Cremer and Eichner 2000; Guadayol et al. 1997; Luning et al. 1994b).

The interaction of taste, odor, and textural feeling when a food is ingested provides an overall sensation, which is defined as flavor. Flavor substances may be divided in two wide groups: compounds responsible for taste and compounds responsible for odors. In the context of flavor, it is clear that the chemistry of pungency and aroma of *capsicum* fruits and related products must be examined, although some biochemical processes and chemical modifications that affect the native carotenoid content (the coloring components) of fruits are also a source of aroma as will be described herein. In pungent *capsicum* varieties, despite the diversity of aroma compounds that can be found in fresh and processed *capsicum* fruits, the main contributors to flavor are capsaicinoids. These compounds provide the characteristic oral heat generated during the consumption of *capsicum* fruits, which has made them very popular, and has earlier attracted the interest of the food industry on using this particular flavoring property to provide or to enhance the taste of foodstuffs, achieving the interest of consumers on new savors and multicultural cuisine. *Capsicum* fruits are not the unique spices able to infer a pungent taste. Black pepper (*Piper nigrum*) contains piperine, piperanine, and piperylin, while ginger (*Zingiber officinale*) contains gingerol and shogaol. However, the relative pungency of capsaicinoids is 150- to 300-fold, considering piperine as reference.

CAPSAICINOIDS

Chemical Features, Biosynthesis, and Biodegradation of Capsaicinoids of Pepper Fruits

Capsaicinoids are a group of alkaloid compounds responsible for the pungent taste of *capsicum* fruits. Chemically, these compounds are amides of vanillyamine and carboxylic acids, with 9–11 carbon atoms (Bennett and Kirby 1968; Leete and Louden 1968). The carboxylic acid chain may or may not present an unsaturation. A methyl side group also may or may not be present. The chemical structures of the capsaicinoid group are depicted in Figure 47.1. Quantitatively, the most important capsaicinoids in *capsicum* are capsaicin and dihydrocapsaicin, responsible of 90% of the pungency (Govindarajan 1986; Iwai et al. 1979a; Kosuge and Furata 1970), compounds that structurally vary in both chain length and chain terminus.

Capsaicinoids are exclusive compounds to the genus *Capsicum* (Govindarajan and Sathyanarayana 1991; Govindarajan et al. 1987) biosynthesized by means of a phenyl propanoid pathway intermediate (Fujikawe et al. 1982; Iwai et al. 1979a). The vanillylamine portion of the molecule of capsaicinoids is biosynthetically derived from L-phenylalanine through the successive conversion of this parent compound in cinnamic, coumaric, caffeic, and ferulic acids. An amine group is added to the last intermediate, vanillin, by means of an amine transferase. The branched fatty acid moiety is derived from valine or leucine (García-Galindo et al. 1995; Iwai et al. 1979a). Vanillylamine and 8-methyl-nonenoic acid are condensed through the capsaicin synthase to form capsaicin. Whether transformation of the fatty acid moiety, which produces the structural diversity of capsaicinoids, occurs before or

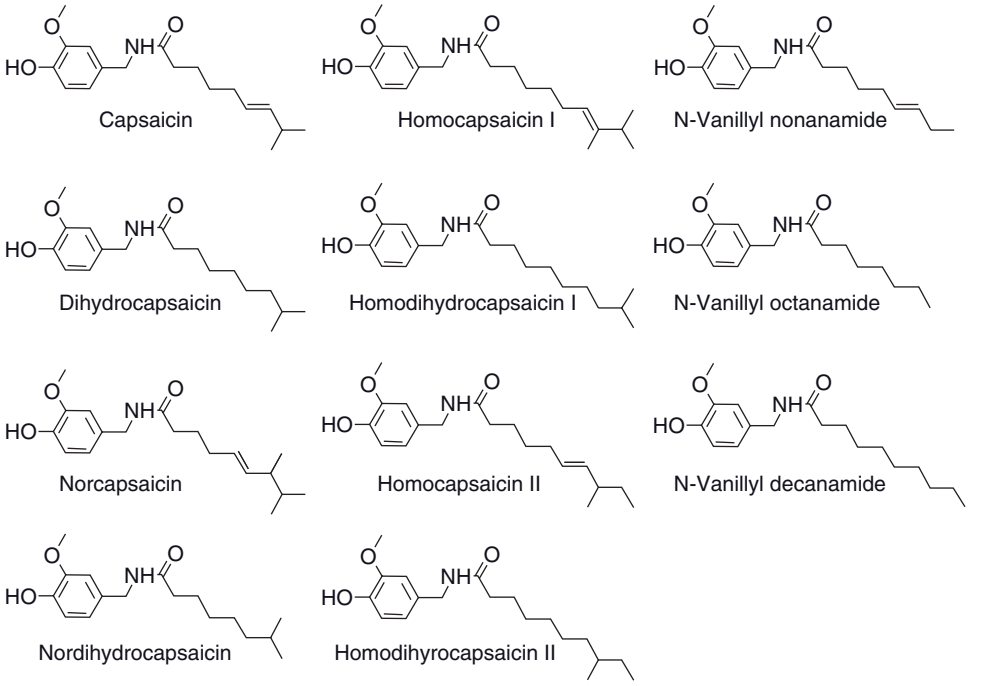

Figure 47.1. Chemical structure of capsaicinoids.

after condensation is unknown. The pathway of capsaicin biosynthesis is represented in Figure 47.2.

Although some studies report the presence of capsaicinoids in vegetative organs and seeds, the biosynthesis and accumulation of capsaicinoids take place along the epidermal cells of the interlocular septum, which defines the fruit locules and is derived from the tissue connecting the placenta to the pericarp (Judd et al. 1999; Stewart et al. 2007). Very little is known about the regulation of the biosynthetic pathways, although it seems that the key enzymes involved in the biosynthesis of capsaicinoids are the caffeic acid *O*-methyl transferase (leading the formation of ferulic acid), keto acyl synthase (involved in the synthesis of 8-methyl-nonanoic acid), and capsaicin synthase (Prasad et al. 2006). Different pungency levels result from genetic diversity among the *Capsicum* species and from environmental factors. It seems that the pungent characteristic of *capsicum* fruits was a dominant feature associated with the locus *Pun1*, formerly named locus *C*, which produces a putative acyltransferase (Stewart et al. 2005). Although this enzyme was initially correlated with the condensation process to form capsaicin, it has been recently demonstrated that the enzyme responsible for this process, capsaicin synthase, is encoded by *csy1* (Prasad et al. 2006). The activity of the acyltransferase enzyme is associated with the development of vesicles where capsaicinoids are accumulated (Stewart et al. 2007). Among environmental factors that affect capsaicinoid accumulation is water stress, although the response to this factor depends on the variety (Sung et al. 2005). The effect of these factors on capsaicin biosynthesis is difficult to predict and control because the capsaicin biosynthetic pathway shares some precursors with

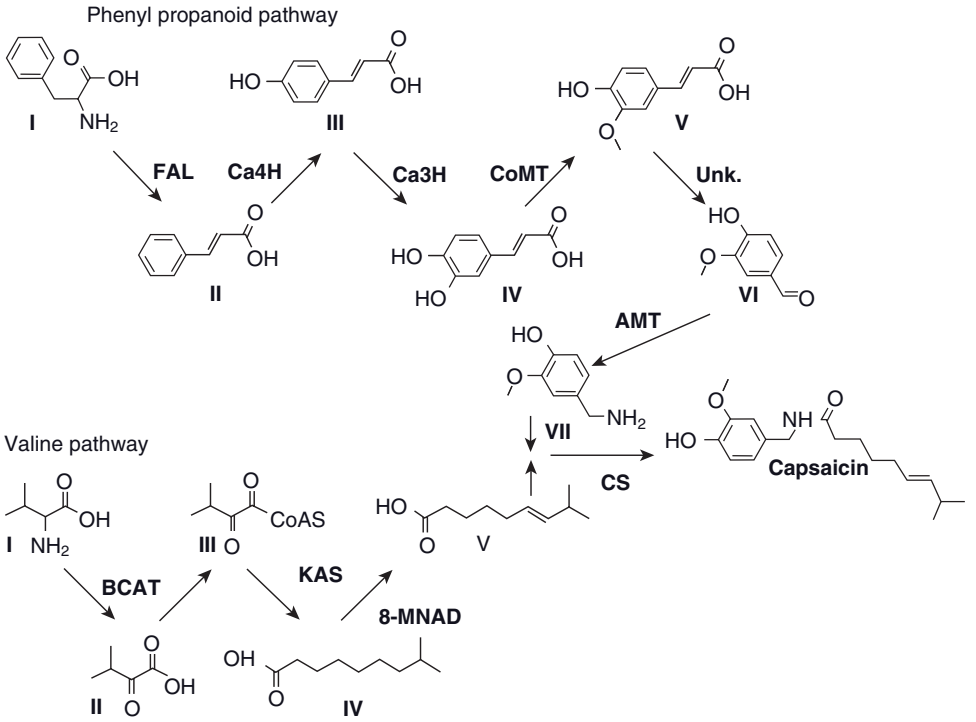

Figure 47.2. Biosynthesis of capsaicin through the phenyl propanoid and valine pathways. The enzymes involved in the routes are phenylalanine ammonia lyase (PAL), cinnamic acid 4 hydroxylase (Ca4H), coumaric acid 3 hydroxylase (Ca3H), caffeic acid *O*-methyl transferase (CoMT), amino transferase (AMT), capsaicin synthase (CS), branched-chain amino acid transferase (BCAT), keto acyl synthase (KAS), and 8-methyl nonanoic acid dehydrogenase (8-MNAD). The intermediate compounds are the following: (phenyl propanoid pathway) I, phenylalanine; II, cinnamic acid; III, coumaric acid; IV, caffeic acid; V, ferulic acid; VI, vanillin; VII, vanillylamine; and (valine pathway) I, valine; II, α-keto isovalerate; III, isobutyryl CoA; IV, 8-methyl nonanoic acid; V, 8-methyl-6-nonenoic acid.

other biosynthesis routes, probably leading to coumarin and lignin derivatives, whose accumulation is also regulated by environmental factors.

The function of capsaicinoids in *capsicum* fruits is related to seed dissemination and the preference of the plant for birds as seed-dispersal agents. Thus, Tewksbury and Nabhan (2001) suggest that birds, insensitive to capsaicinoids, transport seeds in the gut and later excrete them in suitable environments for plant growth. Mammals, extremely sensitive to capsaicinoids, would not properly function in the seed disseminating process due to the damage that the mastication and acid digestion would produce to the plant seeds. With this directed deterrence, the *Capsicum* genus has "selected" the appropriate agents for the seed dispersion.

During ripening of fruits, the capsaicinoid content reaches a maximum level, decreasing later by rapid turnover and degradation as observed by Iwai and others (1979a) to unknown products, perhaps to lignin-like compounds with a dimerization as a first step (Bernal and Ros-Barceló 1996). Monitoring of capsaicinoid accumulation has shown that there is no predetermined time profile for appearance and

maximum levels of pungency, and this is going to strongly depend on environmental factors. Some reports show that the first pungent values appear 15–20 days after flowering, while the time window for maximum levels is more open, 25–50 days after flowering. Decreasing to settle is accomplished during the next 50–70 days. Several *in vitro* studies have shown that peroxidases are involved in the degradation of capsaicinoids. Bernal and others (1993) suggested that peroxidase activity is involved in capsaicin turnover and degradation. Pepper peroxidase is mainly located in the placenta and in the outermost epidermal cell layers, the same accumulation place for capsaicinoids (Bernal et al. 1994b). A peroxidase isoenzyme from *capsicum* fruits was purified and showed a strong capsaicin-oxidizing activity (Bernal et al. 1994a). The authors showed later that this isoenzyme is also involved in the insolubilization of precursors of capsaicinoids, demonstrating that there is an oxidative competitive sink for capsaicin intermediates. *In vivo* studies also associate peroxidase activity with capsaicinoid degradation. Thus, Contreras-Padilla and Yahia (1998) found an inverse relationship between the evolution of capsaicinoids and peroxidase activity in a study that monitored both parameters during the development, maturation, and senescence of chili peppers. Other physical and chemical oxidative conditions also promote capsaicinoid degradation. Kirschbaum-Titze and others (2002) observed an increase of the degradation rate of capsaicinoids when the cellular structure of fruits is disrupted. Further increases were denoted after the application of oxidative conditions (raising the temperature or storage under oxygen atmosphere).

Analysis of Capsaicinoid Content

The capsaicinoid content of *capsicum* fruits is the discriminating variable to distinguish hot varieties, which present a pungent flavor from the sweet ones without such flavoring property. Although capsaicinoid is not a good chemotaxonomic indicator for characterization of chili species (Zewdie and Bosland 2000), according to Richard (1991), four varieties can be distinguished considering their capsaicinoid content:

1. *C. annuum* L. var. annuum (capsaicinoid content: 0.01 ± 0.70%),
2. *C. baccatum* L. var. pendulum (0.11 ± 0.25%),
3. *C. pubescens* (0.12 ± 0.36%), and
4. *C. frutescens* L. (0.26 ± 1.21%).

As mentioned before, these amounts can be substantially modified in the function of environmental and growth factors. Govindarajan and others (1987) state for fresh pepper a range from 0.001% to 0.01% for low pungent varieties and a range from 0.1% to <1% for the hot chili varieties. Parrish (1996), reports a range from 0.1 mg/g in chili pepper to 2.5 mg/g in red pepper and a concentration of 60 mg/g in *capsicum* oleoresins.

Pretreatment of *capsicum* fruits (mincing and blanching) diminishes the original capsaicinoid content of fruits due to temperature-dependent oxidation and activation of oxidative enzymes (Kirschbaum-Titze et al. 2002; Schweiggert et al. 2006). Processing of the fruit material to obtain the dry fruits, the powder, and *capsicum* oleoresins, processing of the food formulation where fruits have been added for

flavoring purposes, and, finally, cooking often imply the application of thermal treatments that negatively affect the original capsaicinoid content (Suresh et al. 2007). It has been shown that during fermentation, the amount of capsaicinoid decreases may be due to microbial degradation (Lee and You 1977; Lee et al. 2005; Todd et al. 1977).

The studies dealing with capsaicinoid content report different values not only depending on variety and ripening stage but also on the sample pretreatment, the capsaicinoid extraction method used, and the analytical tool applied for determination. A wide variety of analytical methodologies have been developed for the analysis of capsaicinoids, which implies pros and cons. Depending on the lab equipment and training possibilities, one may select the straightforward methods or sophisticated ones. However, with the use of different extraction procedures and analytical tools, it is difficult to compare and contrast the data appearing in literature even although several publications transform the obtained data to Scoville units, an organoleptic test often employed as a reference method.

Extraction Procedures The starting material for capsaicinoid analysis presents differences on water content and sample size. Thus, fresh or dehydrated fruits, ground or not, milled paprika, and paprika oleoresins are possible sample presentations. For the analysis of capsaicinoids, after sample homogenization, if required, an extraction procedure is applied. The simplest extraction method is maceration with an organic solvent (acetone, methanol, ethanol, and ethyl acetate) for some time (15–30 min), cleaning the extract from solid residues by filtration. Extraction of capsaicinoids can be improved by the application of ultrasounds, use of Soxhlet extractors, microwave, and use of supercritical fluids. Application of sample pretreatment with enzymes for cell disruption to facilitate extraction has been reported (Santamaría et al. 2000). Pressurized liquid extraction technique has been used with the aims of reducing the amount of organic solvent and extraction time and minimizing the possible oxidative degradation. Moreover, this process can be automatized, increasing the reproducibility of data (Barbero et al. 2006).

Once the extract has been obtained, some procedures described in literature apply a cleanup process using a solid phase extraction column. The aim of this step is to separate the capsaicinoid content from the carotenoid fraction that, irremediably, has been co-extracted with the capsaicinoids and that may interfere in the subsequent analysis of the pungent compounds. Some procedures like liquid–liquid fractionation, separation by thin-layer chromatography and solid phase extraction have been applied (Attuquayefio and Buckle 1987; Jurenitsch et al. 1978; Korel et al. 2002).

Analytical Techniques Applied for Pungency and Capsaicinoid Content Determination Development of methodologies for the assessment of the capsaicinoid content of *capsicum* fruits and related products has been a constant challenge for academic institutions, regulating agencies, and the food industry due to the significance of these compounds on the quality of the product, their use to determine the economical value of the raw material (fruits and their extracts), and the growing market of the pungent flavor that results from increasing popularity and demand for products with such taste. Consequently, it is possible to find a wide variety of methodologies using different approaches to determine the capsaicinoid

content or its equivalent pungent value, including organoleptic tests and chemical, instrumental, and sensory methods.

The method developed by Scoville in 1912 determines the heat (burning sensation or pungent flavor) of *capsicum* samples by extracting the pungent compounds with ethanol and by diluting an aliquot of the extract with a 5% sucrose solution. The final solution is presented to the panel members, who swallow it and note the pungent sensation in the throat. Further dilutions are made and tasted by the panelists until the pungent sensation is detected. The results are recorded as Scoville units. These are defined as the greatest dilution, expressed as the denominator of the dilution factor, at which a pungent sensation is perceived under the stated test conditions. Thus, a limiting dilution of 1 in 50,000 is equivalent to 50,000 Scoville units. This method was adopted by the American Spice Trade Association and the Essential Oil Association (EOA) of the United States and could be considered as convenient for primary evaluation of the pungency level of the fresh *capsicum* fruits, to be performed in the field before submission of the harvest to the industry for processing. It is noteworthy to mention that this measurement deals with the pungency but does not determine the capsaicinoid content, although obviously, both parameters are closely linked. Some equivalency equations have been evaluated to calculate the capsaicinoid content from Scoville units, equations derived from spectrophotometric measurements that will be discussed later. As a rule of thumb, 150,000 Scoville units could represent 1% on capsaicinoid content.

Examples of specification of *capsicum* products based on Scoville units were the EOA specification no. 244, which indicates a minimum value of 480,000 Scoville units for *capsicum* oleoresin and 240,000 for oleoresin red pepper, and the Federal Specification EE- S-00645a (ARMY-GL) for oleoresin of red pepper, with a 12,500–16,500 Scoville unit range (Heath 1981). Nevertheless, modifications and criticisms to this procedure have been published (Gillette et al. 1984; Govindarajan et al. 1977; Rhyu 1978). Sensitization and desensitization effects produce confusion to the measurement of pungency and protocols to predict the necessary interstimulus intervals have been reported (Dowell et al. 2005). However, it is the subjective nature of this method the main drawback that produces variability among values for the same sample and lack of reproducibility. Therefore, chemical, instrumental, and sensory methods have been established as standards in the food industry and academia instead of the oral test of Scoville (1912). These methods have been reviewed by Anu and Peter (2000), Govindarajan (1986), Pruthi (2003), and Wall and Bosland (1998).

Chemical Methods Some colorimetric methods have been developed for the analysis of capsaicinoids. Tice (1933) developed a method, based on the production of a blue color, when capsaicin reacts with vanadium oxytrichloride. This method has been revised and modified in several publications. Thus, the method of North (1949) uses phosphotungstic-phosphomolybdic acid to generate a blue color when reacted with capsaicin. In this measurement, the standard reagent for calibration is vanillin, assuming an equivalency ratio of 1:2 between this compound and capsaicin. As the coloring agent reacts with any phenol group, phenolic compounds interfere in the measurement, so isolation of capsaicinoids in a sufficient degree of purity is required. Ramos-Palacio (1977, 1979) revised the colorimetric method based on the vanadium oxytrichloride reagent in order to determine the quality of the measurements and

the interference level of carotenoid pigments in the measurement of capsaicinoids in low-pungency samples. In the publication of Ramos-Palacio (1979), a correlation equation between Scoville units and capsaicin content is proposed. A method published by Bajaj (1980) performs the colorimetric reaction with an aqueous solution of sodium nitrite and sodium molybdate, which produces a yellow compound when reacted with capsaicin. In this procedure, a pre-cleaning process of the sample, to discard interfering compounds, is made by means of column chromatography on basic alumina as described by Dicecco (1976). A recent modification of the method of Bajaj, published by Gibbs and O'Garro (2004), avoids the purification of the extract by column chromatography and performs a convenient purification of capsaicinoids by liquid–liquid fractionation. Calibration curves are obtained with the use of pure standards of capsaicin in these publications (Bajaj 1980; Gibbs and O'Garro 2004; Ramos-Palacio 1977, 1979).

Instrumental Methods Among instrumental techniques, and considering sample preparation, sophistication level of the instrumentation and skill in operation, spectrophotometric measurements could be considered as convenient if a rapid evaluation of capsaicinoid content is needed. The main handicap is the relatively high amount of interfering substances on the measurement, usually performed at the UV range, which may introduce uncertainty on the results. Rymal and others (1984) described a direct spectrophotometric determination of a capsaicinoid extract obtained with a single injection–extraction procedure with methanol. Absorbance unit measurement is performed at 275 nm and concentration is determined with the calibration curve obtained with capsaicin standard solutions. This measurement is suitable for the measurement of capsaicinoid in fresh *capsicum* fruits. Another strategy to avoid sample purification steps and to perform the measurement of capsaicinoid content spectrophotometrically is to apply statistical algorithms to remove the influence of interfering compounds. This has been the procedure used in the work of Davis and others (2007). They apply a partial least squares regression technique to obtain correlation models between capsaicinoid content values obtained by high-performance liquid chromatography (HPLC) and spectral measurements. The models were validated and the error of prediction estimated, obtaining good results.

The most widely used methods apply HPLC to the isolation and quantification of capsaicinoids in *capsicum* and food samples, which allow achieving sensitivity at submicrogram levels. The source HPLC method was published by Woodbury (1980) to which successive modifications have been applied. The separation is performed in an octadecyl silane column, by reversed phase. Fluorescence detection, excitation at 288 nm, and emission at 320 nm was used. The official analytical method of the American Spice Trade Association (Way 1985) performs the chromatographic separation in similar conditions (reversed phase using a nonpolar stationary phase), but detection is achieved by a UV detector set at 280 nm for samples containing more than 700 ppm or fluorescence detection (excitation at 288 nm, emission at 320 nm) for samples containing less than 700 ppm. As mentioned before, modifications on mobile phase composition, application of isocratic of gradient elution systems, flow rate, and detectors (UV, fluorescence, combination of electrochemical and UV, chemiluminiscent-nitrogen detector) can be found on the wide number of HPLC methods described in literature. Table 47.1 summarizes some recent methods used

TABLE 47.1. Summary of Some Recent Methods for Analysis of Capsaicinoids in *Capsicum* Samples

Sample	Extraction Procedure	Analysis Conditions	Capsaicinoids Measured	Reference
Fresh green and red peppers	Homogenization at high speed with methanol, left standing for 30 min, filtration	Lichrospher RP-18e column, isocratic, fluorescence detection, nonanoic acid vanillylamine as e.s.	Sum of nordihydrocapsaicin, capsaicin, and dihydrocapsaicin	Kirschbaum-Titze and others (2002)
Ground red peppers	Soxhlet extractor, acetone, 5 h, cleaning with SPE C18	μ-Bondapak C18 column, isocratic, UV detection, bisphenol A as i.s.	Capsaicin and dihydrocapsaicin	Korel and others (2002)
Fresh peppers, dry pepper powder, sliced pepper, commercial pepper-containing foods	Maceration with methanol, filtration, extraction assisted by ultrasonic bath	C18 column, 30°C, gradient, UV detection	Sum of nordihydrocapsaicin, capsaicin, dihydrocapsaicin homocapsaicin I and II, and homodihydrocapsaicin I and II	Kozukue and others (2005)
Fresh peppers	Chopping with ethanol for 30 s, heating for 30 min at 78°C, filtration	Inertsil ODS-3v column, 40°C, nonlinear gradient, UV detection	Capsaicin and dihydrocapsaicin	Davis and others (2007)

for the HPLC analysis of capsaicinoids. Most of these methods make use of UV detection at 280 nm, quantifying capsaicin and nordihydrocapsaicin, which represent 90% of the total capsaicinoids, and, in some cases, nonivamide. Quantification of minor capsaicinoid compounds is not generally considered due to low levels of those capsaicinoids analogues, inadequate sensitivity, or poor resolution. Still, separation of minor compounds can be achieved with some of the methodologies previously mentioned. The method of Maillard and others (1997) describes the separation and quantification of almost the complete profile of capsaicinoids of *capsicum* extracts. The mobile phase includes silver nitrate in a final concentration of 37.9 mM, which enhances the separation of the unsaturated compounds from their saturated isomers. UV detection was performed at 281 nm. Up to 13 compounds were separated, including 10 known capsaicinoids and 3 new ones, from which 1 was tentatively identified by nuclear magnetic resonance (NMR).

For the identification and quantification of minor capsaicinoids in *capsicum* fruits and food, the use of more selective detection and identification techniques such as mass spectrometry is sometimes required. HPLC had been used in combination with mass spectrometry off-line mode to improve the identification and quantification of capsaicinoids (Heresch and Jurenitsch 1979), or in combination with field desorption mass spectrometry (Games et al. 1984). Finally, the direct coupling of HPLC with mass spectrometry (MS) by means of ionization techniques, like atmospheric pressure chemical ionization and electrospray interfaces, definitively improved this analytical tool, which has become very fashionable (Kozukue et al. 2005; Reilly et al. 2002; Thompson et al. 2005). Detection limits are in the nanogram range, which is feasible for metabolic studies.

Gas chromatography is another separation method used in the analysis of capsaicin content that, like the HPLC methods, provides rapid and accurate analyses, although preparation of the sample could be in some cases more exhaustive. Gas–liquid chromatographic methods developed by Hartman (1970) and by Todd and others (1977) perform a derivatization process of capsaicinoids through a silylation reaction with trimethylchlorosilane hexamethyldisilzane or N,O-bis(trimethylsilyl)-trifluoreacetamide, respectively. This derivatization process eliminates the previous separation steps of the capsaicinoid fraction from the interfering or inert material. The method of Hartman (1970) focused only on the determination of capsaicin, consuming 6 min per analysis, while the method of Todd and coworkers (1977) accomplished the separation and quantification of seven capsaicinoids, completing the analysis in 20 min. The sequence of elution was nordihydrocapsaicin, vanillyl pelargonamide, capsaicin, dihydrocapsaicin, vanillyl capramide, homocapsaicin, and homodihydrocapsaicin. In this case, calibration is performed through the internal standard technique using vanillyl octanamide. Five capsaicinoids were synthesized and employed in the optimization of the separation conditions. The method of Dicecco (1976) does not make use of derivatization and the separation is achieved in a Teflon column with 0.2% of carbowax. This method only quantifies capsaicin using piperine as internal standard. The analysis is completed in 16 min.

The combination of gas–liquid chromatography with mass spectrometry has satisfactorily enhanced the resolution of the separation and identification of minor capsaicinoids. Lee and others (1976) performed a method for the quantitative microanalysis of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. This method employs cholestane as internal standard and reaches resolution on the nanogram level. Hawer and others (1994) developed a gas chromatography–mass spectrometry (GC-MS) method achieving the isolation of capsaicinoids from the sample matrix with acetone, removing undesirable components by liquid–liquid fractionation, and avoiding interferences on the analysis or contamination of the column. Two major peaks are separated in a fuse-silica capillary column, capsaicin and dihydrocapsaicin, and five minor peaks are also resolved, with the use of squalane as internal standard.

The gas chromatography technique has been frequently used in combination with other analytical procedures for the determination of capsaicinoid composition. Thus, Jurenitsch and others (1978) used a rapid separation procedure of capsaicinoids by thin-layer chromatography, removing pigments and other lipophilic compounds, performing later the gas chromatography analysis. Capsaicinoids, previous purification and separation by HPLC, are subsequently quantified by GC-MS in the method of Iwai and others (1979b).

Physiological Base of the Pungent Flavor of Capsaicinoids

The perception of flavor includes aromatic and taste impressions, but in the case of *capsicum* fruits and related products, it is the pungent sensation the main flavor characteristic, also referred as heat or burning impression, which may produce different acceptance by the consumer, and it has even created a geographic consumption pattern. The sensitization process is mediated by the participation of receptors found in peripheral neurons. The existence of a specific capsaicin receptor was postulated by Szolcsányi and Jancsó-Gábor (1976) based on the structure–activity relationship among different synthetic capsaicin analogues and their irritant actions. At the initial establishment of the function of this capsaicin receptor, it was denoted that the vanillyl moiety was responsible for the activation of the receptor, so this was named vanilloid receptor, member 1 (VR1). Later, it was discovered that there is a complete family of receptors, the transient receptor potential family (TRP), which functions as thermometers on a molecular level, and is activated not only by capsaicin but also by noxious heat, acidic pH, proinflammatory agents and algesic mediators. In fact, the receptor VR1 is a member of the subfamily TRPV (vanilloid), now termed TRPV1. This family is formed by six members from which TRPV1–4 are defined as heat-activated channels that are nonselective for cations and are modestly permeable to Ca^{+2}, and also function as chemosensors for a wide range of ligands.

For a full detailed revision of the sensitizing mechanism of the pair capsaicin/TRPV1 receptor, the reader is referred to comprehensive reviews (Caterina and Julius 2001; Szallasi and Blumberg 1999). It is supposed that capsaicinoids are absorbed and pass through the plasmatic membrane of the cell and then, they bind to the cytosolic part of the receptor, opening the channel what allows the influx of cations. This ionic effect results in an excitatory effect on terminals of primary sensory neurons, with the subsequent depolarization of the nerve fiber and the initiation of action potential propagation (Geppetti and Trevisani 2004). The neuronal excitation is followed by a lasting refractory state, desensitization, in which the cell is refractory to new stimuli (Buck and Burks 1986; Szallasi and Blumberg 1999; Szolcsányi 1989). The desensitization effect is the basis for therapeutic applications of capsaicin analogues and antagonists of the TRPV receptor. Thus, the group of TRPV is nowadays a target for drug development based on its desensitization by antagonist compounds that prevent pain by silencing the nociceptors in the periphery where the pain is located. This strategy is in an advanced stage with promising results, which will produce a new set of drugs for pain management (Szallasi et al. 2006).

Food Applications of *Capsicum* Fruits Based on Pungency Flavor

It is estimated that a quarter of the world's population consumes *capsicum* products in some form daily (Szallasi and Blumberg 1999). The market for food products containing hot pungency has increased in recent years, due to consumer acceptance and the search of ingredients to provide some flavor to foods designed for special dietary uses. Hot sauces and tomato-based salsa containing hot peppers account for an estimated $500 million (United States) in annual sales (Perkins et al. 2002). A search on the Web (http://www.espacenet.com) retrieved 591 hits introducing the term *capsicum* in the title or abstract areas.

Figure 47.3 shows the world's production data during the decade 1995–2005 of dry *capsicum* fruits (FAOSTAT, http://faostat.fao.org/default.aspx). The production levels have continuously increased during that time period. A similar situation is denoted in the balance of imports and exports shown in Figure 47.4. Major challenges in the international trade of *capsicum* products and spices in general are related to safety issues. Thus, the preference for research into food safety in European countries is more of a necessity, since they are importing from producing countries that are still developing. In such countries, spices are normally produced using artisanal techniques, with few or inappropriate hygiene measures, a problem which is normally solved using inadequate decontamination techniques or techniques that are prohibited in developed countries. It is for this reason that a considerable amount of research is dedicated to developing methods for detecting contaminants, food decontamination, and safety techniques.

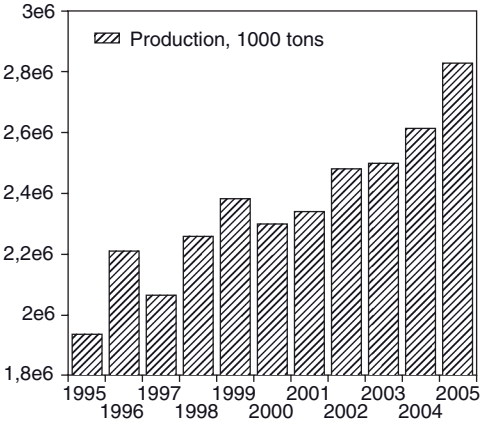

Figure 47.3. World's production data of chili during the decade 1995–2005.

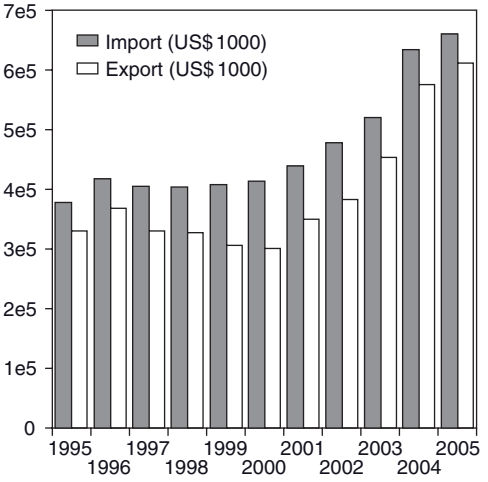

Figure 47.4. Import and export balance of chilies during the decade 1995–2005.

Furthermore, inequalities exist between the quality standards in producing countries and those demanded or desired by the agri-food industry, as a result of production methods applied in exporting countries. This situation has led to the development of methodologies for the characterization of spices, fraud detection, and conservation procedures to preserve the organoleptic characteristics that determine the market value of such products.

The inclusion of *capsicum* products occurs in thousands of recipes. If the reader is also interested on culinary recipes with *capsicum* products, a visit to the website http://www.hotsauceblog.com/ is recommended. There is a geographic pattern of *capsicum* consumption with a higher intake in tropical countries. Thus, López-Carrillo and others (1994) reported a daily consumption of 20 g per person in Mexico, and Monsereenusorn (1983) estimated a consumption of 5.0 g per person per day in Thailand and of 2.5 g per person per day in India. These data imply a daily intake of capsaicin in the range of 25–200 mg. In Europe and in the United States, capsaicin consumption would be in a lower range (1–2.5 mg/day).

Capsicum fruits and related products may be used to improve the presentation (based on the coloring capacity owed to carotenoids) or to impregnate the meal with a characteristic flavor (based on the pungency owed to capsaicinoids). The food industry makes use of *capsicum* for coloring and flavoring purposes in garnishes, pickles, meats, barbecue sauces, ketchup, cheese, snack food, dips, salads, and sausages. Dry fruits may be crushed and directly employed for pizza and pasta production and in the preparation of some flavored oils. To facilitate transport and to enhance handling during the storage and dosage of spices in food formulations, it is usual to obtain the oleoresin from the dry material. An oleoresin is an oily concentrate that contains essential compounds, in the case of *capsicum*, the carotenoid pigments and the capsaicinoids. Two types of *capsicum* oleoresins are distinguished depending on their use: for coloring purposes, paprika oleoresin (10–15% of color content), and for pungency and color uses, *capsicum* oleoresin (3–6% of capsaicinoid content, 1% of color content).

Legislation Issues Concerning *Capsicum* Products and Capsaicin

The general idea that food components are safe when they form part of natural and common foods that have been processed with good manufacturing practices and consumed within a normal diet has been profusely recognized and assumed as valid. Even so, that idea was the starting point to elaborate the initial food safety regulations and positive list of accepted food ingredients and additives. However, food safety and human health is a key issue in our society involving regulating agencies and governmental health bodies, whose implication in this subject has been reinforced and compiled by the common aptitude of the consumer to self-controlling health and demanding safe food products. All this has strengthened the idea that safety claims of food ingredients and additives must both be scientifically assessed and supported, although it is evident that some critical factors are implied in this development, like the economical effort, the standardization of analytical tools and experiments to get reliable results, and the time necessary to perform the studies.

This picture is applied to the *capsicum* fruits and related products, when the safety of the compounds responsible of pungency is strictly considered. Of course

it is possible to find personal experiences of profuse perspiration and digestive problems after the ingestion of foods flavored with *capsicum* fruits or with hot sauces. However, the facts on the safety of these flavoring products are based on the ancient use of *capsicum* fruits in cooking and flavoring foodstuffs together with the absence of toxicological and health problems associated with their intake, within habitual cooking recipes and normal dosage in food products where they are added. Even so, both personal perception and the individual liking for the pungent taste are the basis to take or reject *capsicum* products. Therefore, the consumption of *capsicum* fruits and their use in the food industry has not been specifically regulated; only the already established norms concerning general food safety, food labeling, and consumer protection were applied.

Capsicum fruits and processed products are included in the positive list of food ingredients and present in the generally recognized as safe (GRAS) qualification. In the case of the food legislation of the United States, the Food and Drug Administration (FDA) includes in the Code of Federal Regulations (title 21, section 182.10) *capsicum* fruits as materials generally recognized as safe as well as the *capsicum* oleoresins, obtained from *C. frutescens* L. and *C. annuum* L. fruits, (title 21, section 182.20) with emphasis on the application, in this case, of the normative regarding solvent specifications and allowed solvent traces in the final product (title 21, sections 170–189). Capsaicin is not recognized as a food additive but appears in some drug applications. Thus, capsaicin is considered to be safe and effective as an external analgesic counterirritant but not for fever blister and cold sore treatment.

Within the European Union (EU), legislation has proceeded in a similar way. Directives 88/388/EEC, 88/389/EEC (Directive 1988a,b), and Regulation 2232/96/EC (Regulation 1996) claimed for the establishment of an inventory of the various sources materials and substances that may be used in the preparation of flavorings. This was made to harmonize food legislation of different member countries of the EU. A positive list was published, Directive 1999/217/EC (Directive 1999), but was included on a registry of additive flavoring substances allowed for use in or on foodstuffs, and it did not include the source materials. In the list of additives to be used as flavoring in or on food published in 1999, capsaicin appeared. In addition, the following limits for capsaicin content were suggested: 5 ppm for foods and beverages, 10 ppm for hot foods and beverages, 20 ppm for hot ketchup, and 50 ppm for Tabasco (Council of Europe 2001).

However, the European Commission asked the Scientific Committee on Food to advise if restrictions of use or presence might be necessary to ensure the safety of substances used as flavors or present in food ingredients with flavoring properties. In the case of capsaicin, the opinion was published in 2002, and, on the basis of the available data, the committee concluded that it was impossible to establish a safe exposure level for capsaicinoids in food. The suggested limits are still valid, but the report concluded with the observation of a correlation between high intake of pungent *capsicum* products and an increased risk of cancer of the upper digestive tract. Although the intake levels in Europe are lower than those associated with the cancer risk, the EU adopted the decision of eliminate capsaicin from the list of authorized flavors (Directive 2004).

With regard to the *capsicum* oleoresins, the EU specifications specifically deal with paprika ones, that is, those oleoresins elaborated with *C. annuum* L. fruits

and to be used as coloring agents. It was established a maximum value of 250 mg/kg for capsaicin content (Directive 1995).

A similar situation is denoted in the Codex Alimentarius. *Capsicum* fruits are recognized as safe as food ingredients, but capsaicin does not appear in the list of flavoring substances. The Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) only established specifications for paprika oleoresins (not *capsicum* ones), recognizing in this case their use as coloring and flavoring agents, with a limit of 0.5% to capsaicin content (JECFA 2006).

In summary, the FDA and EU legislation recognize *capsicum* fruits as food ingredients. The legislation is very clear in the FDA and a list of source materials to be used as ingredient flavoring agents should be published in the EU, although some limits have been established for total capsaicinoid content in flavored foods and beverages. A different approach has been adopted for oleoresins, the EU legislation being more restrictive, as oleoresins are recognized only as coloring stuffs limiting their capsaicin content. Capsaicin as additive is not recognized as safe neither by the FDA nor EU.

AROMA

The volatile fraction of foods may be considered as the most complex one due to the high number of compounds that could be detected in a simple food (50–200), usually in a low concentration (trace amounts). A complicating issue on aroma analysis and its relation to the flavor of food is that perception of aroma is determined by the concentration of volatiles in the air phase, and that concentration results from interactions between the food matrix and volatiles. Therefore, the concept of aroma, the chemical description of individual volatiles, the correlation of each compound with an aroma note or an attribute, and its significance to the total aroma of a foodstuff have changed in the same way as analytical procedures and lab equipment available for aroma analysis have evolved. The volatile profile of *capsicum* fruits is described in fresh fruits (distinguishing different ripeness stages), dry fruits or paprika, and *capsicum* oleoresins.

Fresh Fruit

More than 60 volatile compounds were identified by Buttery and others (1969) in fresh bell pepper at different ripening stages, with aliphatic alcohol and carbonyl products, aromatic components, and monoterpenes as the main classes of compounds. Among the determined volatiles, those contributing significantly to the aroma of fresh fruits were 2-methoxy-3-isobutylpyrazine, nona-*trans*, *cis*-2,6-dienal, deca-2,4-dienal, limonene, and methyl salicylate. These were correlated with bell pepper, cucumber, fried chicken, citrus, and oil of wintergreen odor notes, respectively.

Chitwood and others (1983) described the volatiles of three *capsicum* species and correlated the volatile fraction with aroma descriptors. Volatiles were analyzed by GC-MS and sniffing of the GC eluate. Eleven compounds including esters, alcohols,

ketones, and pyrazines were identified and associated with green, fruity, and floral aromas. Thus, *trans*-3-hexenol, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine were related to green aroma characteristics.

Aliphatic esters are the major compounds of the aroma profile of chili peppers (64% of the total volatile content) as described by several authors (Haymon and Aurand 1971; Huffman et al. 1978). However, one of the characteristic compounds of chili pepper aroma is 2-methoxy-3-isobutylpyrazine, the same compound found in fresh bell pepper fruits. A description of the volatiles of chili fruits and of the changes during maturation has been reported recently (Mazida et al. 2005). These authors optimized the solid phase microextraction (SPME) technique to monitor the content of six volatile compounds of chili during ripening. Green chilies showed a higher number of volatiles, although most of the six major volatiles analyzed increased when the fruits turned from green to red. Only hexanal and 2-isobutyl-3-methoxypyrazine (green and grassy notes) decreased during maturation in agreement with previous observations. The main volatile compounds contributing to the aroma of fresh red chili were the sweet and fruity attributes represented by 2,3-butanedione, 3-carene, and *trans*-2-hexenal.

Significant changes on volatile content have been described to occur during the maturation stages of *capsicum* fruits. Luning and others (1994a) showed that most volatiles decreased or even disappeared during ripening. Major compounds in green peppers were toluene, *trans*- and *cis*-β-oximene, 1-hexanol, *trans*-3-hexenol and 6-methylheptyl-2-propenoate, and *trans*- and *cis*-3,4-dimethyl-2,4,6-octatriene. These compounds decrease significantly during maturation. Other minor volatiles, *trans*- and *cis*-2-hexenal and *cis*-2-hexenol, increased significantly from the green ripened stage to the red one. These compounds may appear by the action of oxidative and hydroperoxide cleavage enzyme systems, whose activity changes during maturation (Gaillard et al. 1977). The work of Luning and others (1994a) also reports association of fresh pepper volatiles and odor descriptors as reported elsewhere (Dravnieks 1985; Sheen et al. 1991). At the green stage, bitterness and grassy, cucumber, and green bell pepper aromas were the main attributes, and at the red stage, sweetness, sourness, and red bell pepper aroma were the main ones (Luning et al. 1994b). Recent reports are consistent with these observations (Pino et al. 2006, 2007).

The biochemical routes of aroma generation will not described herein. A lecture of the excellent chapters included in this handbook is advised. However, it must be taken into account that together with the volatiles biogenerated, some other processes may provide particular volatile compounds. Thus, when fresh *capsicum* fruits are infected with fungi, some specific volatiles can be found on the aroma profile. Kim and others (2007) studied the volatile profile of healthy peppers and compared it with fruit either naturally infected or artificially inoculated with *Colletotrichum* spp. Several volatiles appeared in the infected fruits that were not present in healthy ones. Authors suggest that 1H-indole, 4-methylindole, and 1-ethylindole may be considered as major contributors to the aroma of infected *capsicum* fruits. Fermentation of fruits may take place during processing, especially if dehydration is performed with mild conditions. If so, some volatiles could be biologically formed through carbohydrate metabolism by microorganisms like acetic and propionic acids.

Dried and Cooked Fruits

Thermal processing of fresh *capsicum* fruits to obtain dehydrated husks for subsequent industrial processing means loss of most parts of aroma notes, but rearrangements and formation of new volatiles should be taken into account. During the dehydration processing, thermal influence, evaporation, and removal by water or waste will be the main reactions that will remodel the volatile profile of the fruit material. Additionally, activation of oxidative enzyme systems should be considered during the drying period in which the water content of fruits remains high enough to allow this reaction.

The first report, focused on the aroma and sensory evaluation of dried peppers, was published by Van Ruth and Roozen (1994). The study was performed with varieties of different geographic origins, characterizing the volatile fraction by GC-MS/sniffing port (SP) analysis. Several compounds (46) were identified. The profile includes those compounds described previously for fresh peppers (Chitwood et al. 1983; Keller et al. 1981; Wu et al. 1986) together with low-boiling compounds and several others derived from lipid oxidation reactions. Twelve compounds showed odor attributes similar to those described by Chitwood and others (1983). In subsequent studies (Van Ruth et al. 1995a,b), the number of identified compounds increased to 52, from which 16 showed odor attributes. These studies showed that during dehydration, Strecker aldehydes (acetaldehyde, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal) appear as a consequence of Maillard reaction. Other characteristic compounds of dried peppers are dimethyl sulfide and 6-methyl-5-hepten-2-one, also degradation products. The kinetics of volatile formation during heating of paprika powder was determined by Cremer and Eichner (2000). The analyses were performed with headspace GC (for low-boiling compounds) or SPME and GC-MS, and emphasis was made on the analysis of Strecker aldehydes and the degradation products of sugar and carotenoids. Different reaction orders were observed for each group of compounds (zero to first order), and the amount of Strecker aldehydes formed correlated well with concentrations of the corresponding free amino acids present in the samples. Concentration of off-flavors (hexanal, 6-methyl-5-hepten-2-one, and β-ionone) increased during heating of paprika powder, so these compounds could be used as markers of the thermal processing of paprika. The report of Van Ruth and others (2003), where different gas chromatography and mass spectrometry techniques are evaluated for volatile analysis of dried peppers, describes up to 63 volatile compounds from which 11 showed odor attributes. The volatile profile shown in this study is in agreement with previous publications.

Traditional dehydration processing conducted in La Vera County (Spain) furnishes the dry fruit and, consequently, the paprika powder with a distinctive and particular aroma, which makes this product very appreciated by the consumer and the food industry, which uses the La Vera paprika for the elaboration of meat-based products. *Capsicum* fruits are dehydrated in drying houses where the heat source is the burning of oak logs. This traditional and handcrafted process has two significant features: mild and lengthy conditions. Dehydration is completed in 8–10 days at a mean temperature of 40°C (Pérez-Gálvez et al. 2000). Volatiles of paprika processed in such way have been analyzed by Mateo and others (1997). Several groups of volatile compounds were detected, including acids, alcohols, aldehydes, ketones, and

phenols among others. Some of the characteristic volatiles of fresh fruits did not appear in the samples of La Vera paprika, especially those derived from autoxidation processes. Even so, the distinguishing aroma compound of fresh fruit (2-methoxy-3-isobutylpyrazine) was not detected, probably due to evaporation during the dehydration process. La Vera paprika is characterized by a high content of acetic acid, which may be produced either by carbohydrate metabolism and by smoking. Other compounds appeared exclusively due to this particular dehydration process, like phenols, cyclic ketones, furfural, napththalene, and methanal.

Cooked peppers present a volatile profile mainly formed by aliphatic alcohol, carbonyl and aromatic compounds, and furan derivatives (Buttery et al. 1969; Wu and Liou 1986; Wu et al. 1986). The characteristic compound of fresh pepper fruits (2-methoxy-3-isobutylpyrazine) remains in cooked ones together with 1-nonen-4-one, 2-nonen-4-one, 2,6-nonadienal, 2,4-decadienal, and linalool. Qualitative distribution in cooked fruits by stir-frying is the same, but differences are observed on the quantitative content.

Paprika and *Capsicum* Oleoresins

Extraction of the oil material from dry *capsicum* fruits with organic solvents to obtain paprika oleoresins implies the application of thermal processing to the miscella to remove the solvent to achieve statutory requirements regarding maximum residue levels in foodstuffs. The application of unit operations (concentration of the miscella and steam spraying or indirect heating with water) means that an important volatile fraction of the fruit material will be removed and the extract is subjected to a severe thermal stress, so oxidative reactions of the lipid-soluble material and Maillard reactions will take place generating a different and wide volatile profile. Thus, hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, and pyrazines have been identified in paprika oleoresins (Guadayol et al. 1997; Rios et al. 2008). The volatile compounds derived from lipid autoxidation reactions (hexanal, heptanal, 2-heptenal, 2-nonenal, etc.), as well as terpene-derived compounds like β-elemene and carveol, represent an important fraction. Products derived from amino acid transformation (2-phenylethanol) have also been described, as well as pyrazines that significantly contribute to the aroma of paprika oleoresins.

But one of the main fractions of the volatile profile of paprika oleoresin is that formed by thermal degradation products of carotenoids. These products can be grouped in cyclic olefins and linear ketones. Thus, 6-methyl-3,5-heptadien-2-one and 6-methyl-5-hepten-2-one products are generated when oxidation of the polyenoic chain is the promoted reaction process. A scheme of the reactions of the linear ketone generation from carotenoids is depicted in Figure 47.5. Cyclic olefins arise when intramolecular cyclation and reaction of elimination in the chain, or heterolytic fragmentation, are the promoted degradative processes. This degradation route produces *m*-xylene, toluene, and 2,6-dimethylnaphthalene as main products. Figure 47.6 shows the reaction route for 2,6-dimethylnaphthalene formation. Other volatile products arise from the structural rearrangement of oxidized carotenoids. Therefore, 2-methylbenzaldehyde and ethanone, 1-methylphenyl are produced from a previously oxidized carotenoid and the subsequent reorganization and fragmentation of the oxidized intermediate. The scheme reaction for generation of these products is shown in Figures 47.7 and 47.8, respectively.

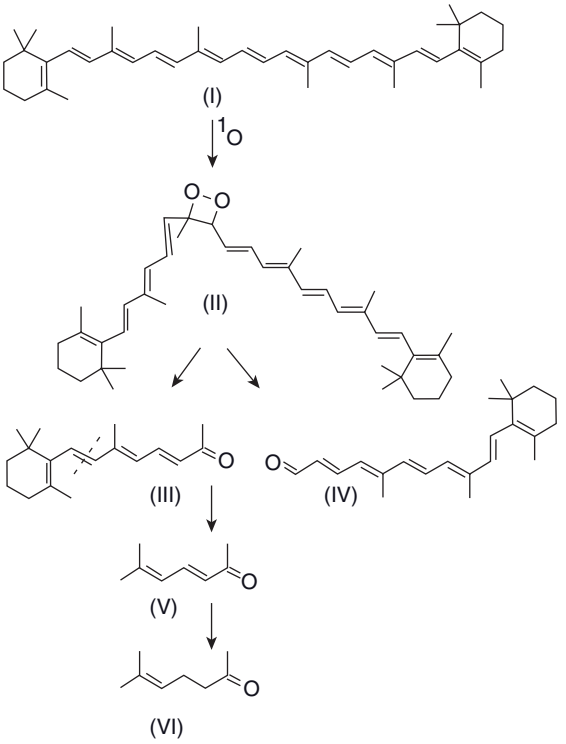

Figure 47.5. Production of 6-methyl-3,5-heptadien-2-one (V) from β-carotene (I) after addition of reactive oxygen species and heterolysis of the carotenoid-endoperoxide (II) to generate trans-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-6-methyl-3,5,7-octatrien-2-one (III), a precursor that fragments to produce the ketone V, and β-apo-14′-carotenal (IV). The hydrogenation of compound (V) may produce 6-methyl-5-hepten-2-one (VI).

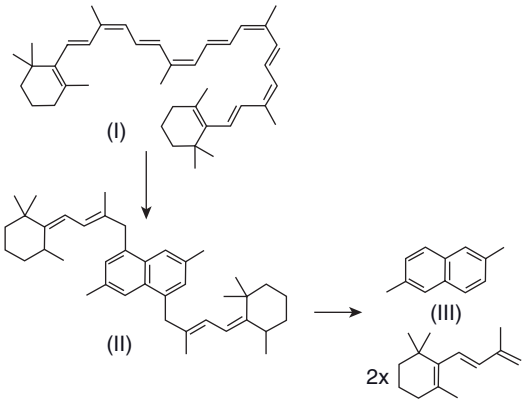

Figure 47.6. Formation of 2,6-dimethylnapthalene (III) by intramolecular cyclation of the polyene chain, starting from β-carotene (I), and subsequent heterolysis of the intermediate (II).

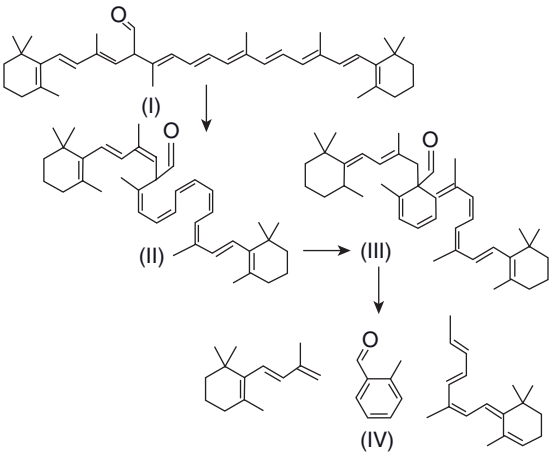

Figure 47.7. Scheme of the proposed reaction for the formation of 2-methylbenzaldehyde (IV) from the precursor 12-formyl-11-nor-β-carotene (I) through cyclation (intermediates II and III) and subsequent heterolysis.

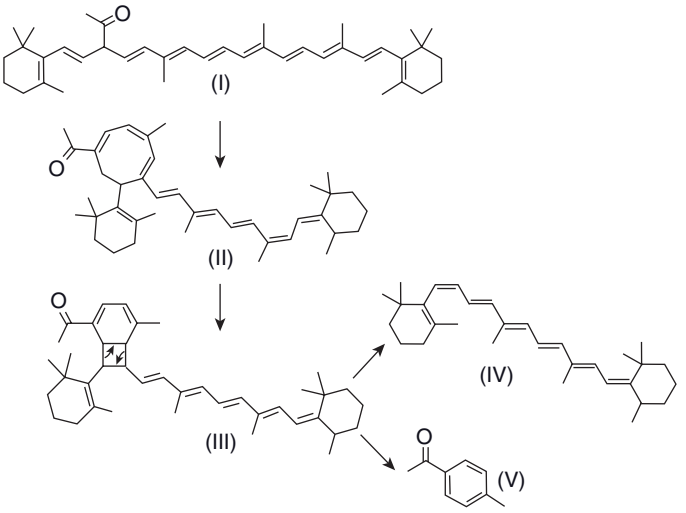

Figure 47.8. Scheme of the reaction proposed for the formation of ethanone-1,(methylphenyl) (V) from the precursor 19-oxomethyl-10-nor-β-carotene through intramolecular cyclation (intermediates II and III) and subsequent in-chain loss unit.

Concentration of volatile degradation products is directly correlated with the intensity (time and/or temperature) of the thermal treatment. From the volatile fraction detected in paprika oleoresins, toluene and *m*-xylene stand out because of their potential toxic effects (Agency for Toxic Substances and Disease Registry 2000, 2005). Lack of regulations on the maximum permitted levels in oleoresins calls for exhaustive studies on the quantitative presence of these products and the establishment of limits for them. Application of extraction procedures that minimize the

intensity of thermal treatments and, consequently, the generation of those volatiles should be considered. Extraction with supercritical fluids is a plausible alternative, and analyses of oleoresins obtained with that extraction procedure should be performed to state whether the volatile fraction resembles the one of dry fruits.

REFERENCES

Agency for Toxic Substances and Disease Registry. 2000. Toxicological profile for toluene. Atlanta, GA: US Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 2005. Draft toxicological profile for m-xylene. Atlanta, GA: US Department of Health and Human Services.

Anu A, Peter KV. 2000. The chemistry of paprika. *Indian Spices* 37:15–8.

Attuquayefio VK, Buckle KA. 1987. Rapid sample preparation method for HPLC analysis of capsaicinoids in capsicum fruits and oleoresins. *J Agric Food Chem* 35(5):777–9.

Bajaj KL. 1980. Colorimetric determination of capsaicin in capsicum fruits. *J Assoc Off Anal Chem* 63(6):1314–6.

Barbero GF, Palma M, Barroso CG. 2006. Pressurized liquid extraction of capsaicinoids from peppers. *J Agric Food Chem* 54(9):3231–6.

Bennett DJ, Kirby GW. 1968. Constitution and biosynthesis of capsaicin. *J Chem Soc C: Organic* 442–6.

Bernal MA, Calderón A, Pedreño MA, Muñoz R, Ros-Barceló A, Medina de Cáceres F. 1993. Capsaicin oxidation by peroxidase from *Capsicum annuum* (var. annuum) fruits. *J Agric Food Chem* 41(7):1041–4.

Bernal MA, Calderon AA, Pedreño MA, Ferrer MA, Merino de Cáceres F, Ros Barceló A. 1994a. Purification of a basic peroxidase isoenzyme from capsicum fruits and the immunoinhibition of its capsaicin oxidation capacity by antibodies raised against horseradish peroxidase. *Z Lebensm Unters Forsch* 199(3):240–2.

Bernal MA, Merino de Cáceres F, Ros Barceló A. 1994b. Histochemical localization of peroxidase in capsicum fruits. *Food Sci Technol-LEB* 27(2):197–8.

Bernal MA, Ros-Barceló A. 1996. 5,5′-Dicapsaicin,4′-*O*-5-dicapsaicin ether, and dehydrogenation polymers with high molecular weights are the main products of the oxidation of capsaicin by peroxidase from hot pepper. *J Agric Food Chem* 44(10):3085–9.

Buck SH, Burks TF. 1986. The neuropharmacology of capsaicin: A review of some recent observations. *Pharmacol Rev* 38(3):179–226.

Buttery RG, Seifert RM, Guadagni DG, Ling LC. 1969. Characterization of some volatile constituents of bell peppers. *J Agric Food Chem* 17(6):1322–7.

Caterina MJ, Julius D. 2001. The vanilloid receptor: A molecular gateway to the pain pathway. *Ann Rev Neurosci* 24:487–517.

Chitwood RL, Pangborn RM, Jennings W. 1983. GC/MS and sensory analysis of volatiles from three cultivars of *Capsicum*. *Food Chem* 11(3):201–16.

Contreras-Padilla M, Yahia EM. 1998. Changes in capsaicinoids during development, maturation, and senescence of chile peppers and relation with peroxidase activity. *J Agric Food Chem* 46(6):2075–9.

Council of Europe. 2001. Committee of experts on flavouring substances. In Scientific Committee on Food, *Datasheet on Capsaicin*. Brussels, Belgium: European Commission, pp. 1–12.

Cremer DR, Eichner K. 2000. Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. *J Agric Food Chem* 48(6):2454–60.

Davis CB, Markey CE, Busch MA, Busch KW. 2007. Determination of capsaicinoids in habanero peppers by chemometric analysis of UV spectral data. *J Agric Food Chem* 55(15): 5925–33.

Dicecco JJ. 1976. Gas-liquid chromatographic determination of capsaicin. *J Assoc Off Anal Chem* 59(1):1–4.

Directive. 1988a. Council Directive 88/388/EEC of 22 June 1988 on the approximation of the laws of the member states relating to flavourings for use in foodstuffs and to source materials for their production. *Official Journal L* 184:61–6.

Directive. 1988b. Council Decision 88/389/EEC of 22 June 1988 on the establishment, by the Commission, of an inventory of the source materials and substances used in the preparation of flavourings. *Official Journal L* 184:67.

Directive. 1995. Commission Directive 95/45/EC of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs. *Official Journal L* 226:1–45.

Directive. 1999. Commission Decision 1999/217/EC of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. *Official Journal L* 84:1–137.

Directive. 2004. Commission Decision 2004/357/EC of 7 April 2004 amending Decision 1999/217/EC as regards the register of flavouring substances. *Official Journal L* 113:28–36.

Dowell KJ, Chambers E, Milliken GA, Chambers DH. 2005. Predicting interstimulus intervals between samples for capsaicin-containing salsa with a range of heat levels. *J Sens Stud* 20(3):187–99.

Dravnieks A. 1985. *Atlas of Odor Character Profiles*. Philadelphia, PA: American Society for Testing and Materials.

Fujikawe H, Susuki T, Iwai K. 1982. Intracellular distribution of enzymes and intermediates involved in the biosynthesis of capsaicin and its analogs in capsicum fruits. *Agric Biol Chem* 46(11):2685–9.

Gaillard T, Matthew JA, Wright AJ, Fishwick MJ. 1977. The enzymatic breakdown of lipids to volatile and non-volatile carbonyl fragments in disrupted tomato fruits. *J Sci Food Agric* 28(9):863–8.

Games DE, Alcock N, van Der Greef J, Nyseen L, Maarse H, Ten Noever de Brauw M. 1984. Analysis of pepper and capsicum oleoresins by high-performance liquid chromatography-mass spectrometry and field desorption mass spectrometry. *J Chromatogr* 294:269–79.

García-Galindo H, Ortega-Martínez J, García-Rosales M, Martínez C, Beristáin C. 1995. La capsaicina, el principio pungente del chile; su naturaleza, absorción, metabolismo y efectos farmacológicos. *Ciencia* 46:84–102.

Geppetti P, Trevisani M. 2004. Activation and sensitisation of the vanilloid receptor: Role in gastrointestinal inflammation and function. *Br J Pharmacol* 141(8):1313–20.

Gibbs HAA, O'Garro LW. 2004. Capsaicin content of West Indies hot pepper cultivars using colorimetric and chromatographic techniques. *HortScience* 39(1):132–5.

Gillette MH, Appel CE, Lego MC. 1984. A new method for sensory evaluation of red pepper heat. *J Food Sci* 49(4):1028–33.

Govindarajan VS. 1986. Capsicum production, technology, chemistry, and quality. Part II. Processed products, standards, world production and trade. *Crit Rev Food Sci* 23(3): 207–88.

Govindarajan VS, Narashimhan S, Dhanaraj S. 1977. Evaluation of spice and oleoresins. II: Pungency of capsicum by Scoville heat units-a standardized procedure. *J Food Sci Technol* 26:435–74.

Govindarajan VS, Rajalakshmi D, Chand N. 1987. Capsicum production, technology, chemistry, and quality. Part IV. Evaluation of quality. *Crit Rev Food Sci* 25(3):185–282.

Govindarajan VS, Sathyanarayana MN. 1991. Capsicum production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. *Crit Rev Food Sci* 29(6):435–73.

Guadayol JM, Caixach J, Ribé J, Cabañas J. 1997. Extraction, separation and identification of volatile organic compounds from Paprika oleoresin (Spanish type). *J Agric Food Chem* 45(5):1868–72.

Hartman KT. 1970. A rapid GLC determination for capsaicin in capsicum spices. *J Food Sci* 35(5):543–7.

Hawer WS, Ha J, Hwang J, Nam Y. 1994. Effective separation and quantitative analysis of major heat principles in red pepper by capillary gas chromatography. *Food Chem* 49(1):99–103.

Haymon LW, Aurand LW. 1971. Volatile constituents of tobacco peppers. *J Agric Food Chem* 19(6):1131–4.

Heath BH. 1981. Natural flavoring material. In Heath BH (ed.), *Source Book of Flavors*. Westport, CT: Avi Publishing Company, pp. 148–287.

Heresch F, Jurenitsch J. 1979. Off-line mass spectrometric monitoring of HPLC-effluents–An improved identification and quantitation method for mixtures of similar compounds: Natural capsaicinoids. *Chromatographia* 12(10):647–50.

Huffman VL, Schadle ER, Villalon B, Burns EE. 1978. Volatile components and pungency in fresh and processed Jalapeño peppers. *J Food Science* 43(6):1809–11.

Iwai K, Susuki T, Fujiwaki H. 1979a. Formation and accumulation of pungent principle of hot pepper fruits, capsaicin and its analogues, in *Capsicum annuum* var. annuum cv. Karayatsubusa at different growth stages after flowering. *Agr Biol Chem* 43(12): 2493–8.

Iwai K, Suzuki T, Fujiwake H. 1979b. Simultaneous microdetermination method of capsacin and its four analogues by HPLC and GC/MS. *J Agric Food Chem* 172:303–11.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2006. *Monograph on Paprika Oleoresin*. Rome, Italy: FAO.

Judd WS, Campbell CS, Kellogg EA, Stevens PF. 1999. *Plant Systematics: A Phylogenetic Approach*. Sunderland, MA: Sinauer Associates.

Jurenitsch J, Kubelka W, Jentzsch K. 1978. Gaschromatographische bestimmung des einzel— und gesamtcapsaicinoidgehaltes in Fructus Capsici nach dünnschichtchromatographischer vorreinigung. *Sci Pharm* 46:307–18.

Keller U, Flath RA, Mon TR, Teranishi R. 1981. Volatiles from red pepper (*Capsicum* spp.). In Teranishi R, Barrera-Benitez H (eds.), *Quality Selected Fruits and Vegetables of North America, ACS Symposium Series 31*. Washington DC: American Chemical Society, pp. 137–46.

Kim IK, Abd El-Aty AM, Shin HC, Lee HB, Kim IS, Shim JH. 2007. Analysis of volatile compounds in fresh healthy and diseased peppers (Capsicum annuum L.) using solvent free solid injection coupled with gas chromatography-flame ionization detector and confirmation with mass spectrometry. *J Pharm Biomed Anal* 45(3):487–94.

Kirschbaum-Titze P, Hiepler C, Mueller-Seitz E, Petz M. 2002. Pungency in paprika (*Capsicum annuum*). 1. Decrease of capsaicinoid content following cellular disruption. *J Agric Food Chem* 50(5):1260–3.

Korel F, Bagdatlioglu N, Balaban MO, Hisil Y. 2002. Ground red peppers: Capsaicinoids content, scoville scores, and discrimination by an electronic nose. *J Agric Food Chem* 50(11):3257–61.

Kosuge S, Furata M. 1970. Studies on the pungent principle of *Capsicum*. Part XIV: Chemical constitution of the pungent principle. *Agric Biol Chem* 34(2):248–56.

Kozukue N, Han JS, Kozukue E, Lee SJ, Kim JA, Lee KR, Levin CE, Friedman M. 2005. Analysis of eight capsaicinoids in peppers and pepper-containing foods by high performance liquid chromatography and liquid chromatography mass spectrometry. *J Agric Food Chem* 53(23):9172–81.

Lee JH, Lee KT, Kim MR. 2005. Effect of gamma-irradiated red pepper powder on the chemical and volatile characteristics of kakdugi, a Korean traditional fermented radish kimchi. *J Food Sci* 70(7):441–7.

Lee KR, Suzuki T, Kobashi M, Hasegawa K, Iwai K. 1976. Quantitative microanalysis of capsaicin, dihydrocapsaicin and nordihydrocapsaicin using mass fragmentography. *J Chromatogr A* 123(1):119–28.

Lee SS, You IS. 1977. Biodegradation mechanism of capsaicin and its analogues. I. Microbial-w-hydroxylation on capsaicin mixture. *Korean J Biochem* 1:135–46.

Leete E, Louden MCL. 1968. Biosynthesis of capsaicin and dihydrocapsaicin in *Capsicum frutescens*. *J Am Chem Soc* 90(24):6837–41.

López-Carrillo L, Avila HM, Dubrow R. 1994. Chili pepper consumption and gastric cancer in Mexico: A case-control study. *Am J Epidemiol* 139(3):263–71.

Luning PA, de Rijk T, Wichers HJ, Roozen JP. 1994a. Chromatography, mass spectrometry, and sniffing port analyses of volatile compounds of fresh bell peppers (*Capsicum annuum*) at different ripening stages. *J Agric Food Chem* 42(4):977–83.

Luning PA, van der Vuurst de Vries R, Yuksel D, Ebbenhorst-Seller T, Wichers HJ, Roozen JP. 1994b. Combined instrumental and sensory evaluation of flavor of fresh bell peppers (*Capsicum annuum*) harvested at three maturation stages. *J Agric Food Chem* 42(12): 2855–61.

Maillard MN, Giampaoli P, Richard HMJ. 1997. Analysis of eleven capsaicinoids by reversed-phase high performance liquid chromatography. *Flav Frag J* 12(6):409–13.

Mateo J, Aguirrezábal M, Domínguez C, Zumalacárregui JM. 1997. Volatile compounds in Spanish paprika. *J Food Compost Anal* 10(3):225–32.

Mazida MM, Salleh MM, Osman H. 2005. Analysis of volatile aroma compounds of fresh chilli (*Capsicum annuum*) during stages of maturity using solid phase microextraction (SPME). *J Food Compost Anal* 18(5):427–37.

Monsereenusorn Y. 1983. Subchronic toxicity studies of capsaicin and capsicum in rats. *Res Commun Chem Pathol Pharmacol* 41(1):95–110.

North H. 1949. Colorimetric determination of capsaicin in oleoresin of capsicum. *Anal Chem* 21(8):934–6.

Parrish M. 1996. Liquid chromatographic method of determining capsaicinoids in capsicums and their extractives: Collaborative study. *J Assoc Off Anal Chem* 79(3):738–45.

Pérez-Gálvez A, Garrido-Fernández J, Mínguez-Mosquera MI. 2000. Carotenoid content of the varieties Jaranda and Jariza (*Capsicum annuum* L.) and response during the industrial slow drying and grinding steps in paprika processing. *J Agric Food Chem* 48(7):2972–6.

Perkins B, Bushway R, Guthire K, Fan T, Stewart B, Price A, Williums M. 2002. Determination of capsaicinoids in salsa by liquid chromatography and enzyme immunoassay. *J AOAC Int* 85(1):82–5.

Pino J, González M, Ceballos L, Centurión-Yah AR, Trujillo-Aguirre J, Latournerie-Moreno L, Sauri-Duch E. 2007. Characterization of total capsaicinoids, color and volatile compounds of Habanero chilli pepper (*Capsicum chínense* Jack) cultivars grown in Yucatan. *Food Chem* 104(4):1682–6.

Pino J, Sauri-Duch E, Marbot R. 2006. Changes in volatile compounds of Habanero chile pepper (*Capsicum chinense* Jack.cv. Habanero) at two ripening stages. *Food Chem* 94(3):394–8.

Prasad BCN, Gururaj HB, Kumar V, Giridhar P, Ravishankar GA. 2006. Valine pathway is more crucial than phenyl propanoid pathway in regulating capsaicin biosynthesis in *Capsicum frutescens* Mill. *J Agric Food Chem* 54(18):6660–6.

Pruthi JS. 2003. Chemistry and quality control of capsicum and capsicum products. In Krishna De A (ed.), *Capsicum*, Vol. 33. London: Taylor and Francis, pp. 25–70.

Ramos-Palacio JJ. 1977. Spectrophotometric determination of capsaicin. *J Assoc Off Anal Chem* 60(4):970–2.

Ramos-Palacio JJ. 1979. Further study of the spectrophotometric determination of capsaicin. *J Assoc Off Anal Chem* 62(5):1168–70.

Regulation. 1996. Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996 laying down a Community procedure for flavouring substances used or intended for use in or on foodstuffs. *Official Journal L* 299:1–4.

Reilly CA, Crouch DJ, Yost GS, Fatah AA. 2002. Determination of capsaicin, nonivamide, and dihydrocapsaicin in blood and tissue by liquid chromatography-tandem mass spectrometry. *J Anal Toxicol* 26(6):313–9.

Rhyu HY. 1978. Some comments on the estimation of sensory threshold by Kaerber and Scoville method. *J Food Sci* 43(5):1632–3.

Richard HMJ. 1991. Spices and condiments I. In Maarse H (ed.), *Volatile Compounds in Foods and Beverages*. New York: Marcel Dekker, pp. 411–48.

Rios JJ, Fernández-García E, Mínguez-Mosquera MI, Pérez-Gálvez A. 2008. Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins. *Food Chem* 106(3):1145–53.

Rymal KS, Cosper RD, Smith DA. 1984. Injection-extraction procedure for rapid determination of relative pungency in fresh jalapeño peppers. *J Assoc Off Anal Chem* 67(3):658–9.

Santamaría RI, Reyes-Duarte MD, Bárzana E, Fernando D, Gama FM, Mota M, López-Munguía A. 2000. Selective enzyme-mediated extraction of capsaicinoids and carotenoids from chili Guajillo Puya (*Capsicum annuum* L.) using ethanol as solvent. *J Agric Food Chem* 48(7):3063–7.

Schweiggert U, Schieber A, Carle R. 2006. Effects of blanching and storage on capsaicinoid stability and peroxidase activity of hot chili peppers (*Capsicum frutescens* L.). *Innov Food Sci Emerg Technol* 7(3):217–24.

Scoville WL. 1912. Note on capsicums. *J Am Pharm Assoc* 1:453–4.

Sheen LY, Tsai Ou YH, Tsai SJ. 1991. Flavor characteristic compounds found in the essential oil of *Ocimum basilicum* L.with sensory evaluation and statistical analysis. *J Agric Food Chem* 39(5):939–43.

Stewart C Jr., Kang BC, Liu K, Mazourek M, Moore SL, Yoo EY, Kim BD, Param I, Jahn MM. 2005. The Pun1 gene for pungency in pepper encodes a putative acyltransferase. *Plant J* 42(5):675–88.

Stewart C Jr., Mazourek M, Stellari GM, O'Connell M, Jahn M. 2007. Genetic control of pungency in *C. chinense* via the Pun1 locus. *J Exp Bot* 58(5):979–91.

Sung Y, Chang YY, Ting NL. 2005. Capsaicin biosynthesis in water-stressed hot pepper fruits. *Bot Bull Acad Sinica* 46(1):35–42.

Suresh D, Manjunatha H, Srinivasan K. 2007. Effect of heat processing of spices on the concentrations of their bioactive principles: Turmeric (*Curcuma longa*), red pepper (*Capsicum annuum*) and black pepper (*Piper nigrum*). *J Food Compost Anal* 20(3–4):346–51.

Szallasi A, Blumberg PM. 1999. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacol Rev* 51(2):159–211.

Szallasi A, Cruz F, Geppetti P. 2006. TRPV1: A therapeutic target for novel analgesic drugs? *Trends Mol Med* 12(11):545–54.

Szolcsányi A, Jancsó-Gábor A. 1976. Sensory effects of capsaicin congeners. II. Importance of chemical structure and pungency in desensitizing activity of capsaicin-type compounds. *Arzneimittelforschung* 26:33–7.

Szolcsányi J. 1989. Capsaicin, irritation and desensitization. Neurophysiological basis and future perspectives. In Geen BG, Mason JR, Kare MR (eds.), *Chemical Senses, Irritation* Vol. 2. New York: Marcel Dekker, pp. 141–68.

Szolcsányi J, Jancsó-Gábor A. 1975. Sensory effects of capsaicin congeners. I. Relationship between chemical structure and pain-producing potency. *Arzneimittelforschung* 25:1877–81.

Tewksbury J, Nabhan GP. 2001. Directed deterrence by capsaicin in chillies. *Nature* 412(6845):403–4.

Thompson RQ, Phinney KW, Welch MJ, White E. 2005. Quantitative determination of capsaicinoids by liquid chromatography-electrospray mass spectrometry. *Anal Biochem* 381(7):1441–51.

Tice LF. 1933. Simplified method for extracting capsaicin with colorimetric method for its determination. *Am J Pharm* 105:320.

Todd PH, Beninger MG, Biftu T. 1977. Determination of pungency due to capsicum by gas-liquid chromatography. *J Food Sci* 42(3):660–8.

Van Ruth S, Boscaini E, Mayr D, Pugh J, Posthumus M. 2003. Evaluation of three gas chromatography and two direct mass spectrometry techniques for aroma analysis of dried red bell peppers. *Int J Mass Spectrom* 223(1–3):55–65.

Van Ruth SM, Roozen JP. 1994. Gas chromatography/sniffing port analysis and sensory evaluation of commercially dried bell peppers (*Capsicum annuum*) after rehydration. *Food Chem* 51(2):165–70.

Van Ruth SM, Roozen JP, Cozijnsen JL. 1995a. Volatile compounds of rehydrated French beans, bell peppers and leeks. Part 1. Flavour release in the mouth and in three mouth model systems. *Food Chem* 53(1):15–22.

Van Ruth SM, Roozen JP, Cozijnsen JL, Posthumus MA. 1995b. Volatiles compounds of rehydrated French beans, bell peppers and leeks. Part II. Gas chromatography/sniffing port analysis and sensory evaluation. *Food Chem* 54(1):1–7.

Wall MM, Bosland PW. 1998. Analytical methods for color and pungency of chiles (capsicums). *Dev Food Sci* 39:347–73.

Way RM. 1985. *Official Analytical Methods of the American Spice Trade Association*, Vol. 3. Washington DC: American Spice Trade Association, pp. 51–2.

Woodbury JE. 1980. Determination of capsicum pungency by high-pressure liquid-chromatography and spectrofluorometric detection. *J Am Oil Chem Soc* 63(3):556–8.

Wu C, Liou S. 1986. Effect of tissue disruption on volatile constituents of bell peppers. *J Agric Food Chem* 34(4):770–2.

Wu CM, Liou SE, Wang MC. 1986. Changes in volatile constituents of bell peppers immediately and 30 minutes after stir frying. *J Am Oil Chem Soc* 63(9):1172–5.

Zewdie Y, Bosland PW. 2000. Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica* 111(3): 185–90.

CHAPTER 48

Potato Flavor

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INTRODUCTION

Potato is one of the most popular vegetables worldwide and is the most important vegetable crop in the United States, accounting for nearly one-third of per-capita vegetable consumption (Lin and Yen 2004). Potatoes can be prepared in many ways, including baking, boiling, roasting, frying, and microwaving, allowing for a diversity of uses. Most people find potatoes to be an agreeable food, and very few (less than 1%) actually dislike potatoes (Harper 1963). Potato flavor results from the combination of taste, aroma, and texture. Flavor precursors synthesized by the plant are present in raw potatoes and consist mainly of sugars, amino acids, RNA, and lipids (Table 48.1). Plant genotype, production environment, and storage environment influence the levels of these compounds and the enzymes that react with them to produce flavor compounds. During cooking, flavor precursors react to produce the Maillard reaction compounds and the sugar, lipid and RNA degradation products that contribute to flavor (Duckham et al. 2001).

Domestication has led to a reduction in flavor compounds in fruits such as tomatoes (Goff and Klee 2006) and strawberries (Aharoni et al. 2004). Presumably, selection by breeders for yield, appearance, and disease resistance has resulted in an unintended loss of flavor compounds. Fruits emit volatile compounds in order to attract seed dispersal agents, which seek them out as a source of nutrition (Goff and Klee 2006). Essential nutrients contribute to most volatiles produced by tomato fruits. The volatiles released during fruit ripening act as flavor compounds that signal desirable taste and high nutrient availability. In contrast, potato tubers and other vegetables are not seed dispersal organs. They do not emit volatile flavor compounds at maturity. Instead, elements of flavor develop from compounds in tuber tissues when they are sliced and/or heated. Since flavor compounds per se are not necessary for the function of potato tubers as asexual reproductive structures, it would be interesting to know whether tubers of wild and cultivated potato relatives differ in types and concentrations of flavor compounds. This is an open area for research.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

TABLE 48.1. Approximate Levels of Potato Tuber Components

Compound	Percent Fresh Weight
Starch	18.0
Protein	2.0
Fiber (suberin, lignin)	1.3
Sugars (glucose, fructose, and sucrose)	1.0
Minerals (K, Mg, Ca, P, Na)	1.0
Free amino acids	0.8
Non-starch polysaccharides (hemicelluloses and pectins)	0.7
Organic acids (citric, oxalic, malic, and chlorogenic)	0.2
Lipids (fatty acids include linoleic, linolenic, and palmitic)	0.1
Pigments (anthocyanins and carotenoids)	0.01
Glycoalkaloids (solanine and chaconine)	0.01
Nucleotides, RNA	0.01

Note: Actual values vary depending on cultivar, production environment, and storage conditions.

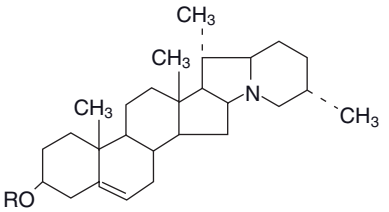

Figure 48.1. Chemical structure of α-solanine and α-chaconine, the most common glycoalkaloids in potato tubers. The R group for α-solanine consists of glucose and two moieties of rhamnose. The R group for α-chaconine consists of glucose, galactose, and rhamnose.

TASTE

Human taste receptors monitor bitter, sour, sweet, salty, and umami flavors. In vegetables, bitterness is considered a deterrent and sweetness is a stimulant for consumption (Dinehart et al. 2006). Unlike fruits, potato tubers have evolved mechanisms to deter consumption. Toxic glycoalkaloids in wild potato tubers produce a strong bitter taste, providing protection against pests and disease (Valkonen et al. 1996). Domestication has selected for low levels of bitterness in potato tubers (Johns and Alonso 1990), but they still contain glycoalkaloids. The major glycoalkaloids in commercial potato cultivars are α-solanine and α-chaconine (Bushway and Ponnampalam 1981). Each is composed of the alkaloid solanidine plus three monosaccharides (Fig. 48.1). Glycoalkaloids are concentrated in the skin of tubers, so when consumed with the skin on, small tubers may taste more bitter than large tubers. Although the upper limit allowed for a new cultivar release is 20 mg/100 g fresh weight, bitterness can be tasted in tubers with glycoalkaloid levels higher than 14 mg/100 g (Sinden et al. 1976). At low levels (below 10 mg/100 g), glycoalkaloids may make a positive contribution to flavor (Ross et al. 1978).

Organic acids determine the acidity of potato tubers. They are produced by the incomplete oxidation of sugars and deamination of amino acids, ascorbic acid, and

polyphenolic acids (Lisinska and Aniolowski 1990). They are generally not considered to be major flavor components (Vainionpaa et al. 2000). However, a positive correlation between levels of phenolic compounds and bitterness/astringency has been reported (Mondy et al. 1971). Sinden and others (1976), on the other hand, did not find a strong relationship between phenolic content and bitterness. They did note that tubers containing 120 mg/100 g chlorogenic acid tasted slightly sour to some panelists.

Starch is the main carbohydrate in potato tubers. Although starch is tasteless, it influences texture and can also form stable complexes with flavor compounds during cooking (Solms and Wyler 1979). Potato tubers also contain low levels of sugars such as glucose, fructose, and sucrose, which are not typically considered to directly contribute to taste (Solms and Wyler 1979). In fact, sweetness has historically been considered to be an undesirable flavor component in potatoes (Burton 1966). However, the consumption of sugars in developed countries has increased over the past 30 years (Pereira and Simin 2003). Today's consumers are likely to have a strong preference for sweet foods. In fact, we have found that sweetness of baked potatoes is significantly correlated with desirable flavor (Jansky 2008). Similarly, sucrose and reducing sugar levels have been found to be important factors in determining potato flavor attributes (Vainionpaa et al. 2000).

Ribonucleotides act as precursors for flavor potentiators, known as umami compounds, which are associated with desirable flavor. Potato tubers have higher levels of 5′ ribonucleotides than any other plant food (Solms and Wyler 1979). While they are present in low quantities in raw potatoes, 5′ ribonucleotides are liberated by enzymatic hydrolysis of RNA as tubers are heated during cooking. Steamed or boiled tubers of *Solanum tuberosum* Phureja group cultivars (South American landraces) with higher levels of glutamate and guanosine 5′-monophosphate (GMP) than *S. tuberosum* cultivars had higher acceptability scores in taste tests (Morris et al. 2007). The most important ribonucleotides for flavor enhancement are inosine 5′-monophosphate (IMP) and GMP. Both levels and types of ribonucleotides vary among potato cultivars (Maga and McNeill 1986). This is probably due to differences in the activities and types of enzymes that break down RNA. A synergistic effect is detected when 5′ ribonucleotides interact with amino acids, especially glutamate. In fact, the products of interactions between amino acids and 5′ ribonucleotides are considered to be mainly responsible for boiled potato flavor (Halpern 2000; Solms 1971). Sugars may also contribute to umami taste characters in the form of glutamate glycoconjugates (Beksan et al. 2003). In addition, potassium salts have been found to enhance umami taste intensity (Ugawa and Kurihara 1994). Significant levels of potassium leach out of potatoes during boiling (Bethke and Jansky 2008). It would be interesting to know whether tuber potassium levels enhance umami flavor intensity more effectively in baked potatoes, which retain potassium, than in boiled potatoes, which lose potassium.

AROMA

Cooked potatoes contain a complex array of aroma compounds. In one study, 228 volatile compounds were found to contribute to baked potato flavor (Coleman et al. 1981). The most important aroma compounds are produced by lipid

degradation and by the Maillard reaction and/or sugar degradation during the heating of potato tubers (Oruna-Concha et al. 2002). In the nonenzymatic Maillard reaction, reducing sugars (glucose and fructose) interact with amino acids at high temperature.

During baking, the surface temperature of the tuber increases first and water evaporates from the skin. As the temperature of the skin rises over 100°C, a crust develops and the tuber gradually warms from the outside toward the interior. At these high temperatures, baked potatoes produce a complex array of volatile compounds including lipid degradation products, Maillard reaction products, sulfur compounds, and methoxypyrazines (Oruna-Concha et al. 2001). Pyrazines are considered to be among the most important and characteristic components of baked potato flavor (Buttery et al. 1973). There is a strong positive relationship between pyrazines and organoleptic quality in both baked potatoes (Maga and Holm 1992) (Fig. 48.2) and potato chips (Maga and Sizer 1973). Pyrazines are produced by the Maillard reaction, which is the same reaction that causes dark fries and chips. It is interesting that industry standards require light-colored chips. While there is some visual preference for light-colored chips, blindfolded taste panelists clearly prefer the taste and odor of dark-colored chips (Maga 1973).

In contrast to baked potatoes, water loss in boiled potatoes is minimal and the interior warms quickly. However, the tuber temperature never rises above 100°C, as it does during baking. The major aroma components of boiled potatoes include methional, aliphatic alcohols and aldehydes, thiols and sulfides, and methoxypyrazines (Maga 1994; Mutti and Grosch 1999; Ulrich et al. 2000). Boiled tubers contain higher levels of lipid degradation products than do baked potatoes (Oruna-Concha et al. 2002). The disruption of tissues during slicing in preparation for boiling

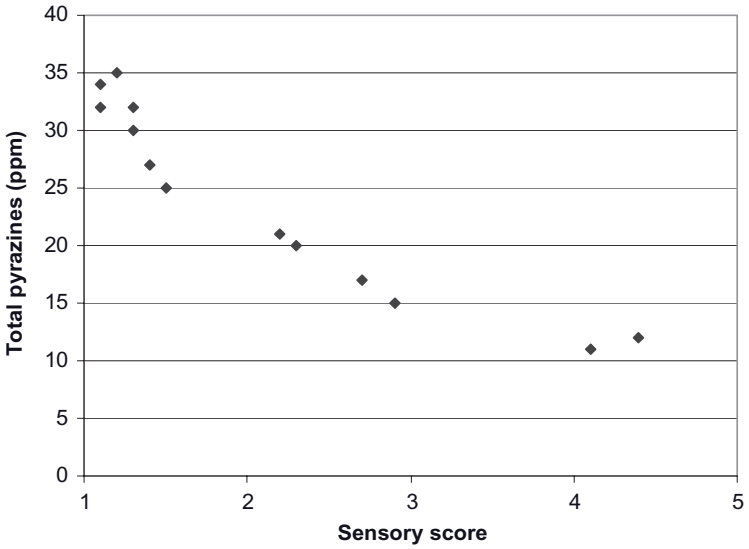

Figure 48.2. Relationship between sensory score (1 = positive, 5 = negative) and total pyrazines in baked potatoes of 13 cultivars and breeding clones. Derived from Maga and Holm (1992).

provides more opportunities for lipoxygenase to come into contact with its substrates. In addition, during boiling, tuber tissues heat up more gradually, allowing time for lipoxygenase to oxidize lipids. Lipid-derived flavor components in boiled tubers, then, are expected to include both products of enzymatic oxidation and thermal degradation, while those from baking are primarily products of thermal degradation. Fatty acids degrade to produce aldehydes and ketones, which contribute to fatty, fruity, and floral flavor notes (Duckham et al. 2002). Another major contributor to boiled potato aroma is the lipid oxidation product c4-heptanal (Josephson and Lindsay 1987). It produces an earthy aroma at low levels. However, the higher levels found in dehydrated potatoes following storage result in stale flavors. The aldehydes produced by lipid oxidation have been implicated in the off-flavor of boiled potatoes after they are refrigerated (Peterson and Poll 1999).

During microwave baking, the tuber temperature increases relatively uniformly, with all parts reaching 100°C within a few minutes of each other (Oruna-Concha et al. 2002). In contrast to oven baking, though, the skin remains cooler than the interior of the tuber due to evaporative cooling, and a crust does not develop. Because there is no crust, the rate of water loss is higher. Microwave-baked potatoes have lower levels of volatiles than oven-baked or boiled potatoes, probably due to evaporative cooling at the tuber surface and the loss of volatile compounds through co-distillation as water evaporates.

Methional is a major aroma compound that is formed by the Strecker degradation reaction, in which intermediates in the Maillard reaction interact with the amino acid methionine. "Russet Burbank" plants transformed with a gene to enhance methionine biosynthesis produced tubers with two to four times more methional than those of untransformed plants (Di et al. 2003). Levels of methional vary among cultivars and production environments (Duckham et al. 2002; Oruna-Concha et al. 2001). It is interesting that this compound is not detectable in all potato cultivars. In fact, in one study, it was found in only 5 out of 11 cultivars evaluated (Duckham et al. 2001).

Unlike most flavor compounds, methoxypyrazines are present in raw tubers, so heating is not required for their production. They are products of free amino acids and are responsible for subtle earthy flavor notes. They may be produced by the tuber and have been found in higher amounts in boiled potatoes than in baked potatoes (Oruna-Concha et al. 2002). The production of methoxypyrazines may be increased by the cell damage that results from peeling tubers in preparation for boiling. Alternatively, methoxypyrazines may be produced by soil bacteria (*Pseudomonas taetrolens*) and then absorbed by the tuber (Buttery et al. 1973). Consequently, the production environment may make an important contribution to this flavor component. The odor threshold of these compounds is very low. For example, the odor threshold of a common methoxypyrazine, 2-methoxy-3-isopropylpyrazine, in water is two parts in 10^{12}. Consequently, methoxypyrazines are said to have a high aroma impact value (Duckham et al. 2002). 2-Methoxy-3-isopropylpyrazine has been found in tubers at one part in 10^{10} (Buttery et al. 1973). Therefore, even though it is present at very low levels, it is detectable when a potato is eaten. In biochemical analyses, methoxypyrazines are not detectable in many cultivars and are present at very low levels in others. Because of their high aroma impact values, though, small changes in methoxypyrazine levels are expected to have large effects on flavor.

TEXTURE

Texture is one of the most important quality attributes of potato tubers. It is determined mainly by cultivar, but with effects of production environment and storage. This component of flavor is easily recognizable by consumers, who tend to have distinct preferences. While potato texture is a complex trait, much variation can be explained by determining the degree of a tuber's mealiness or, at the opposite end of the spectrum, waxiness (Faulks and Griffiths 1983; van Marle et al. 1997a). A mealy potato is dry and granular, while a waxy potato is moist and gummy. Mealiness has been found to be associated with high dry matter content (Jansky 2008; Leung et al. 1983; van Dijk et al. 2002). As a result of selection by breeders for market class qualities, red-skinned potatoes are typically waxy, while white and russet processing potatoes are mealy. Mealiness is one of the readily described components of flavor for taste panelists. In fact, detection thresholds for mealiness by taste panelists have been identified (Murphy et al. 1967). However, dry matter content does not always explain mealiness. In one sensory analysis study, the cultivar "Ontario" was judged to be less mealy than other cultivars in the trial, but its total solids content was similar to some of those cultivars (True and Work 1981).

Another component of texture is the size and structure of starch grains in raw tuber tissue (Thybo and Martens 1999). Starch gelatinizes during cooking and creates pressure in cells as it expands. The proportion of each tuber cell occupied by gelatinized starch influences the moistness components of texture (Martens and Thybo 2000). A large volume of gelatinized starch is associated with a mealy texture, while cells that contain less starch and more loosely held water produce a waxy texture (Martens and Thybo 2000; McComber et al. 1994). The loosely held water in the latter cell type is released upon chewing, producing a moist mouthfeel in sensory analyses. The gelatinized starch in the mealy types retains water, creating a dry mouthfeel. Cell size has also been found to be associated with mealiness (Barrios et al. 1963). Tubers with high mealiness scores by taste panelists were found to contain more starch and have larger cells than less mealy tubers.

Cell wall characteristics can also affect texture (Jarvis and Duncan 1992; Jarvis et al. 1992; Martens and Thybo 2000; McComber et al. 1994; van Marle et al. 1997a). Pectin methyl esterase activity is important for creating firm tissue during cooking by cross-linking pectin in the middle lamella, which binds cells together (Faulks and Griffiths 1983; Thybo and Martens 1999). Cultivars vary in cell wall density and in the degree of solubilization of the middle lamella and cell walls, which are believed to influence texture (van Marle et al. 1997a). The mealy cultivar "Irene" was found to have more cell wall material per unit cell surface area than the non-mealy cultivar "Nicola." The cell wall and middle lamella were also found to be thicker in the mealy cultivar "Russet Burbank" than in the waxy cultivar "Red Pontiac" (McComber et al. 1994 [#362]). The thicker cell walls and middle lamellae, along with stronger pectic substances, may result in more resistance to shearing and a hard, particulate mouthfeel. Both cohesiveness and adhesiveness of cells in tuber tissue influence texture. In one study, while either factor alone did not correlate with mealiness of boiled potatoes, a strong negative correlation (–0.87) was detected between mealiness and the product of the two parameters (Leung et al. 1983).

EFFECT OF PRODUCTION ENVIRONMENT ON FLAVOR

Production environment may affect sensory quality. Levels of methional vary among tubers harvested from different production environments (Duckham et al. 2002; Oruna-Concha et al. 2001). Because methionine contains sulfur, it has been suggested that sulfur application rates in the field may account for some differences in methional levels (Duckham et al. 2002). Similarly, potassium application in the field may influence umami flavor intensity (Morris et al. 2007). Methoxypyrazines may be produced by soil bacteria (*P. taetrolens*) and then absorbed by the tuber, so soil microbe populations may influence flavor (Buttery et al. 1973). In a study involving three U.K. cultivars, the influence of production site on texture (as measured by physical properties and sensory analyses) was stronger than that of cultivar (Faulks and Griffiths 1983).

The levels of off-flavors may be influenced by the production environment as well. As nitrogen levels increase, sensory quality decreases, probably due to the production of acrid-tasting amides and amines (Cieslik 1997; Jansky 2008; Thybo et al. 2006). Differences in production environment were found to influence the development of off-flavor in precooked vacuum-packed potatoes, but specific environmental parameters influencing off-flavor were not determined (Jensen et al. 1999). A musty off-flavor detected in cooked potatoes was found to be due to the presence of 2,4,6-trichloroanisole (TCA). While TCA is not a known metabolite of potato, it was found in tubers harvested from pesticide-treated soils after an exceptionally warm production year (Daniels-Lake et al. 2007). Glycoalkaloid levels and, consequently, bitter flavor may increase in tubers grown under stressful conditions and in tubers that are exposed to light during harvest, storage, and/or marketing (Percival et al. 1994; Sinden and Webb 1972; Uppal 1987; Valkonen et al. 1996). In addition, bruising during harvest can result in significant increases in levels of glycoalkaloids and chlorogenic acid (Dale et al. 1998), both of which may contribute to bitterness.

Some studies have evaluated the effects of organic versus conventional production systems on sensory attributes. Using triangle tests, Wszelaki and others (2005) found that taste panelists were able to distinguish between conventionally and organically grown boiled red potatoes if the skin was left on the tubers while boiling. If the skin was removed during boiling, then differences could not be detected. Hajslova and others (2005) determined that cultivar and production year are important influences on the sensory quality of boiled potatoes, but organic production systems are not consistently superior or inferior to conventional. Jansky (2008) also reported that no flavor differences were detected between organic and conventionally grown potatoes. Minor effects of organic fertilizer treatments have been reported to influence moistness, mealiness, color, and odor (Thybo et al. 2002). However, a comprehensive review of studies conducted to evaluate organoleptic quality in organic versus conventional systems found that no clear statements could be made regarding the superiority of one type over the other (Woese et al. 1997).

EFFECT OF STORAGE ENVIRONMENT ON FLAVOR

Changes in sensory quality have been reported following the storage of potato tubers. In a sensory analysis study, tubers for 6 months at 5.5°C were found to be mealier,

sweeter, and more flavorful than fresh tubers (Jansky 2008). In addition, levels of off-flavors have been reported to decrease during storage (Jansky 2008; Thybo et al. 2006). True and Work (1981) noted that "Russet Burbank" ranked high and "Ontario" ranked low for flavor preference in fresh baked potatoes, but differences were not detected in tubers stored at 8.2°C for 6 months. In a study of pre-peeled boiled potatoes, cultivar and storage time (0, 1.5, and 6.0 months at 4°C) explained a major portion (68%) of taste, color, and texture attributes (Thybo et al. 2006). Interestingly, in another study, while taste panelists were not able to detect the sprout inhibitor isopropyl-N-chlorophenyl carbamate (CIPC), they were able to taste residual levels of the alternative sprout inhibitor 1,8-cineole (Boylston et al. 2001).

When potato tubers are cooked, fatty acids degrade to produce aldehydes and ketones, which contribute to flavor (Duckham et al. 2002). The total levels of fatty acids and their flavor products increase during storage (Duckham et al. 2002). It is interesting to note that tubers alter their fatty acid profiles as they acclimate to cold storage temperatures. Consequently, both levels and types of fatty acids change. Cultivars vary in the way they change their fatty acid profiles during cold storage (Mondy et al. 1963). However, levels of linoleic acid typically decrease and α-linolenic acid increases (Dobson et al. 2004). Variation exists among cultivars in levels of fatty acids at harvest and after storage.

Lipoxygenase activity in potato tubers increases during cold storage, but the concentrations of off-flavor products, such as aldehydes, were found to decrease when these tubers were boiled and then chilled (Peterson et al. 2003). Since an increase in lipoxygenase activity does not necessarily result in a corresponding increase in aldehydes, further research is needed to determine the availability of substrates in the pathways leading to the production of aroma compounds, including those leading to off-flavor.

The types and levels of Maillard reaction components of flavor also change during storage, presumably due to changes in enzyme activities and levels of flavor precursors, such as sugars (Duckham et al. 2002). Levels of reducing sugars and free amino acids increase during cold storage (Blenkinsop et al. 2002; Fitzpatrick and Porter 1966; Sowokinos 2001). Consequently, aroma intensity due to pyrazines increases during cold storage (Duckham et al. 2002). In addition, Maillard-derived glutamate glycoconjugates, which act as umami compounds, are expected to increase with the accumulation of sugars during storage.

Cultivar-dependent effects of storage on texture have been reported (Faulks and Griffiths 1983; Martens and Thybo 2000; Thybo and Martens 1999). In general, though, tuber mealiness decreases during storage (Ridley and Lindsay 1984; Shetty et al. 1992; van Marle et al. 1997b). Starch is broken down during storage, leading to pitted starch granules (Cottrell et al. 1993). The degradation of starch and the breakdown of the middle lamella during storage may lead to less mealy tubers (Martens and Thybo 2000). In addition, potato tubers lose water during storage and become less turgid. As a result, cells lose their elasticity and are ruptured during cooking, resulting in a less mealy texture (Shetty et al. 1992).

CONCLUSIONS

Flavor is an important marketing trait for any vegetable crop, but potato breeders have not historically focused on selection for superior flavor. The development of

potato varieties with enhanced flavor has the potential to increase consumer interest in fresh market potatoes and to boost consumption. Breeding progress for improved flavor requires both genetic diversity for the trait and an effective way to identify superior clones. The first requirement is not a problem because tremendous diversity is found in cultivated and wild relatives of potato. The second requirement is more problematic because flavor evaluation based on sensory panels is extremely time-consuming and can be carried out on only a few samples at a time. Breeders must to be able to characterize specific components of flavor to allow them to identify superior clones in their programs. In addition, the effects of production environment and storage environment on flavor must be understood. While the biochemical components of potato flavor are well characterized, the connection to taste panel data is often missing. As we increase our knowledge base, our goal is to reach a point where growers can optimize growing, harvest, and storage conditions, while breeders can select for varieties with appropriate levels of flavor compounds, resulting in a highly desirable, nutritious food crop.

REFERENCES

Aharoni A, Giri AP, Verstappen FWA, Bertea CM, Sevenier R, Sun Z, Jogsma MA, Schwab W, Bouwmeester HJ. 2004. Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16:3110–31.

Barrios EP, Newsom DW, Miller JC. 1963. Some factors influencing the culinary quality of Irish potatoes. II. Physical characters. *Am Potato J* 40:200–8.

Beksan E, Schieberle P, Robert F, Blank I, Fay LB, Schlichtherle-Cerny H, Hofmann T. 2003. Synthesis and sensory characterization of novel umami-tasting glutamate glycoconjugates. *J Agric Food Chem* 51(18):5428–36.

Bethke PC, Jansky S. 2008. The effects of boiling and leaching on the content of potassium and other minerals in potatoes. *J Food Sci* 73(5):H80–H85.

Blenkinsop RW, Copp LJ, Yada RY, Marangoni AG. 2002. Changes in compositional parameters of tubers of potato (*Solanum tuberosum*) during low-temperature storage and their relationship to chip processing quality. *J Agric Food Chem* 50:4545–53.

Boylston TD, Powers JR, Weller KM, Yang J. 2001. Comparison of sensory differences of stored Russet Burbank potatoes treated with CIPC and alternative sprout inhibitors. *Am J Potato Res* 78:99–107.

Burton WG. 1966. *The Potato*. Wageningen: H. Veenan and Zonen.

Bushway RJ, Ponnampalam R. 1981. Alpha-chaconine and alpha-solanine content of potato products and their stability during several modes of cooking. *J Agric Food Chem* 29(4): 814–7.

Buttery RG, Guadagni DG, Ling LC. 1973. Volatile components of baked potatoes. *J Sci Food Agric* 24:1125–31.

Cieslik E. 1997. Effect of the levels of nitrate and nitrites on the nutritional and sensory quality of potato tubers. *Hyg Nutr Foodserv Catering* 1:225–30.

Coleman EC, Ho C-T, Chang SS. 1981. Isolation and identification of volatile compounds from baked potatoes. *J Agric Food Chem* 29:42–8.

Cottrell JE, Duffus CM, MacKay GR, Allison MJ. 1993. Changes in the surface morphology of starch granules of the cultivated potato, *Solanum tuberosum* L. during storage. *Potato Res* 36:119–25.

Dale MFB, Griffiths DW, Bain H. 1998. Effect of bruising on the total glycoalkaloid and chlorogenic acid content of potato (*Solanum tuberosum*) tubers of five cultivars. *J Sci Food Agric* 77:499–505.

Daniels-Lake BJ, Prange RK, Gaul SO, McRae KB, de Antueno R, McLachlan D. 2007. A musty "off" flavor in Nova Scotia potatoes is associated with 2,4,6-trichloroanisole released from pesticide-treated soils and high soil temperature. *J Am Soc Hortic Sci* 132(1): 112–19.

Di R, Kim J, Martin MN, Leustek T, Jhoo J, Ho C-T, Tumer R. 2003. Enhancement of the primary flavor compound methional in potato by increasing the level of soluble methionine. *J Agric Food Chem* 51:5695–702.

Dinehart ME, Hayes JE, Bartoshuk LM, Lanier SL, Duffy VB. 2006. Bitter taste markers explain variability in vegetable sweetness, bitterness and intake. *Physiol Behav* 87:304–13.

Dobson G, Griffiths DW, Davies HV, McNicol JW. 2004. Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum* and *Solanum phureja*. *J Agric Food Chem* 52(20):6306–14.

Duckham SC, Dodson AT, Bakker J, Ames JM. 2001. Volatile flavour components of baked potato flesh: A comparison of eleven potato cultivars. *Food/Nahrung* 45:317–23.

Duckham SC, Dodson AT, Bakker J, Ames JM. 2002. Effect of cultivar and storage time on the volatile flavor components of baked potato. *J Agric Food Chem* 50:5640–8.

Faulks RM, Griffiths NM. 1983. Influence of variety, site and storage on physical, sensory and compositional aspects of mashed potato. *J Sci Food Agric* 34:979–86.

Fitzpatrick TJ, Porter WL. 1966. Changes in the sugars and amino acids in chips made from fresh, stored and reconditioned potatoes. *Am Potato J* 43:238–48.

Goff SA, Klee HJ. 2006. Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311:815–19.

Hajslova J, Schulzova V, Slanina P, Janne K, Hellenas KE, Andersson C. 2005. Quality of organically and conventionally grown potatoes: Four-year study of micronutrients, metals, secondary metabolites, enzymic browning, and organoleptic qualities. *Food Addit Contam* 22(6):514–34.

Halpern BP. 2000. Glutamate and the flavor of foods. *J Nutr* 130(4):910s–14s.

Harper R. 1963. Some attitudes to vegetables, and their implications. *Nature* 200:14–18.

Jansky S. 2008. Genotypic and environmental contributions to baked potato flavor. *J Sci Food Agric Rev* 85:455–65.

Jarvis MC, Duncan HJ. 1992. The textural analysis of cooked potatoes. 1. Physical properties of the separate measurement of softness and dryness. *Potato Res* 35:83–91.

Jarvis MC, MacKenzie E, Duncan HJ. 1992. The textural analysis of cooked potatoes. 2. Swelling pressure of starch during gelatinisation. *Potato Res* 35:93–102.

Jensen K, Petersen MA, Poll L, Brockhoff PB. 1999. Influence of variety and growing location on the development of off-flavor in precooked vacuum-packed potatoes. *J Agric Food Chem* 47:1145–9.

Johns T, Alonso JG. 1990. Glycoalkaloid change during the domestication of the potato, *Solanum* section Petota. *Euphytica* 50:203–10.

Josephson DB, Lindsay RC. 1987. C4-heptenal: An influential volatile compound in boiled potato flavor. *J Food Sci* 52(2):328–31.

Leung HK, Barron FH, Davis DC. 1983. Textural and rheological properties of cooked potatoes. *J Food Sci* 48:1470–4.

Lin B-H, Yen ST. 2004. U.S. potato consumption: Looking ahead to 2020. *J Food Prod Market* 10(2):49–65.

Lisinska G, Aniolowski K. 1990. Organic acids in potato tubers: Part 1—The effect of storage temperatures and time on citric and malic acid contents of potato tubers. *Food Chem* 38:255–61.

Maga JA. 1973. Influence of freshness and color on potato chip sensory preferences. *J Food Sci* 38(7):1251–2.

Maga JA. 1994. Potato flavor. *Food Rev Int* 10(1):1–48.

Maga JA, Holm DG. 1992. Subjective and objective comparison of baked potato aroma as influenced by variety/clone. In Charalambous G (ed.), *Food Science and Human Nutrition*. Amsterdam: Elsevier Science Publishers, pp. 537–41.

Maga JA, McNeill JG. 1986. Nucleotide composition of incubated potatoes from five potato (*Solanum tuberosum*) cultivars. *Lebenson Wiss Technol* 19(1):31–3.

Maga JA, Sizer CE. 1973. Pyrazines in foods. A review. *J Agric Food Chem* 21(1):22–30.

Martens HJ, Thybo AK. 2000. An integrated microstructural, sensory and instrumental approach to describe potato texture. *Lebenson Wiss Technol* 33(7):471–82.

McComber DR, Horner HT, Chamberline MA, Cox DF. 1994. Potato cultivar differences assoiciated with mealiness. *J Agric Food Chem* 42:2433–9.

Mondy N, Mattick LR, Owens E. 1963. The effects of storage on total lipides and the fatty acid composition of potatoes. *J Agric Food Chem* 11(4):328–9.

Mondy NI, Metcalf C, Plaisted RL. 1971. Potato flavor as related to chemical composition. 1. Polyphenols and ascorbic acid. *J Food Sci* 36(3):459–61.

Morris WL, Ross HA, Ducreux JM, Bradshaw J, Bryan GJ, Taylor MA. 2007. Umami compounds are a determinant of the flavor of potato (*Solanum tuberosum* L.). *J Agric Food Chem* 55:9627–33.

Murphy EF, True RH, Hogan JM. 1967. Detection threshold of sensory panels for mealiness of baked potatoes as related to specific gravity differences. *Am Potato J* 44:442–51.

Mutti B, Grosch W. 1999. Potent odorants of boiled potatoes. *Food/Nahrung* 43:302–6.

Oruna-Concha MJ, Bakker J, Ames JM. 2002. Comparison of the volatile components of two cultivars of potato cooked by boiling, conventional baking and microwave baking. *J Sci Food Agric* 82:1080–7.

Oruna-Concha MJ, Duckham C, Ames JM. 2001. Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *J Agric Food Chem* 49:2414–21.

Percival GC, Dixon GR, Sword A. 1994. Glycoalkaloid concentration of potato tubers following continuous illumination. *J Sci Food Agric* 66:139–44.

Pereira MA, Simin L. 2003. Types of carbohydrates and risk of cardiovascular disease. *J Womens Health* 12(2):115–22.

Peterson MA, Poll L. 1999. Identification of compounds contributing to boiled potato off-flavour ("POF"). *Lebenson Wiss Technol* 32:32–40.

Peterson MA, Poll L, Larsen LM. 2003. Changes in flavor-affecting aroma compounds during potato storage are not associated with lipoxygenase activity. *Am J Potato Res* 80:397–402.

Ridley SC, Lindsay RC. 1984. Evaluation of factors affecting predictability of mealy textures in potatoes. *J Food Qual* 7:1–16.

Ross H, Pasemann P, Nitzsche W. 1978. Glycoalkaloid content of potatoes and its relationship to location, year and taste. *Z Pflanzenzucht* 80:64–79.

Shetty KK, Dwelle RB, Fellman JK. 1992. Sensory and cooking quality of individually film wrapped potatoes. *Am Potato J* 69:275–87.

Sinden SL, Deahl KL, Aulenbach BB. 1976. Effect of glycoalkaloids and phenolics on potato flavor. *J Food Sci* 41(3):520–3.

Sinden SL, Webb RL. 1972. Effect of variety and location on the glycoalkaloid content of potatoes. *Am Potato J* 49:334–8.

Solms J. 1971. *Nonvolatile Compounds and Flavour*. London: Academic Press.

Solms J, Wyler R. 1979. Taste components of potatoes. In Boudreau JC (ed.), *Food Taste Chemistry*. Washington, DC: American Chemical Society, pp. 175–84.

Sowokinos JR. 2001. Biochemical and molecular control of cold-induced sweetening in potatoes. *Am J Potato Res* 78:221–36.

Thybo AK, Christianson J, Kaack K, Peterson MA. 2006. Effect of cultivars, wound healing, and storage on sensory quality and chemical components in pre-peeled potatoes. *LWT Food Sci Technol* 39(2):166–76.

Thybo AK, Martens M. 1999. Instrumental and sensory characterization of cooked potato texture. *J Texture Stud* 30:259–78.

Thybo AK, Mølgaard JP, Kidmose U. 2002. Effect of different organic growing conditions on quality of cooked potatoes. *J Sci Food Agric* 82(1):12–18.

True RH, Work TM. 1981. Sensory quality of Ontario potatoes compared with principal varieties grown in Maine. *Am Potato J* 58:375–9.

Ugawa T, Kurihara K. 1994. Enhancement of canine taste responses to umami substances by salts. *Am J Physiol* 266:944–9.

Ulrich D, Hoberg E, Neugebauer W, Tiemann H, Darsow U. 2000. Investigation of the boiled potato flavor by human sensory and instrumental methods. *Am J Potato Res* 77:111–17.

Uppal DS. 1987. Varietal and environmental effect on the glycoalkaloid content of potato (*Solanum tuberosum* L.). *Qual Plant* 37(4):333–40.

Vainionpaa J, Kervinen R, De Prado M, Laurila E, Kari M, Mustonen L, Ahvaneainen R. 2000. Exploration of storage and process tolerance of different potato cultivars using principal component and canonical correlation analyses. *J Food Eng* 44:47–61.

Valkonen JPT, Keskitalo M, Vasara T, Pietila L. 1996. Potato glycoalkaloids: A burden or a blessing? *Crit Rev Plant Sci* 15(1):1–20.

van Dijk C, Fischer M, Holm J, Beekhuizen JG, Stolle-Smits T, Boeriu C. 2002. Texture of cooked potatoes (*Solanum tuberosum*). 1. Relationships between dry matter content, sensory-perceived texture, and near-infrared spectroscopy. *J Agric Food Chem* 50(18): 5082–8.

van Marle JT, Stolle-Smits T, Donkers J, van Dijk C, Voragen AGJ, Recourt K. 1997a. Chemical and microscopic characterization of potato (*Solanum tuberosum* L.) cell walls during cooking. *J Agric Food Chem* 45(1):50–8.

van Marle JT, van Der Vuurst De Vries R, WIlkinson EC, Yuksel D. 1997b. Sensory evaluation of the texture of steam-cooked table potatoes. *Potato Res* 40:79–90.

Woese K, Lange D, Boess C, Bögl KW. 1997. A comparison of organically and conventionally grown foods—Results of a review of the relevant literature. *J Sci Food Agric* 74(3): 281–93.

Wszelaki AL, Delwiche JF, Walker SD, Liggett RE, Scheerens JC, Kleinhenz MD. 2005. Sensory quality and mineral and glycoalkaloid concentrations in organically and conventionally grown redskin potatoes (*Solanum tuberosum*). *J Sci Food Agric* 85:720–6.

PART VIII

OTHER VEGETABLES AND VEGETABLE PRODUCTS

CHAPTER 49

Mexican Pickled Jalapeño Pepper

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INTRODUCTION

Peppers are vegetables belonging to the genre *Capsicum* of Middle American origin; it was domesticated in Mexico, a country that possesses the biggest genetic *Capsicum* diversity, more than 6000 years ago. The genre *Capsicum* is provided with wild species. It has approximately 30 identified and classified species, of which the domesticated species of *Capsicum annuum, Capsicum chinense, Capsicum frutescens, Capsicum baccatum*, and *Capsicum pubescens* are highly appreciated for their pungency, flavor, aroma, and characteristics such as food additives, pigments, and uses in the pharmaceutical industry (Cisneros-Pineda 2007; Luna-Ruiz 2007).

The species *C. annuum* includes different pepper varieties such as paprika, cayenne, Jalapeños, and the chiltepin; inside the species *C. frutescens* are the Tabasco chilis, while inside the species *C. chinense* are the hottest chilis such as, naga, habanero, and Scotch bonnet. The species *C. pubescens* and *C. baccatum* include the South American peppers rocoto and aji, respectively.

On a global scale, there is about 24.9 million tons of pepper (*C. annuum)*, pepper being the ninth most produced vegetable in the world, with an average annual growth of 6.26% in the last 10 years. China is the number one world producer (60.6%), followed by Turkey (8.4%), Mexico (7.8%), Spain (5.0%), EUA (4.3%), Indonesia (3.4%), Nigeria (2.8%), Egypt (2.2%), Korea (2.0%), and Italy (1.7%). Mexico is the principal consumer of peppers in the world, with an annual average of 8 kg/person (SAGARPA 2007).

Jalapeño pepper (*C. annuum* L) is a vegetable that is green to obscure green in color, and in its completely mature state it presents an intense red coloration (ASERCA 2005). In addition to water, the most important components of Jalapeño pepper are 1.2% of protein, 5.3% of carbohydrates, 2.3% of fiber, and 0.1% of fat. With regard to mineral content its most important contribution is its potassium content with 340 mg of potassium for every 100 g of fresh product; it also contains

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25 mg of calcium, 25 mg of magnesium, 7 mg of sodium, 2 mg of iron, and 0.3 mg of zinc. Ascorbic acid, retinol, and folic acid are the most important vitamins with concentrations of 72, 20, and 23 mg/100 g, respectively. It contains thiamine, riboflavin, niacin, and pyridoxine. In its amino acids content, lysine, methionine, and valine are in concentrations of 252, 40, and 23 mg/100 g protein (Muñoz of Chávez et al. 1996).

In Mexico, Jalapeño pepper is mostly consumed fresh, nevertheless, and since this product is a part of the basic diet of the Mexican, majority of the consumers either acquire canned pickled peppers (named "en escabeche") or prepare their own products through pickling and/or homemade fermentation. The increase of the consumption of the slides of Jalapeño pepper, the so called "nachos," has generated an increase in the small and medium industries dedicated to the production of these products for fermentation and pickling processes. Other forms of conserving Jalapeños are refrigeration, freezing, and drying (to produce the so-called "chipotles").

This chapter is oriented to review the components responsible for the flavor and aroma of the Jalapeño pepper; the modification of the above-mentioned components as well as the flavor and aroma due to the processes of pickling and fermentation is discussed here.

PEPPER PUNGENCY

Pungency is one of the characteristics of flavor of the named like hot peppers, which is the result of the accumulation of capsaicin and other related, well-known compounds as capsaicinoids. All the capsaicinoids are acid amides of C9–C11 branched-chain fatty acids and vanillylamine. The major differences between the various capsaicinoids are in the length of the aliphatic side chain, the presence or absence of a double bond, the branching point, and the relative pungency. Most of the above-mentioned compounds show pungency, with the exception of ω-hydroxycapsaicin (Díaz et al. 2004).

The different pepper species may not be pungent with zero Scoville units (e.g., sweet peppers), or very pungent, at the levels of 445,000 Scoville units, like the habanero, red of Havana, and Caribbean.

Capsaicinoids content in Jalapeño peppers may be affected by (1) stage of maturation, (2) cultivar, and (3) techniques used to preserve it. Capsaicinoids accumulation is related in a particular way to the stage of development of the fruit, although it is also related to its age and size (Díaz et al. 2004). Like other vegetable alkaloids, they accumulate in the first stages of the development of the fruit and gradually increase up to a maximum; later they undergo a rapid scarcity and degradation due to processes like chemical decomposition such as photooxidation and/or enzymatic reactions. It was proposed that the synthesis of capsaicin coincides with the softening of the fruit, and such a softening can provide an abundant substrate supplement for the synthesis of capsaicin (Estrada et al. 2000). Table 49.1 shows the increase in the capsaicin concentration of three pepper varieties in the green and mature state. In the case of the Padron variety, the green state corresponds to 14 days of maturation, while the mature one to 42 days.

Differences in capsaicinoids content due to cultivars are also illustrated in the following data: Harris (1998) reported that Jalapeño pepper presents values of

TABLE 49.1. Capsaicin Content of Three Pepper Varieties of *Capsicum annum* L

Compound	Ripeness State	Variety		
		Padron[a]	Bronowicka Ostra[b]	Cyklon[b]
Capsaicin (mg/g)	Green	0.10	0.44	0.29
	Red	0.85	0.53	0.343

[a]Estrada and others (2000).
[b]Materska and Perucka (2005).

Scoville units between 2500 and 5000, equivalent to 166–333 ppm of capsaicin. Acero-Ortega and others (2005) obtained (using high-performance liquid chromatography [HPLC]) higher contents of capsaicin for Jalapeño Don Pancho and Aji Jalapeño, being of 648 and 556 ppm, respectively. Nevertheless, and in accordance with FLAGSTAFF Pungency Scale, Jalapeño variety is classified as medium-power pungency pepper (Harris 1998).

Techniques to prepare or preserve Jalapeño peppers may include changes in capsaicinoids content. Pickling influence is analyzed later. Peppers called "chipotles" are mature Jalapeños that are dehydrated and smoked, and that acquire flavors and very special aromas, giving a warm-spicy-smoky flavor to the food. The pungency is retained in Jalapeño peppers after the processing.

Molecular Basis for Human Sensitivity to Capsaicinoids

One of the characteristics of Jalapeño pepper is that it presents a medium pungency; therefore, it is important to understand the mechanism of action of capsaicinoids to produce the sensation of pungency. The molecular basis for species-specific sensitivity to hot chilli peppers was studied by Jordt and Julius (2002). When ingested, capsaicin and other pungent vanilloid compounds evoke a sensation of tingling and burning pain by activating a nonselective cation channel on sensory nerve endings that is called vanilloid receptor subtype one (VR1) because a vanilloid moiety constitutes an essential chemical component of capsaicin,. The mechanism includes the interaction of capsaicinoids with the protein receptor, modifying its quaternary structure, and resulting in the opening of the channel. When capsaicin-activated currents exhibit a nonselective cation permeability with outwardly rectifying current-voltage relationship, VR1 can be activated by at least three different pain-producing stimuli: capsaicin, heat (higher than 43°C), or protons (acidification).

Role of the Phenolic Compounds in Pepper Pungency (Flavor—Aroma)

Different research work has showed the behavior of the phenolic compounds during the *C. annuum* maturation. Free and combined phenolic compounds have been analyzed in different peppers to understand how the capsaicinoid synthesis is regulated. Among the phenolic compounds identified by Road and others (2000), the major content found was the protocatehuic acid, followed by chlorogenic acid, and finally by coumaric acid. The ferulic acid was present only in the last maturation stage, scarcely 10 ng/g of solid dry. Lie-EJ and others (2008) quantified 73.91 mg/g of total

phenolic compounds as GAE/g. Estrada and others (2000) observed that as the pepper ripeness process proceeded, the concentration of free phenolic compounds was reduced from 215 ng/g of dry weight to 63 ng/g of dry weight. Materska and Perucka, (2005) reported as principal phenolic compounds the *trans*-p-feruloyl-β-D-glucopyranoside, *trans*-p-sinapoyl-β-D-glucopyranoside, and quercetin, whose contents ranged in green pepper from 0.766 to 0.723 mg/g in mature fruit (dry weight), and of a total concentration of phenolic compounds of 0.836 mg/g of dry weight for the green pepper to 0.589 mg/g of dry weight for the mature. Guzmán-Maldonado and others (2004) found 35 different compounds in the Jalapeño pepper, emphasizing the presence of chlorogenic acid, cafeic acid, and 4, hydroxy-3-methoxy-benzoic acid.

Alkylphenols present in peppers, potatoes, and other food possess sour, bitter, astringent, and/or phenolic-like flavor characteristics. For example, *p*-coumaric acid tastes bitter, and astringent, having a recognition threshold of 48 ppm. Ferulic acid was perceived as having a sour taste at 90 ppm. Combination of phenolic acids may result in much more sensitive detection thresholds than the individual acids. In this way the combination of *p*-coumaric and ferulic acids resulted in sour and bitter taste at a 20 ppm concentration. The lower taste thresholds for combination of phenolics was due to a synergistic effect (Shahidi and Naczk 1995).

The alkylphenols content of two cultivars of Jalapeño and other nine different varieties of *Capsicum* (provided by National Institute of Agriculture and Forestry Researches INIFAP, Tamaulipas and San Luis Experimental Centers, México) was determined by Acero and others (2005). Considering a yield of 8.5% (extract weight relative to fresh weight of peppers), the concentration of capsaicin, *o*-coumaric, and ferulic acids was calculated for the fresh peppers, and the values obtained are shown in Table 49.2. Since the ripening stage of the peppers was controlled, the concentration differences may be due to the variety.

It may be observed that in the two Jalapeño varieties, *o*-coumaric acid was not detected and ferulic acid was present in considerable amounts (higher than the mean of all the varieties). As mentioned above, these two hydroxicinnamic acids may influence the flavor of food.

Huang and Zayas (1991) found that *o*-coumaric, *p*-coumaric, and ferulic acids were the principal phenolic acids in the free and soluble esters fractions of corn germ protein flour. Three regression models were established to predict taste characteristics from phenolic content. These models were applied to calculate the sourness intensity of the peppers, considering the concentration of ferulic and *o*-coumaric acids in ppm. In this case sourness intensity is incremented (positive coefficient) by ferulic acid and decreased (negative coefficient) by *o*-coumaric acid, as may be deduced from the following equation:

$$\text{Sourness Intensity} = 1.18 + 0.083(\text{Ferulic}) - 0.019(o - \text{Coumaric})$$

Jalapeño, Don Pancho, Costeño, Ají chili, and Serrano varieties, resulted in the highest sourness intensity, and the Ancho variety had the lower intensity, as may be appreciated from Table 49.3.

It is noteworthy that the reason these varieties have been processed in México, for many years, as pickled, may be because the sourness intensity they possess make them more suitable for this process, matching their sensorial characteristics with the sourness of the pickling solution.

TABLE 49.2. Concentration of Capsaicin, *o*-Coumaric and Ferulic Acids (ppm) in *Capsicum annuum* L

Variety	Capsaicin	*o*-Coumaric Acid	Ferulic Acid
Guajillo San Luis	356	85	15
Guajillo INIFAP	395	31	7.6
Morrón guardián	ND	17	ND
Decayenne	340	ND	4.2
Ancho Carmín	540	95	6
Serranito	552	15	0.8
Serrano Coloso	574	145	40
Costeño Chilli	551	ND	17
Serrano Tampico	569	ND	4.2
Jalapeño don Pancho	648	ND	17
Ají Jalapeño	556	ND	12.7

Adapted from Acero-Ortega and others (2005).

ND, not detected.

TABLE 49.3. Sourness Intensity Calculated from the Ferulic and *o*-Coumaric Acid Content of Peppers

Pepper Variety	Equation	Sourness Intensity
Guajillo San Luis	1.18 + 0.083(15) – 0.019(85)	0.81
Guajillo INIFAP	1.18 + 0.083(0.09) – 0.019(0.37)	1.22
Morrón Guardian	1.18 + 0.083(0) – 0.019(0.2)	0.86
Decayenne	1.18 + 0.083(0.05) – 0.019(0)	1.53
Ancho Carmín	1.18 + 0.083(0.07) – 0.019(1.12)	0
Serranito	1.18 + 0.083(0.009) – 0.019(.18)	0.97
Serrano Coloso	1.18 + 0.083(0.47) – 0.019(1.71)	1.75
Costeño	1.18 + 0.083(0.020) – 0.019(0)	2.59
Serrano Tampiqueño	1.18 + 0.083(0.05) – 0.019(0)	1.53
Jalapeño don Pancho	1.18 + 0.083(0.2) – 0.019(0)	2.59
Ají chili	1.18 + 0.083(0.15) – 0.019(0)	2.23

Volatile Compounds and Their Influence in the Pepper Flavor

The flavor of the food is the result of chemical and biochemical reactions of biosynthetic pathways, which have not been elucidated in most cases. The most important factors that affect the flavor of the peppers are the aromatic substances, generally not pungent and present in very small quantities (Huffman et al. 1978).

The compounds *trans*-2-hexenol, 2 sec-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine have been considered descriptors of the aroma of *C. annuum*, varieties Jalapeño, Anaheim, and Fresno. Other compounds characterized in the mature Jalapeño pepper were 2-isobutyl-3-methoxyphenol and linalool, both give fresh sweet and floral caramel notes as well as other compounds derived from lipoxigenase reactions (Cadwallader et al. 2007).

Also, Huffman and others (1978) reported that the flavor of the Jalapeño pepper is due primarily to 2-isobutyl-3-methoxypyrazine in concentrations of 121.34 ng/g (dry weight) in fresh pepper and 21.37 ng/g (dry weight) in pickled pepper.

Koh and others (2004) studied the flavors of six different varieties of Jalapeño peppers (Grande, Veracruz, Tula, Mitla, JM, and Coyame), whose profiles were similar and were described as wood, burning, pungent, plastic, roasted peanut, and muddy flavor, with the principal volatile compounds belonging to the groups of alcohols, acids, and aldehydes. The levels of the principal volatile compounds of the peppers Grande, Veracruz, Tula, and Coyame were similar, while for the varieties JM and Mitla they were much minor.

Kollmannsberger and others (2006) studied the contents of eight different volatile compounds of 20 different pepper species. Figure 49.1 shows the comparison of the contents of 2-methoxy-3-isobutylpyrazine that produces an intense herbaceous aroma; 2-heptanetiol that provides herbaceous/sulfurous aroma; 2,6-*trans, cis* nonadienal known as aroma to immature pumpkin; esters that are related to sweet/fruit aroma; iononas, of sweet, floral, and fruit-bearing aroma; methyl salicylate sweet/herbaceous aroma; oxigen monoterpens floral/fruit-bearing aroma, for example, the linalool; and 1,3,5,8-undecatetraeno of fruit-bearing and balsamic aroma. Figure 49.1d shows the esters characteristic of different groups of peppers. It is possible to observe that Jalapeño pepper has one of the lowest concentrations of such esters, behavior very similar to the content of ionones. Athough in the case of the ionones the content expressed like percentage relative to the content with other peppers, is much major that of esters. In the case of methyl salicylate content, the Jalapeño pepper possesses an important content of this compound, just like the Arbol pepper, although minor compared with that of the Tabasco, and major compared with that of the Poblano and Serrano peppers. The Jalapeño pepper has one of the major contents of the oxygenated monoterpens compared with the other peppers (Fig. 49.1f). It is possible to see that the pirazines and the methyl salicylate are the compounds that prevail in the generation of the aroma/flavor of the Jalapeño pepper, along with 2-heptanetiol and of oxygenated monoterpens, like the linalol.

Lie and others (2008) found in *C. annum* 35 different volatile compounds, of which they quantified 15 acids and 2 esters to be the most.

FERMENTATION AND PICKLING OF THE JALAPEÑO PEPPER

Fermentation and pickling are the preservation methods most commonly used to extend the shelf life of the Jalapeño pepper, since they are relatively simple and accessible technologies. The processed pepper is well accepted by the consumer, as can be confirmed by the increasing demand for pickled Jalapeño for "nachos" and other similar products.

According to Pederson and Luh (1988), pickled products are produced by lactic or acetic acid (vinegar) being incorporated into the raw material to modify its sensory properties and to extend its shelf life. On the other hand, fermented products modify their physical-chemical and sensory properties due to the generation of lactic acid for the bacterial action from the sugar originally present in the fresh product Both processes preserve the Jalapeño fundamentally for the reduction of pH, due to the incorporation or generation of acid compound that improves the

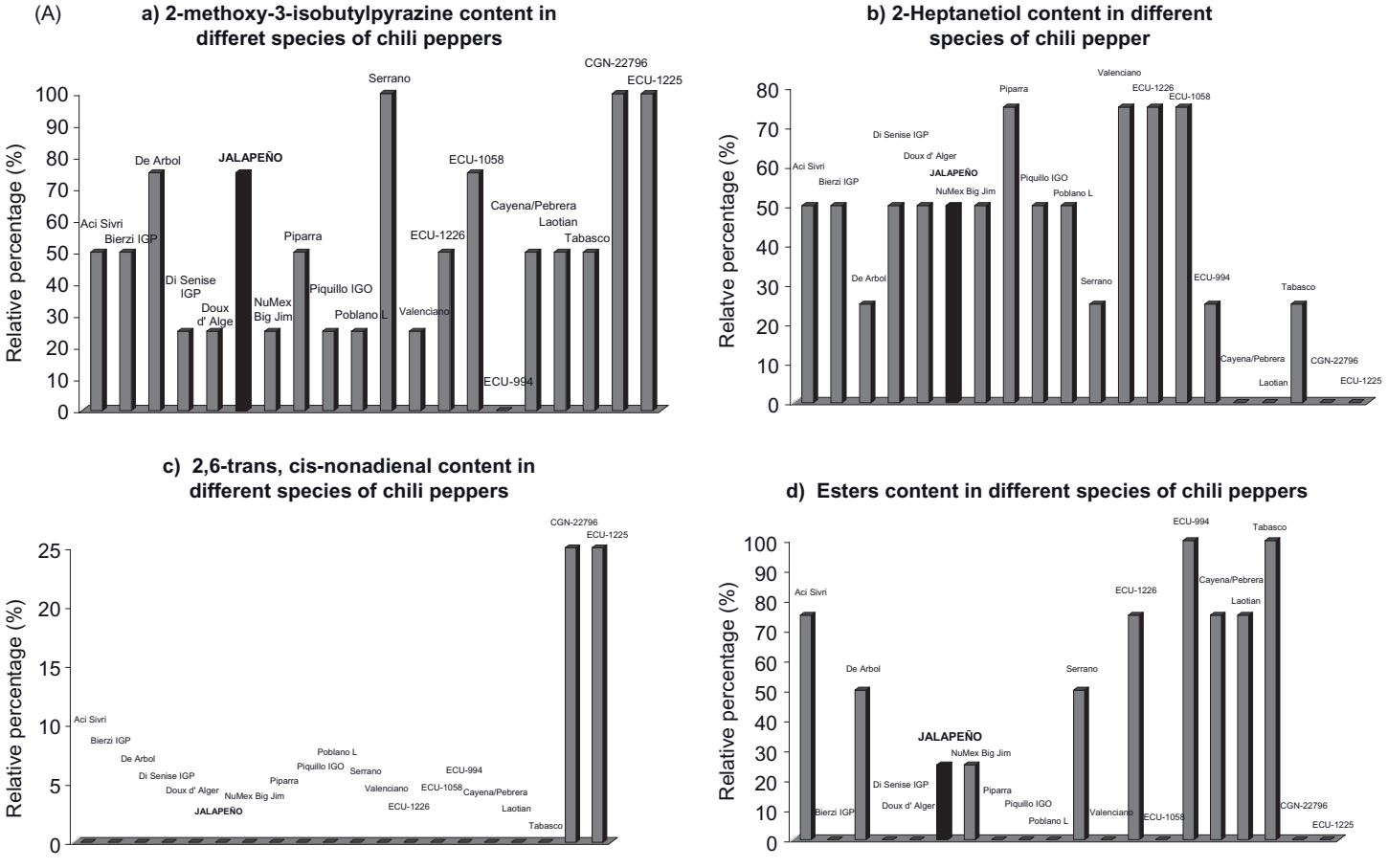

Figure 49.1A. Relative percentage of volatile compounds in different species of *Capsicum annum*. (a) 2-methoxy-3-isobutylpyrazine, (b) 2-heptanetiol, (c) 2,6- *trans*, *cis* nonadienal, (d) esters. (Adapted from Kollmannsberger and others [2006])

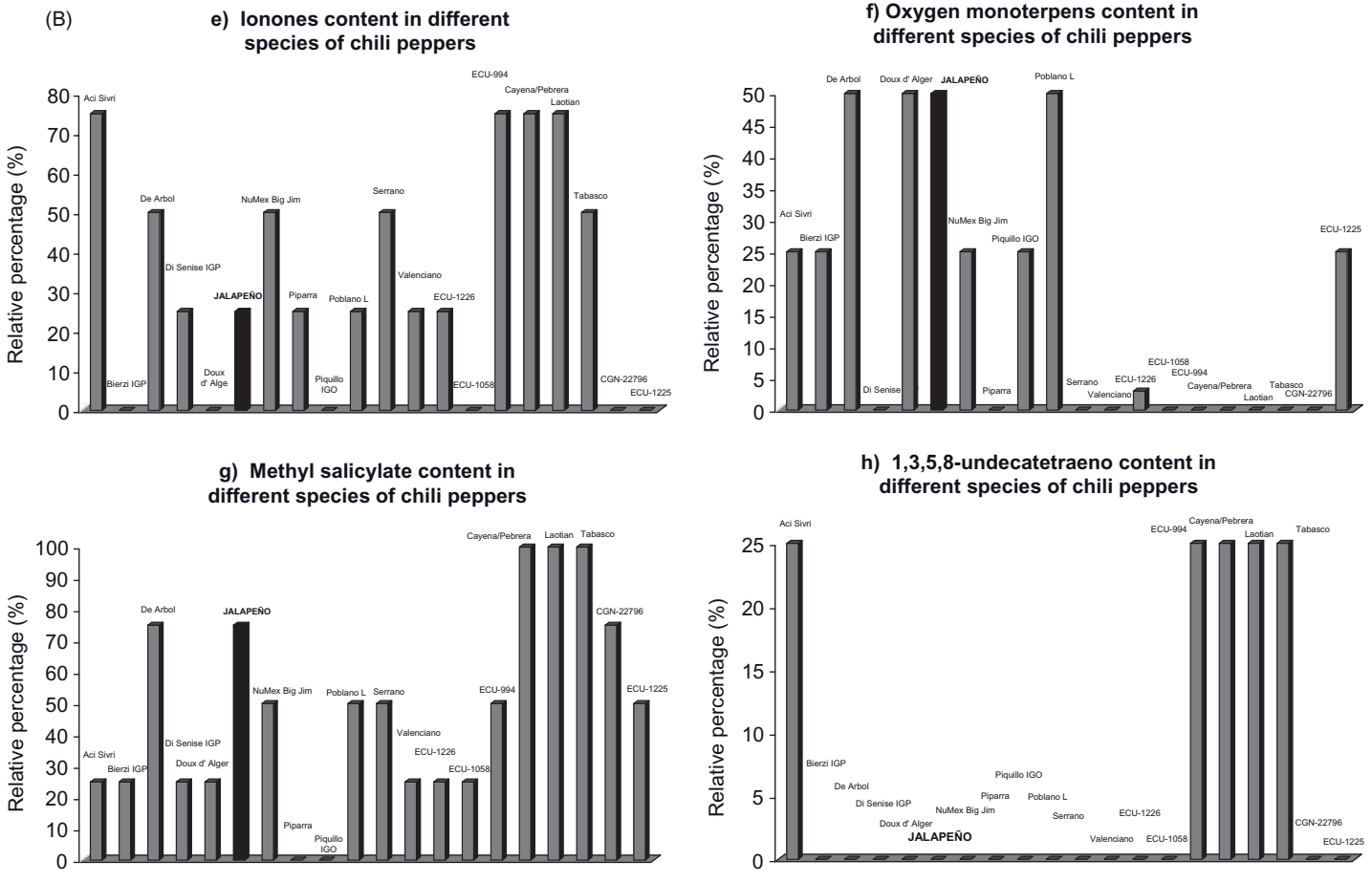

Figure 49.1B. Relative percentage of volatile compounds in different species of *Capsicum annum*. (e) ionones; (f) methyl salicylate; (g) oxygen monoterpens; (h) 1,3,5,8-undecatetraene. (Adapted from Kollmannsberger and others [2006])

sensory characteristics of the product, and possibly its nutritional value. The Jalapeño pepper pickled or fermented is prepared without seeds, in halves cut along or in rings.

Fermentation Process

The fermentation of the Jalapeño pepper is carried out by the immersion of the vegetable in pickles with concentrations of chloride of sodium between 18% and 20%. The biochemical fermentation process is performed by the action of facultative anaerobic bacteria such as *Lactobacillus plantarum* and *Pediococcus cerevisea*.

L. plantarum is originally present in the peppers and transforms the sugars via glucolysis generating acetic acid, ethanol, and gas. During this process the peppers transfer cellular fluids to the brine, so the brine tends to be diluted. For this reason it is necessary to add 1% of salt everyday during the first week, and three times a week during the remainder of the immersion to have the pickle concentration at between 18% and 20% (Galicia-Cabrera 2004). The fermentation takes place for 4 or 6 weeks. Traditionally, it is realized in closed tanks, with a valve that allows the elimination of the gas formed during the process. After the period of fermentation, the peppers that were originally luminous green change their color to olive green. This process normally intensifies the pepper characteristics of flavor and aroma, and avoids the proliferation of pathogenic microorganisms obtaining a stable product (Adams and Nout 2001; Enachescu 1995).

As soon as the fermentation process is finished the acid concentration in the product increases from 0.8 to 1.5% (expressed like lactic acid), diminishing the pH; then the peppers are washed to eliminate the salt excess, classified in accordance with the size, placed in glass recipients or plastic bags, mixed with other vegetables (usually carrots and onions), and finally covered with vinegar. The fermented peppers are highly perishable if the brine contains less than 3% of the acetic acid, in this case a pasteurization process is necessary. Finally the product is labeled, packed, and stored (Galicia-Cabrera 2004).

Pickling

In Mexico, Jalapeños are commonly commercialized like canned pickled products (not fermented), mixed by carrots and onions, in vinegar, in different presentations. The primary difference between this type of products and the fermented ones is the raw material; in this case the fresh pepper is used. In accordance with the acidity of the raw material, the products is submitted to thermal treatment (Galicia-Cabrera 2004); Figure 49.2 shows the flowchart for this process.

The pickled Jalapeño peppers may or may not be submitted to thermal treatment and they have physical-chemical and sensory characteristics different from those of the fermented products, and of fresh products.

The flavor of the pickled Jalapeño is the result of the contribution of multiple compounds such as capsaicinoids, volatile compounds (described previously), sodium chloride, acetic acid, carotenoids and derivatives, sucrose, glucose and fructose that are the principal sugars used in case of fermentation, organic acids like the citric one, malic, and ascorbic in addition to the phenolic acids, such as ferulic, cinnamic, and chlorogenic acids, and all the volatile compounds mentioned above.

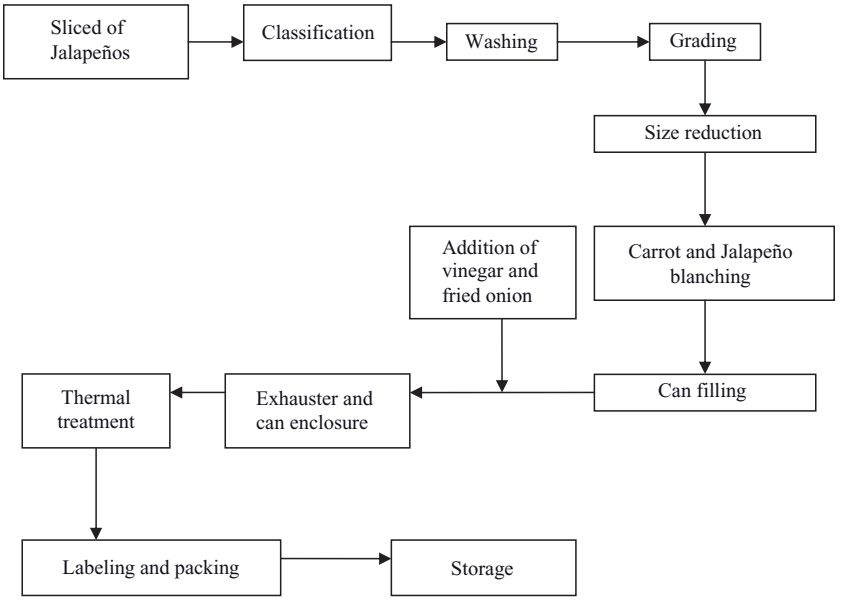

Figure 49.2. Flowchart for pickled Jalapeño peppers.

PROCESSING EFFECTS ON THE FLAVOR OF JALAPEÑO PEPPERS

During the preservation processes of Jalapeño pepper, for pickling and/or fermentation, a series of changes is developed in the physical-chemical and sensory properties that affect the quality of the product, so it is important to analyze some of the changes related to the type of process applied to the pepper.

Pickling of Jalapeño pepper is a process that includes impregnation with brine solutes of the pepper matrix, as well as osmotic dehydration of the peppers that includes the migration of fluid from the pepper to the brine. Therefore, changes in components concentration are probably due to modification of pepper matrix, diffusion of soluble compounds from the pepper to the brine and impregnation phenomena (Martínez-Monteagudo et al. 2006; Mújica-Paz et al. 2006; Valdez-Fragoso et al. 2007).

Influence on the Content of the Capsaicinoids

Determinations of capsaicin content were realized to evaluate the changes in composition of Jalapeño and red peppers due to the processing (García-Martínez 2006; Huffman et al. 1978). In Table 49.4 it is observed that the capsaicin content during the fermentation process is not modified, while in the case of the pickled pepper (2% acetic acid, 2% vegetable oil, and 0.2% salt) thermally processed, an important increase in the capsaicin levels is observed, probably due to the rupture of the tissues and cellular lysis that produces a major diffusion of this compound to the liquid phase of this product. Nevertheless, in the case of red pepper, the authors reported that after the thermal treatment (boiling to 93°C, 10–20 min), the capsaicin was lost between 18% and 22%. These results can be attributed primarily to the difference

TABLE 49.4. Capsaicin Content of Raw and Processed *Capsicum annum* L

	Fresh	Fermented	Fresh	Thermal Processed
Jalapeño pepper	0.34[a]	0.34[a]	0.268[b2]	7.36[b]
Red pepper	—	—	2.93[c]	2.29–2.39[c]

Capsaicin content: mg/g de peso seco.
[a]García-Martínez and others (2006).
[b]Huffman and others (1978).
[c]Suresh and others (2007).

TABLE 49.5. Free Phenolic and Flavonoid Concentration Before and After the Pickling Process

	Free Phenolic Compounds (mg/g)			
Sample	Jalapeño	Carrot	Onion	Pickle
Fresh	0.932 ± 0.02	0.128 ± 0.03	0.283 ± 0.02	—
Pickled	0.674 ± 0.01	0.493 ± 0.01	0.469 ± 0.01	0.330 ± 0.01[a]
	Flavonoids (μg/g)			
Fresh	350 ± 4	286 ± 5	377 ± 2	—
Pickled	641 ± 4	217 ± 4	390 ± 4	248 ± 2[b]

[a]mg/mL.
[b]μg/mL.

in both peppers in terms of chemical composition and texture, as well as to the brine composition used in each case, as was demonstrated for carotenoid retention. Migration of carotenoids during pickling was observed by Guerra-Vargas and others (2001).

The composition and pH of the brine may influence the sensory response to capsaicin, since it may be considered that in the case of pickled Jalapeño, two different stimuli are activating the VR1 channel, the protons of the acetic acid and the capsaicinoids from pepper, and so are affecting the level of pungency. Liu and others (2003) revised the activations by capsaicin and pH, and reported that these stimuli probably do not act on the same site on the channel, since pH works only from the outside of the cell, whereas capsaicin appears to bind the intracellular side. Therefore, different stimuli are likely to interact in an allosteric manner in the protein receptor of sensory nerve endings. The authors observed that very low pH prolongs channel openings, indicating that pH has additional effects besides activating the channel and potentiating capsaicin responses. Based on these observations, a lower pH should be used, when a higher capsaicin response is desired.

Influence on the Phenolic Content.

The phenolic content of Jalapeño peppers, carrots, onions, and brine was determined before and after the process of pickling in our laboratory. In Table 49.5 it is observed that due to the process of pickling, the phenolic compounds content diminishes in the Jalapeño and increases in the carrot and onion incorporated into the product.

On the contrary, the flavonoids increase significantly in the Jalapeño after the pickling process, with small changes in these compounds in the onion as well as in the carrots. It is likely to that these modifications in composition affect the sensory characteristics and the pungency of the Jalapeño and the other ingredients.

Influence on the Volatile Compounds of the Jalapeño

Huffman and others (1978) studied the changes in the volatile compounds of fresh and processed Jalapeño peppers by means of the combination of chromatography of gases and of mass spectrometry. The principal quantified compound was 2-isobutil-3-metoxy pyrazine; this compound is distributed irregularly across the pepper tissue, and could be an important contribution to the pepper flavor. On the other hand, the processed peppers presented major capsaicin levels compared with fresh peppers, and it could be assumed that during heating the capsaicin evaporated and expanded freely across the pepper tissues, so that after the thermal process all the parts of the Jalapeño presented this typical "hot" flavor.

FINAL COMMENTS

It may be concluded that during the preservation of Jalapeño pepper, by pickling and/or fermentation processes, a series of changes is developed in the physicalchemical and sensory properties that affect the quality of the product, so it is important to analyze some of the changes related to the type of process applied to the pepper.

ACKNOWLEDGMENTS

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REFERENCES

Abegaz EG, Kerr WL, Koehlñer PE. 2004. The role of moisture in flavor changes of model peanut confections during storage. *Lebenson Wiss Technol* 37(2):215–25.

Acero-Ortega C, Dorantes-Alvarez L, Hernandez-Sanchez H, Gutierrez LG, Aparicio G, Jaramillo ME. 2005. Evaluation of phenylpropanoids in the Capsicum annuum L varieties and their inhibitory effects on Listeria monocytogenes Murray, Webb and Swam Scott A. *Food Sci Tech Int* 11(1):5–6.

Alasalvar C, Grigor JM, Zhang D, Quantick PC, Shadini F. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. *J Agric Food Chem* 49(3):1410–6.

Adams M, Nout MJR. 2001. *Fermentation and Food and Safety*. Maryland: Aspen Publishers.

ASERCA. 2005. Claridades Agropecuarias: Abriendo surcos 56(1):1–17. http://www.ASERCA.GOB.MX.

Cadwallader KR, Gnadt TA, Jasso L. 2007. *Aroma compounds of chipotle peppers*. ACS Symposium Series, 946, pp. 57–66, American Chemical Society.

Cisneros-Pineda O, Torres-Tapia LW, Gutiérrez-Pacheco LC, Contreras-Martín F, González-Estrada T, Peraza-Sánchez SR. 2007. Capsaicinoids quantification in chilli peppers cultivated in the satet of Yucatán, Mexico. *Food Chem* 104(4):1755–60.

Díaz J, Pomar F, Bernal A, Merino F. 2004. Peroxidases and the metabolism of capsaicin in *Capsicum annum* L. *Phytochem Rev* 3(1–2):141–57.

Enachescu DM. 1995. *Fruit and Vegetable Processing*. Rome: FAO Agricultural Services Bulletin No. 119.

Estrada B, Bernal MA, Díaz J, Pomar F, Merino F. 2000. Fruit development in Capsicum annum: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J Agric Food Chem* 48(12):6234–9.

Galicia-Cabrera RM. 2004. Jalapeno pepper preservation by fermentation or pickling. In Hui YH, Ghazala S, Graham DM, Murrell KD, Nip WK (eds.), *Handbook of Vegetable Preservation and Processing*. New York: Marcel Dekker, pp. 179–88.

García-Martínez I, Miranda-González NG, González-Gonzáles LR, Nieto-Pineda F. 2006. Estudios preliminares de la fermentación de chile jalapeño (*Capsicum annum* L). Ed. Tecnológico de Estudios Superiores de Ecatepec. *Investigación Universitaria Multidisciplinaria* 5(5):36–42.

Guerra-Vargas M, Jaramillo-Flores, ME, Dorantes-Alvarez L, Hernández-Sánchez H. 2001. Carotenoid retention in canned pickled jalapeño peppers and carrots as affected by sodium chloride, acetic acid, and pasteurization. *J Food Sci* 66(4):620–6.

Guzmán-Maldonado SH, Torres-Pacheco I, Gonzálea-Chavira M, Mora-Avilés MA, Herrera-Hernández MG, Hernández-López D. 2004. Análisis preliminar de compuestos fenólicos y capsaicinoides en variedades de chile con diferente capacidad pungente. Biotecnología. Consejo Nacional de Productores de Chile. Proceedings of the First World Pepper Convention, pp. 115–22, Zacatecas, México.

Harris LJ. 1998. Peppers. Safe methods to store, preserve, and enjoy. Publication 8004. University of California, Davis. http//anrcatalog.ucdavis.edu.

Huang CA, Zayas JF. 1991. Phenolic acid contribution to taste characteristics of corn germ protein flour products. *J Food Sci* 56(55):1308–15.

Huffman VL, Schadle ER, Villalón B, Burns EE. 1978. Volatile components and pungency in fresh and processed jalapeño peppers. *J Food Sci* 43(6):1809–11.

Jordt S, Julius D. 2002. Molecular basis for species-specific sensitivity to "hot" chilli peppers. *Cell* 108(3):421–30.

Kollmannsberger H, Rodríguez-Burruezo A, Nitz A, Nuez F. 2006. Compuestos volátiles implicados en el aroma de las especies de Capsicum. Ed.Universidad Politécnica de Valencia, España. Proceeding of III Congreso de Mejora Genética de Plantas. *Acta Hortic* 45:89–90.

Koh FM, Xu, Z, Wilson, PW, Prinyawiwatkul W. 2004. Identifying major volatile compounds of six varieties of jalapeño peppers harvested in Louisiana. Flavor Chemistry. Book of Abstracts. 2004 IFT Annual Meeting, Las Vegas, NV.

Lie-EJ, Qing C, Ke-Chang X. 2008. Antioxidant activities and composition of extracts from chilli. *Int J Food Sci Technol* 43(4):666–72.

Liu B, Hui K, Qin F. 2003. Thermodynamics of heat activation of single capsaicin ion channels. *Biophys J* 85(5):2988–3006.

Luna-Ruiz JJ, Pérez-Chávez MS, Martínez de Anda J, Kraft K, Gepts P, Sosa-Ramírez J, Peralta-Hernández AR. 2007. Biogeography of wild populatios of chile-pepper (Capsicum Nahum var glabriusculum) in Mexico: Applications, risks, and opportunities for conserva-

tion. Genetic Biosources. Consejo Nacional de Productores de Chile. Proceedings of the Fourth World Pepper Convention, Querétaro, México.

Martínez-Monteagudo SE, Salis-Fierro F, Pérez-Carrillo JR, Valdez-Fragoso A, Welti-Chanes J, y Mújica-Paz H. 2006. Impregnation and infiltration kinetics of isotonic solution in whole jalapeño pepper using a vacuum pulse. *J Food Sci* 71(3):E125–31.

Materska M, Perucka I. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annum* L.). *J Agric Food Chem* 53(5):1750–6.

Mújica-Paz H, Argüelles-Piña LD, Pérez-Velázquez LC, Valdez-Fragoso A, y Welti-Chanes J. 2006. Vacuum pulse and brine composition effect on pickling kinetics of whole jalapeño pepper. *Innov Food Sci Emerg Technol* 7:195–202.

Muñoz de Chávez M, Chávez-Villasana A, Roldán-Amaro JA, Pérez-Gil Romo F, Ledesma-Lozano JA, Hernández-Cordero SL, Mendoza-Martínez E, Chaparr-Flores AG. 1996. *Tablas de Valor Nutritivo de los Alimentos de Mayor Consumo en México*, 1st Ed. México, Pax.

Pederson CS, Luh BS. 1988. Pickling and fermenting of vegetables. In Luh BS, Woodroof JG (eds.), *Comercial Vegetables Processing*, 2nd Ed. Westport: AVI, pp. 475–501.

SAGARPA. 2007. Producción anual de chile fresco y seco. http://www.SAGARPA.GOB.MX.

Shahidi F, Naczk M. 1995. *Food Phenolics Sources, Chemistry, Effects, Applications*. Lancaster, PA: Technomic Publishing, pp. 199–234.

Sinden SL, Deahl KL, Aulenbach BB. 1976. Effect of glycoalkaloids and phenolics on potato flavor. *J Food Sci* 41(3):520–3.

Suresh D, Manjunatha H, Srinivasan K. 2007. Effect of heat processing of spices on the concentrations of their bioactive principles :Turmeric (Curcuma longa), red pepper (Capsicum annum) and black pepper (Piper nigrum). *J Food Compost Anal* 20(3–4):346–51.

Valdez-Fragoso A, Martínez-Monteagudo SI, Salais-Fierro F, Welti-Chanes J, Mújica-Paz, H. 2007. Vacuum pulse-assisted pickling whole jalapeño pepper optimization. *J Food Eng* 79(4):1261–8.

CHAPTER 50

Dried Western Vegetable Products

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DRYING VEGETABLES

Why?

Drying is one of the oldest methods of food preservation. Drying preserves foods by removing sufficient water from the food to prevent decay and spoilage. The water content of dried foods is between 5% and 25%. When foods are sufficiently dehydrated, microorganisms cannot grow and foods will not spoil.

Drying Methods

Vegetables to dry are selected at maximum flavor and good eating quality. This stage is when vegetables reach maturity. Sweet corn and green peas should be slightly immature; their sweet taste may be retained before sugars change into starch.

Vegetables are dried immediately after harvesting because harvesting activates enzymes that change color, flavor, texture, sugar content, and nutrient content in vegetables. Harvested vegetables are cleaned and washed to thoroughly remove dirt. The washed vegetables are drained thoroughly. Vegetables with decay or mold are discarded.

Foods can be dried or dehydrated by means of the sun, a conventional oven, an electric dehydrator, a microwave oven, or a more sophisticated system. Drying needs energy. Unless sun drying is possible, the energy cost of dehydrating foods is higher than that of canning, and in some cases more expensive than freezing.

Food is exposed on trays. The materials of the trays can be different, simple, or complex. Good air circulation between trays and food without any reaction with the food is important. Wooden or plastic trays with slatted, perforated, or woven bottoms may be used.

Drying times in conventional ovens or dehydrators vary considerably depending on the amount of food dried, its moisture content, and room temperature and humidity (and the use of fans, for oven drying).

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

It is important to control air temperature and circulation during the drying process. If the temperature is too low or the humidity is too high the food will dry more slowly and microbial growth can occur. If the temperature is too high at first a hard shell may develop on the outside, trapping moisture on the inside, a process known as case or surface hardening. Temperatures that are too high at the end of the drying period may cause food to scorch. Different foods requiring similar drying times and temperatures can be dried together. Vegetables with strong odors or flavors (garlic, onion, and pepper) should be dried separately.

Most vegetables are steam- or water-blanched before drying. Blanching helps to slow down or to stop enzyme activity; it also helps in the destruction of bacteria during drying. Vegetables may also be blanched in, for example, citric acid solution. Blanching and drying times for some vegetables are given in Table 50.1.

Sun drying is the oldest known method of food preservation. Climate can affect sun drying. The ideal climate is one with low humidity and bright, strong sunshine.

A fan will help air circulation. Turning vegetables is advisable. Covering vegetables with cloth or netting keeps insects out.

Solar drying is a modification of sun drying. The sun's rays are collected inside a specially designed unit with ventilation for removal of moist air. The temperature in the unit is usually 20–30°C higher than in open sunlight, resulting in shorter drying times. While solar drying has many advantages over sun drying, lack of control over the weather is the main problem with both methods. Control of mold is obvious.

There is less risk of spoilage because of the speed of drying. The product is protected against flies, pests, rain, and dust. The product can be left in the dryer overnight or during rain. The quality of the product is better in terms of nutrients, hygiene, and color.

Al-Juamily and others (2007) tested a vegetable drying system. The system consisted of three parts: a solar collector, a solar drying cabinet, and an air blower. The cabinet was divided in six divisions separated by five shelves. Water content of beans was reduced from 65% to 18% in only 1 day.

Pumpkin, green pepper, green bean, and onion were dried in thin layers in a solar cabinet consisting of a solar air heater and a drying cabinet (Yaldyz and Ertekyn

TABLE 50.1. Blanching and Drying Times of Some Vegetables

	Blanching	Drying
Beets	Cook before drying	Sun drying: 8–10 h Oven drying: 3.5–5 h
Carrots	Steam blanching: 3–3.5 h Water blanching: 3.5 h	Sun drying: 8 hours Oven drying: 3.5–5 h
Onions	Not necessary	Sun drying: 8–11 h Oven drying: 3–6 h
Peppers	Not necessary	Sun drying: 6–8 h Oven drying: 2.5–5 h
Squash	Steam blanching: 2.5–3 h Water blanching: 1.5 h	Sun drying: 6–8 h Oven drying: 4–6 h
Tomatoes	Steam blanching: 3 h	Sun drying: 8–10 h

2001). Drying time varied between 30 and 90 h for solar drying and 48.5 and 122 h for sun drying.

Shanmugam and Natarajan (2007) studied the effect of a reflective mirror or not on the drying of green peas and pineapple slices.

Tomatoes and onions were sensory-evaluated after solar and natural drying. Solar drying altered the sugar content more than natural drying did. Solar drying is much better for sensory quality than sun drying for onions but nut for tomatoes (Gallali et al. 2000).

El-Sebaii and others (2002) constructed an indirect type natural convection dryer. The system drastically reduces drying times.

Oven drying is the most practical way to dehydrate vegetables. One advantage is that it does not depend on the weather. A disadvantage is that it is difficult to maintain a low drying temperature in the oven and foods are more susceptible to scorching at the end of the drying period. Oven-dried foods are usually darker, more brittle, and less flavorful than foods dried by a dehydrator. The lowest temperature in the oven must be 60°C.

Lewicki (2006) discussed the design of a hot air dryer to increase the quality of the final product.

Janjai and Tung (2005) evaluated the performance of a solar dryer using hot air from roof-integrated solar collectors for drying herbs and spices.

Foods can be dried on trays in an electric dehydrator, a unit with a heat source and ventilation system. Electric dehydrators are used to dry foods indoors. The quality of the product is better than with any other method of drying. As with oven drying, there is no dependence on weather conditions.

Herbs were dried in a heat pump-assisted dryer by Fatouh and others (2006).

Paakkonen (2002) applied a combined infrared/heat pump drying technology to a rotary dryer. This technology should be suitable for heat-sensitive materials as such herbs and vegetables such as red beet and carrot.

Microwave ovens are used for drying foods. Microwave-related combination drying is a fast drying technique applicable to fruits and vegetables. The advantages are shorter drying time and improved product quality, while the disadvantages are high start-up costs and relatively complicated technology (Zhang et al. 2006).

Infrared drying is a technique of drying and curing with heat radiation, a radiation that has wavelengths greater than 780 nm.

Freeze drying is freezing the vegetables, reducing the surrounding pressure or applying a vacuum, and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas.

In osmotic dehydration, the vegetables, fruits, or herbs are subjected to osmosis by dipping or spreading them in an osmotic solution: an aqueous sugar-salt syrup under specific conditions. Examples of treatments are 5-mm slices of carrots during 4 h in 60% sugar, 10% salt, and 400 ppm SO_2 or 2-mm slices of onions for 2 h in 60% sugar, 10% salt, and 4000 ppm SO_2.

The osmotic solutions of other treatments include sucrose, dextrose, maltose, honey, maltodextrin, and salt.

Vegetables are dried by contact with hot air when the vegetables are in a fluidized state in a column: fluidized bed drying. An intense contact between heat and vegetables occurs. This versatile drying method can be used in batch or in a continuous mode for drying various materials.

How to Store Dried Vegetables?

Dried food should be kept in a cool, dry, and dark place. Dried foods are used within 3 to 6 months.

NUTRITIONAL VALUES OF DRIED VEGETABLES

The nutritive value of food is affected by the dehydration process.

- The calorie content does not change; it is concentrated in a smaller mass.
- The fiber content is not affected.
- Vitamin A content does not change much under controlled circumstances. Vitamin A is destroyed by heat and air but blanching reduces loss of vitamin A.
- Vitamin C content is mostly destroyed during blanching and drying of vegetables.
- Vitamin B, like thiamin, riboflavin, and niacin, is lost to some extent during blanching but if the rehydration water is consumed the losses are limited.

Maeda and Salunkhe (1981) studied the retention of ascorbic acid and total carotene in tropical solar dried vegetables. Potato and other vegetables were dried in open sunshine and in solar driers with and without shade. The maximum retention of vitamins was in solar drier with shade. Vegetables that were directly exposed to the sun had almost no vitamins.

The effect of drying was assessed on the retention of β-carotene, ascorbic aid, and chlorophyll (Negi and Roy 2001). Higher losses of β-carotene, ascorbic acid, and chlorophyll of green leaves were observed in solar drying as in cabinet drying. Storage increased the decline in vitamins and chlorophyll.

After blanching vegetables were dried sun- and solar-dried (Onayemi and Badifu 1987). Drying times were 6–7 h for sun drying (ambient temperature: 28–31°C) and 2–3 h for solar drying (70–75°C).

The results indicated higher levels of ascorbic acid and carotene in solar-dried leafy vegetables than in sun-dried ones.

Minerals may be lost during drying and rehydration if the water is not used. Iron is not destroyed during drying.

How dried foods are stored is important for an optimal retention of nutrients: store in a cool, dark, and dry place and use the dried vegetables within 1 year.

USING DRIED VEGETABLES

Vegetables are rehydrated in cold water prior to use. Dried vegetables may also be added to boiling water or to liquids such as soup.

When soaked in water or bouillon or vegetable juice, for additional flavor, they usually rehydrate within 1–2 h. Using boiling liquids shortens the soaking time. Adding dried vegetables directly to soups or stews is the simplest way to rehydrate vegetables.

Thinly sliced and dehydrated vegetables are consumed as nutritious and low-calorie snacks. Examples of such vegetable chips are tomato, parsnip, beet, or carrot.

By crushing dehydrated vegetables, vegetable flakes are produced, and by milling, vegetable powders.

DRIED VEGETABLES

Tomatoes (*Solanum lycopersicum* L.)

Osmotic dehydration and air drying technology represent a technique that can reduce post-harvest loss of fruits and vegetables. Souza and others (2007) studied the influence of osmotic solution composition (water/sugar/salt) and temperature on the osmotic dehydration of tomatoes. Tomatoes with and without skin were studied.

The effects of different pretreatments and dehydration methods on quality characteristics and storage stability of tomato powder were investigated in Davoodi and others (2007).

Dehydration process was carried out for tomato slices of var. *Avinash* after giving pretreatments with calcium chloride ($CaCl_2$), potassium metabisulfite, $CaCl_2$ + potassium metabisulfite, and sodium chloride (NaCl). Untreated samples served as control. Solar drier and continuous conveyor drier were used for dehydration. Quality characteristics of tomato slices viz. moisture content, sugar, titratable acidity, lycopene content, dehydration ratio, rehydration ratio and, non-enzymatic browning (NEB), affected by the dehydration process, were studied. Storage effects were also studied for a period of 6 months for tomato powder packed in different types of packaging materials viz. metalized polyester (MP) film and low density polyethylene. Changes in lycopene content and NEB were estimated during storage at room temperature. Pretreatment of 5-mm thick tomato slices with $CaCl_2$ in combination with potassium metabisulfite and drying using a tunnel drier with subsequent storage of product in MP bags was selected as the best process.

The combination of osmotic dehydration and microwave drying is a potential new process that could improve the quality of dried tomatoes. Heredia and others (2007) used various osmotic solutions formulated with salt, sugar, and calcium lactate in an osmotic treatment prior to microwave-assisted air drying. The influence of microwave energy on the kinetics was analyzed and correlated with the dielectric properties of the samples. The results showed that osmotic dehydration with ternary solutions (27.5% sucrose, 10% salt and water [w/w]) and the addition of 2% calcium lactate combined with microwave-assisted air drying makes it possible to obtain dried and intermediate moisture tomato products.

Doymaz (2007) investigated the drying characteristics of tomatoes at 55, 60, 65 and 70°C with air flow rate of 1.5 m/s. The tomatoes were dried to the final moisture content of 11%. The pretreatment (dipping in alkaline ethyl oleate solution) and air temperature affect the course and rate of drying.

Chang and others (2006) compared the antioxidant properties of two fresh, freeze-dried, and hot air-dried varieties of tomato *Lycopersicon esculentum* Mill. Fresh samples had the highest ascorbic acid contents. Air-dried samples had the highest content of total phenolics and lycopene.

The influence of pre-drying treatments on quality and safety of sun-dried tomatoes was studied by Latapi and Barrett (2006a). The quality of the tomatoes was evaluated by the determination of moisture, color, rehydration ration, mold, yeast, sulfur dioxide, and/or salt content. Four pre-drying treatments were investigated: steam blanching, boiling brine blanching followed by gas sulfuring, dipping in salt (concentrations from 0% to 20%), and dipping in sodium metabisulfite (concentrations from 0% to 8%).

One conclusion was that dipping tomatoes in 6% or 8% sodium metabisulfite for 5 min before drying gave the best color. The same authors (Latapi and Barrett 2006b) evaluated the affects of pre-drying treatments on the quality and safety of sun-dried tomatoes. Untrained consumers ranked gas-sulfured sun-dried tomatoes higher than sun-dried tomatoes after dipping in either sodium metabisulfite alone or sodium metabisulfite plus salt.

Kerkhofs and others (2005) followed changes in color and antioxidant content of tomato cultivars. Tomatoes were dried by forced air drying. Color (CIELAB L*a*b* values), ascorbic acid, total phenolics, lycopene, and total antioxidant activity were measured. After drying at 42°C for 48 h, ascorbic acid, total phenolics, and total antioxidant activity decreased significantly. Extractable lycopene contents increased.

In a study of Toor and Savage (2006) same results were obtained. Three tomato cultivars were semidried at 42°C. Total phenolics and ascorbic acid of semidried tomatoes were significantly lower than that of fresh plants. In this study lycopene content was lower after semidrying.

The stability of lycopene during spray drying of tomato pulp was investigated by Goula and Adamopoulos (2005). Lycopene losses varied from 8.07% to 20.93% depending on the drying conditions. Air inlet temperature proved to be an important factor.

Lewicki and others (2002) reported the effects of pretreatment conditions, soaking in $CaCl_2$ solution followed or not by osmotic dewatering or treating by osmosis in a solution of sucrose and calcium chloride, on convective drying of tomatoes. Treatment with $CaCl_2$ followed by osmotic dewatering was most effective.

Giovanelli and others (2002) studied the water sorption, drying, and antioxidant properties of dried tomato products: tomato pulp, tomato halves, and insoluble solids-rich tomato. Lycopene and ascorbic content and the antioxidant activity of hydrophilic and lipophilic extracts were measured both in fresh and dried products. The insoluble solids-rich tomato product had the highest lycopene content on lipophilic antioxidant activity.

Beans (*Phaseolus vulgaris* L.)

The influence of storage conditions on the flavor of stored French beans after rehydration was evaluated by gas chromatography-sniffing port analysis (GC-SP) and GC-mass spectrometry (MS) of the volatile compounds, and by quantitative descriptive analysis (QDA) and hedonic sensory evaluation (Van Ruth et al. 1995). The dried beans were stored at three water activities (a[w]), two different temperatures, and in the presence or absence of light. At elevated temperature and a[w] 0.3 and 0.5, GC-SP showed an increase in the number of assessors perceiving chemical, rotten odors.

Al-Juamily and others (2007) constructed a drying system consisting of a solar collector, a solar drying cabinet, and an air blower. The performance of the system was tested with grapes, apricots, and beans. The most effective factor on drying rate was the temperature of the air inside the cabinet.

Beets (*Beta vulgaris* L.)

The volatile constituents of cooked beets, obtained by distillation-extraction of the beets, were separated by GC and subjected to infrared and mass spectral analysis, and 17 components were identified. A unique feature of this vegetable is the high concentration of 4-methylpyridine and pyridine, which constitutes about 60% of the total volatiles. Other components present at greater than 1% level include dimethyl sulfide, isovaleraldehyde, ethanol, isopentanol, and furfural. The occurrence of geosmin and 2-methoxy-3-secbutylpyrazine was confirmed; the low flavor thresholds of these compounds suggest that they play a role in beet flavor (Parliament et al. 1977).

Carrots (*Daucus carota* L.)

The stability of β-carotene and lycopene was investigated during convective air and inert gas drying, microwave vacuum drying, and freeze drying for lycopene-containing carrots (*D. carota* L. cv. *Nutri Red*; Regier et al. 2005). After convection drying at temperatures below 70°C, β-carotene and lycopene contents remained unchanged independent of the drying medium. Microwave vacuum drying gave dry products with high carotenoid retention in a very short drying time of about 2 h.

A consumer study by Marabi and others (2006) on the influence of drying method and rehydration time included two commercial carrot particulates (air-dried and vacuum-puffed-dried) used regularly in ready-to-eat soups. Vacuum-puffed-dried carrots showed a higher overall acceptability than air-dried carrots. Rehydration time had a significant effect only on the overall acceptability of the air-dried samples.

Carrots were dried using a solar cabinet drier, fluidized bed drier, and microwave oven drier (Prakash et al. 2004). Carrots dried by fluidized bed drying showed better color and rehydration properties, greater β-carotene retention, and better overall sensory acceptability.

Sanjuan and others (2005) studied the effects of an alternative method of blanching at low temperature (65°C, 10 min).

Carrot slices were dried by osmo-hot air drying method (Ghosh et al. 2006). Osmo-hot air-dried carrot slices received higher scores in both its dried and rehydrated forms compared with conventional air-dried carrots.

Mdziniso and others (2006) investigated the physical quality and carotene content of solar-dried carrots, sweet potatoes, and collard greens. β-carotene contents of dehydrated carrot was 10.9–17.4% (dry basis). Carrot slices of 5 mm thickness packed at a load of 715 g/m^2 contained the highest β-carotene content (17.4%) and vitamin A activity (362 IU/g). β-losses after solar dehydration was 48.9–67.5% in carrots.

The effects of the six varieties (Kazan, Maxima, Nandor, Nektarina, Simba, and Tito) on drying characteristics, color, and water absorption were investigated (Markowski et al. 2006). Carrot cubes were dried under forced convection

conditions. Color was determined for fresh, dried, and dehydrated samples by measuring L*, a*, and b* values. All investigated parameters were influenced by the variety.

Arevalo-Pinedo and Murr (2007) studied the influence of pretreatments, freezing, and blanching on the drying kinetics of carrot and pumpkin during vacuum drying. Carrots were dried by solar drying, hot air cabinet drying, and direct sunlight. Sun-dried carrots had the maximum loss of β-carotene (71%; Suman and Kumari 2002).

Machewad and others (2003) studied dehydration of carrots. Drying resulted in losses of reducing sugars, total sugars, and β-carotene.

Sweet Corn (*Zea mais* L.)

Hatamipour and Mowla (2003) investigated the drying behavior of maize and green peas in fluidized bed dryer.

Onion (*Allium cepa* L.)—Garlic (*Allium sativum* L.)—Leek (*Allium porrum* L.)

Maw and others (2004) studied the feasibility of a heat-treating system of sweet onions under controlled commercial conditions. Onions (4 m^3) were passed through a continuous-flow dryer.

Kaymak-Ertekin and Gedik (2005) investigated the kinetics of non-enzymatic browning and thiosulfinate loss in onion slices during drying at different temperatures and different air velocities.

The combination of infrared and hot air drying of onion slices was studied (Kumar et al. 2005). Processing parameters were drying temperature, slice thickness, air temperature, and air velocity.

A laboratory dryer was used by Adam and others (2000) to evaluate the color, pyruvate, and chemical and sensory parameters of sliced onion. Temperatures above 65°C had effects on color. Pyruvate content decreased with increasing temperature and had effect on the odor of onion. The temperature also affected the sugar content and ascorbic acid.

The concentration of allicin in garlic was analyzed after hot air drying and freeze drying (Ratti et al. 2007). Allicin content decreased with an increase in drying temperature in both convective hot air drying or freeze drying. Drying at 40 to 50°C resulted in a better allicin retention than at 60°C. The best retention was in garlic samples freeze-dried at 20°C.

Li and others (2007) checked the conditions of microwave-vacuum and vacuum drying on the contents of allicin.

Garlic cloves were dried by a microwave-hot air combination (Sharma and Prasad 2001). The color and flavor strength of dried garlic cloves were used to evaluate the method. Combined microwave-hot air resulted in a better end product.

Ambrose and Sreenarayanan (1998) evaluated four dehydration methods: sun drying, solar cabinet drying, mechanical drying, and fluidized bed drying. Drying at 60°C for 4 h in a fluidized bed dryer resulted in good-quality powdered garlic.

The volatile compounds of leek were extracted by microwave technique and adsorbed on an apolar resin and further analyzed by GC/MS-single ion monitoring (SIM) (Di Cesare et al. 1999). Two cultivars of leek, Atal, and San Giovanni were analyzed. All volatile compounds were detected in the raw cultivars. For the cultivar Atal,

volatiles are quite the same in the dehydrofrozen and dried state. The dehydrofreezing technique better preserves the aroma compounds in the San Giovanni cultivar.

Peas (*Pisum sativum* L.)

Uma and Sharma (2007) determined the effects of cooking, drying using fluidized bed (FBD), and freeze-thaw (FT-FBD) on the quality of peas. Maximum changes in peroxide, free fatty acid, thiobarbituric acid, browning index values, chlorophyll and vitamin C contents, and sensory parameters were observed in FT-FBD samples when stored at ambient temperatures (14–35°C).

Hatamipour and Mowla (2003, 2006) studied the drying behavior of maize and green peas in a fluidized bed dryer.

Peppers (*Capsicum annuum* L.)

Peppers often become hotter as they ripen, and hotter still when they are dried. Dried peppers tend to have a richer, more concentrated flavor.

An improved high-performance liquid chromatography (HPLC) method for analysis of capsaicinoids in dried *Capsicum* fruit powder is reported (Collins et al. 1995). Extraction of *Capsicum* fruit powder using acetonitrile proved to be the best capsaicinoid extractor in the shortest time interval. Solvents used for HPLC separation and quantification of capsaicinoids include methanol and water at 1 mL/min flow rate. Instrument sensitivity is enhanced by altering the fluorescence detector excitation and emission wavelengths. Two analytical methods have been developed. One method determines the total amount of heat units in 7 min, while the other provides the total amount of heat units as well as the separation of all present major and minor capsaicinoids in 20 min.

Color, L-ascorbic acid, and sugar retention in green bell pepper were adversely affected by temperature and relative humidity during drying. Optimum quality, highest levels of L-ascorbic acid, and best color were attained at drying conditions of 55 and 60°C (15–40% relative humidity) and at 65 and 70°C (15% relative humidity; Sigge et al. 1999).

Joy and others (2001) dried red chilis in a solar tunnel. The overall quality was better in tunnel-dried samples than in conventional dried red chilis.

Changes in fatty acids composition and antioxidative activity of pigment extracts from Korean red pepper powder after processing were investigated (Kim et al. 2002). The ratios among fatty acids varied according to the processing conditions but the fatty acids compositions remained approximately the same. Different drying methods resulted in significant differences in the antioxidative activity. Different storage conditions had little effects.

The same authors (Kim et al. 2004) studied the composition of the main carotenoids and changes of pigment stability during drying and storage. The identified pigments were capsanthin, zeaxanthin, β-cryptoxanthin, β-carotene, capsorubin, myristoylcapsanthin, lauroylmyristoylcapsanthin, and myristoylpalmitoylcapsanthin. Drying had the most influence on capsanthin until 2 months storage.

Dehydration of the sweet pepper *Fresno de la Vega* resulted in an 88% loss of ascorbic acid, whereas freeze drying did not cause significant losses (Martinez et al. 2005).

The changes of the aromatic fraction of red peppers during the La Vera region traditional drying process was studied by Aragon and others (2005). This traditional drying process is a mild, slow drying process.

Ninety-six compounds were identified by dynamic headspace and GC-MS. Fifty-five compounds presented significant changes during the drying process.

Perez-Galvez and others (2005) studied the effect of temperature on carotenoid content at La Vera County.

Fresh and sun- and oven-dried red peppers were analyzed for volatile components (Jun et al. 2005). Odor-active compounds were determined using GC-olfactometry (GC-O). Other volatile components, such as aldehydes, ketones, acids, and esters, were found in the dried samples. They included hexanal, ethyl acetate, α-ionone, and P-ionone. Some Strecker aldehydes, 2-methyl butanal, and 3-methyl butanal were found only in dried red peppers, otherwise, only in fresh red peppers were detectedmore hydrocarbons of high volatility and terpene-type components, such as γ-terpinene and aromadendrene. A considerable amount of naphthalene was formed during sun drying, whereas 2-furancarboxaldehyde, 1-methyl-1H-pyrrole, and benzeneethanol were detected only in oven-dried red peppers. Characteristic odor of fresh ones could be attributed to 3-penten-2-ol, 2-methyl-2-butenal, 2-methoxy phenol, 2-hydroxy-methyl-benzoate, and 2-phenoxy ethanol, whereas some odorants, including 2-pentyl furan, naphthalene, hexyl hexanoate, and α-ionone, could be responsible for distinctive odor property of sun-dried red peppers. 2-furancarboxaldehyde, benzeneethanol, 4-vinyl-2-methoxy phenol, and an unknown component played important roles in the odor property of oven-dried red peppers.

Red chili variety *CH-3* was sun-dried and dried in a batch-type dryer (Oberoi et al. 2005). The color was better and capsaicin content was also higher in chilis dried in batch-type dryer. The oleoresin was the same after both drying methods.

Ergunes and Tarhan (2006) studied the color retention of red peppers after chemical pretreatments during greenhouse and open sun drying.

Schweiggert and others (2006) investigated changes of capsaicin, dihydrocapsaicin, and norhydrocapsaicin in chili powders after blanching and storage. Heating and drying resulted in a 21.7% to 28.3% loss of capsaicinoid content. A further loss of 6.8% to 11.9% occurred during storage at ambient temperature for 6 months.

Kim and others (2006) monitored the effect of drying and storage on the antioxidant activity, ascorbic content, and color of Korean red pepper.

Daood and others (2006) examined the effects of drying temperature, endogenous antioxidants, and capsaicinoids on the carotenoid stability in paprika.

Park and Kim (2007) studied the stability of color and antioxidants in paprika powder during drying and storage. Paprika was dried by freeze drying, vacuum drying, far infrared-ray drying, and hot air drying. Freeze drying was found to be the most suitable drying method for the stability of the antioxidants.

Kozukue and others (2005) analyzed the capsaicinoid content in peppers by HPLC and LC-MS. Eight capsaicinoids were analyzed: capsaicin, dihydrocapsaicin, homocapsaicin-I, homocapsaicin-II, homodihydrocapsain-I, homodihydrocapsain-II, novivamide, and nordihydtocpasaicin.

Topuz and Ozdemir (2004) studied the changes in the pungent components (capsaicin, dihydrocapsaicin, homodihydrocapsaicin, isodihydrocapsaicin, and nordihydrocapsaicin) of paprika after sun drying, dehydration, gamma irradiation, and storage period. Levels of all capsaicinoids were higher in the dehydrated paprika than in sun-dried paprika.

Pordesimo and others (2004) investigated the capsaicin levels in jalapeno peppers in relation with harvest conditions and drying parameters. Drying temperatures, from ambient up to 85°C, did not affect the concentration of total capsaicinoids.

Tiwari and Pandey (2007) found that osmotic dehydration prior to fluidized bed drying improved the organoleptic characteristics of sweet pepper slices in both dried and rehydrated forms.

Potato (*Solanum tuberosum* L.)

A study was conducted by Khraisheh and others (2004) to evaluate the quality and structural changes in potatoes during microwave and convective drying. Ascorbic loss was dependent on air temperature, microwave power, and moisture content.

Bondaruk and others (2007) studied the influence of drying conditions on color, starch content, sugar content, mechanical properties, and microstructure of dried potatoes. Drying methods were microwave and forced convection drying.

Pumpkin (*Cucurbita maxima* L.)

Alibas (2007) investigated the effects of microwave, air, and combined microwave-air drying on pumpkin slices.

Wang and others (2007) studied the behavior of microwave drying in pumpkins.

HERBS

The quality of dried herbs depends mainly on three parameters: color, aroma, and absence of off-flavor elements (Bohm et al. 2002). The quality evaluation of dried products was related to the properties of the original fresh product. Especially convective hot air drying (HAD) caused heat damage of the plant tissue and therefore affected the quality of the final product. This study involved the investigation of vacuum-microwave drying (VMD) as a processing method for drying herbs. Color, aroma, and off-flavor of vacuum-microwave-dried parsley was evaluated and compared with corresponding properties of hot air-dried parsley (at 75°C). Vacuum-microwave-dried parsley was greener in color, exhibited a higher content of essential oils, and was less off-flavors than those prepared by HAD. Color degradation was observed in all samples, but was faster for hot air-dried parsley. Investigation of the impact of drying methods on the flavor quality showed that VMD preserved over 90% of the essential oils, while HAD preserved only 30%. Furthermore, vacuum-microwave-dried samples were rated better than HAD samples by a sensory panel.

Basil (*Ocimum basilicum* L.)

The various basils have different scents because the herb has a number of different essential oils in different proportions for the various breeds. The volatile oils of basil contain estragole, linalool, cineole, eugenol, sabinene, myrcene, and limonene. Basil contains also flavonoids and tannins. Other chemicals that produce the distinctive scents of many basils, depending on their proportion in each specific breed, include

cinnamate, citronellol, geraniol, linalool, methyl chavicol, myrcene, pinene, ocimene, and terpineol.

Baritaux and others (1992) discuss the effects of drying and storage on the essential oil of basil.

The changes of the chemical composition of basil caused by different drying techniques were discussed by Di Cesare and others (2003). Leaves were dried by a microwave oven, by air drying at 50°C, and by freeze drying. Raw and dried basil was analyzed for selected aroma compounds by GC/MS-selected-ion-monitoring, chlorophyll a and b by HPLC, and the color by a colorimeter.

The percentages of eucalyptol, linalool, eugenol, and methyl eugenol in samples dried by microwave were higher than in samples dried by traditional methods.

The influence of drying (oven drying at 45°C, air drying at room temperature, and freeze drying) on the aroma compounds of basil was evaluated (Diaz-Maroto et al. 2004). The volatile compounds (mainly linalool and eugenol) of fresh and dried leaves were extracted and concentrated by simultaneous distillation/extraction and analyzed by GC/MS. The total quantity of the volatiles of fresh basil decreased considerably during oven drying and freeze drying. Air drying resulted in only small losses of volatile components.

Basil leaves were dried by either conventional hot air (50, 60, and 70°C) or low-pressure superheated steam dryers (LPSS; Barbieri et al. 2004). Extraction from fresh and dried products was performed by simultaneous distillation-extraction. Further analysis was by GC and GC-MS. Twenty-three compounds were identified, some of which were 1,8-cineole, methyl chavicol, methyl cinnamate, and linalool. In the LPSS-dried product the aroma profile is almost the same as that of the fresh product. Bowes and Zheljazkov (2004) found that air drying and freeze drying resulted in an altered composition of the essential oil of *Ocimum sanctum* L. and *Ocimum basilicum* L. cultivars.

Sage (*Salvia officinalis* L.)

Like rosemary, also a member of the *Labiatae* family, sage contains a variety of volatile oils, which include cineole, borneol, and thujone. Sage also contains flavonoids (including apigenin, diosmetin, and luteolin), and phenolic acids, including the phenolic acid named after rosemary—rosmarinic acid. Sage leaf contains tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavone glycosides, and estrogenic substances.

Venskutonis (1997) studied the effects of drying on the volatile constituents of thyme and sage.

Effects of particle size, temperature, contact time, solvent-to-sage ratio, and the ethanol–water ratio on the extraction of the active compounds rosmarinic acid, carnosic compounds, and essential oil from dried sage were studied (Durling et al. 2007). Optimal extraction conditions giving highest yield of all three active compounds were a particle that was 1 mm in diameter, extraction temperature of 40°C, solvent-to-sage ratio of 6:1, and 55–75 wt% ethanol for up to 3 h. This gave an extract equivalent to 14.9% of dry sage, containing 6.9% rosmarinic acid (55% recovery), 10.6% carnosic compounds (75% recovery), and 7.3% essential oil (42% recovery). Scale-up of the process by a factor of 100 demonstrated that the optimized labora-

tory scale process can be carried out without any loss of efficiency at an industrial scale.

The effects of blanching, drying, and storage on the volatiles of sage leaves were studied by Di cesare and others (2001). The volatile extracts were analyzed by GC/MS-SIM. α- and β-thujone, camphor, borneol, and bornyl acetate concentrations were the same in the unblanched freeze-dried samples. In blanched and dried samples the concentration of α-thujone decreased.

Thyme (*Thymus vulgaris* L.)

The volatile oil components of thyme include carvacrol and geraniol, but most importantly, they include thymol. Further components in the essential oil are thymol methyl ether, cineol, cymene, α-pinene, borneol, and esters of the latter two.

Venskutonis (1997) studied the effects of drying on the volatile constituents of thyme and sage. The content of aroma compounds was the highest when the herb was dried at 60°C.

The percentages of essential oils (thymol and carvacrol) extracted after oven drying and drying by the wire basket solar dryer were 0.5% and 0.6%, respectively (Balladin et al. 1999).

Parsley (*Apium petroselinum* L.)

Parsley contains two different types of components with health benefits. The first type, the volatile oil components, includes myristicin, limonene, eugenol, and α-thujene, and the second type, the flavonoids, includes apiin, apigenin, apiol, crisoeriol, and luteolin.

The drying of dill and parsley leaves was investigated in a laboratory dryer (Doymaz et al. 2006). The effect of temperature on the drying rate of samples at constant air velocity (1.1 m/s) and various temperatures (50, 60 and 70°C) was studied. The color of the dried samples was determined with a Hunterlab in terms of Hunter L*, a*, and b* values. From the results of color quality, drying at 60°C was found to be the optimum temperature for dill and parsley leaves.

Air drying at room temperature resulted in minor losses of volatile compounds compared with the fresh herb. Oven drying at 45°C and freeze drying resulted in losses of most of the volatiles, especially p-meritha-1,3,8-triene, and apiole (Diaz-Maroto et al. 2002).

The same group investigated the evaluation of the effect of drying on the aroma of parsley by free choice profiling (FCP; Diaz-Maroto et al. 2003). Air drying at ambient temperature produced less changes in volatiles.

Parsley leaves were dried in a microwave under different conditions to determine the effects on the color of the leaves (Soysal 2004).

Cilantro (*Coriander sativum* L.)

Coriander's volatile oil contains carvone, coriandrol, geraniol, limonene, borneol, p-cimene, camphor, pinenes, myrcene, camphene, phellandrenes, α-terpinene, limonene, cymene, γ-terpinene, trans-tridec-2-enale, elemol, and linalool. Coriander's flavonoids include quercitin, kaempferol, rhamnetin, and epigenin. Coriander also

contains active phenolic acid compounds, including caffeic acid and chlorogenic acid.

Drying of methi (Fenugreek leaves) and coriander (Pande et al. 2000) leaves was performed in a forced circulation solar hot air dryer. Coriander was dried at 40, 45, and 50°C. Organoleptic quality attributes like color, appearance, and taste of these dried samples were found acceptable, and solar-dried samples were appreciated for retaining fragrance during off-seasons.

Ahmed and others (2001) studied the drying characteristics and product quality of coriander leaves. Water blanching at 80°C for 3 min resulted in greater chlorophyll retention. The best drying temperature for blanched leaves was 45°C.

Kaur and others (2006) evaluated the pretreatment and drying of coriander leaves. The best pretreatment was sipping for 15 min in a solution of 0.1% magnesium chloride, 0.1% sodium bicarbonate, and 2.0% potassium metabisulfite in water at room temperature. The mini multi-ack solar dryer was the best drying method.

Green leafy vegetables, such as coriander, were microwave-dried at 2450 mHz for 10 to 16 min (Fathima et al. 2001). Coriander and mint had the lowest score for flavor and color.

Marjoram (*Origanum majorana* L.)

Compounds found in marjoram are flavonoids, caffeic acid, rosmarinic acid, triterpenoids, linaloool, sabinene, sabinene hydrate, caracrol, and terpenes.

The impact of convection and microwave drying on the flavor quality of marjoram was studied (Raghavan et al. 1997). Cis-sabinene hydrate, trans-sabinene hydrate, and terpinen-4-ol were found to be the major components responsible for the characteristic flavor of the herb. The Indian marjoram contained 23.6% cis-sabinene hydrate. This compound was retained to a great extent in the convection- and microwave-dried (175 W) samples. Convection drying at 45°C preserved the flavor quality of marjoram better than microwave drying.

Marjoram and rosemary are herbs that are valued for their flavor and color (Singh et al. 1996). The effect of microwave blanching as compared with water and steam blanching on the volatile oil, color, texture (by compression), chlorophyll, and ascorbic acid contents of these herbs are reported. There was maximum retention of ascorbic acid in microwave-blanched marjoram and rosemary (21.5, 33.0 mg/100 g) that is, 79.4% and 49.9% compared with fresh herbs. Blanching resulted in better retention of the original green color of the fresh herb compared with direct drying of the herbs. There was greater loss of volatile oil in all the blanched samples.

Oregano (*Origanum vulgare* L.)

Active compounds are tannins, sterols, flavonoids, resin, volatile oil (including carvacrol, thymol, and borneol), rosmarinic acid, and triterpenoids (e.g., ursolic and oleanolic acid).

Raw and blanched oregano (*Origanum vulgare* L. ssp. *prismaticum* Gaudin) were air-dried at 35 and 50°C in two-drier pilot plants; raw oregano was also dried at room temperature in a shady place. Oregano leaves were analyzed for volatile phenolic compounds by GC/MS-SIM, for chlorophyll by HPLC, and for color by

reflected-light colorimetry. The highest contents of thymol and carvacrol were found in unblanched oregano dried at 35°C, followed by oregano dried at room temperature and by both samples dried at 35 and 50°C after blanching. The highest chlorophyll content was identified in oregano dried at room temperature, followed by unblanched oregano dried at 50°C and blanched oregano dried at 35°C. All unblanched, air-dried oregano leaves had color attributes similar to the raw ones, while blanched-dried leaves were greener. The least damaging treatment was air drying at 35 and 50°C without blanching (Di Cesare et al. 2004).

The maximum geographical fluctuations in Croatia found for the main components from fresh plant material of *Origanum vulgare* ssp. *hirtum* were thymol (149.2–1124.4 mg/100 g), carvacrol (51.6–564.3 mg/100 g), p-cymene (20.2–220.9 mg/100 g), and γ-terpinene (50.1–217.5 mg/100 g). The season of collecting affected the qualitative and quantitative composition of the essential oil. The most important fact was the increase of p-cymene content in August. After the drying of the plant material, all samples showed a minor decrease in essential oil yields when compared with fresh plants.

Drying, at room temperature, had no effect on the qualitative composition of oregano oil (Jerkovic et al. 2001a). The same authors in another study (Jerkovic et al. 2001b) evaluated the effect of air drying on glycosidically bound volatiles. Air drying had more effects on these compounds than seasons.

Capecka and others (2005) studied the changes in antioxidant activity of some *Laminaceae* species, including oregano, in fresh and dried condition. Parameters analyzed were antioxidant activity, total phenolics, L-ascorbic acid, and carotenoids. Drying of oregano resulted in an increase in total phenolics and great losses of ascorbic acid and carotenoids.

Rosemary (*Rosmarinus officinalis* L.)

The active compounds of the leaves are flavonoids, rosmarinic acid, diterpenes, rosmaricine, and tannins.

The effect of drying treatment on the aroma characteristics of rosemary was studied using Quantitative Descriptive Analysis (QDA) with a trained panel and by FCP analysis with a consumer group by Diaz-Maroto and others (2007). The highest differences were found between fresh rosemary samples and dried samples. However, dried samples (obtained by oven drying at 45°C and commercial samples purchased from the markets and stored for 6 months) showed significant differences in their sensory characteristics.

In another study Rao and others (1998) studied the impact of microwave drying at 175, 385, and 595 W on the flavor quality of rosemary. Flavor compounds of fresh and dried plants were identified by GC and GC-MS analysis. The greatest loss of flavor was after microwave drying (Rao et al. 1998).

MUSHROOMS

Chandra (2006) reviewed all the parameters of pretreatment and drying of edible mushrooms. Several drying methods may be used: sun drying, solar drying, hot air drying, tray and cabinet drying, fluidized bed drying, vacuum drying, infrared drying,

microwave drying, freeze drying, and osmotic dehydration. Pretreatments may be washing in water, washing in metabisulfite, blanching, sulfating, and steeping.

Ultrasound was used as a pretreatment method prior to drying of mushrooms to reduce drying time. Results were compared with blanched (80°C/3 min) and untreated samples. Mushrooms were freeze-dried or conventionally dried. Drying time was shortened and rehydration properties were better (Jambrak et al. 2007).

Walde and others (2006) evaluated different pretreatment and drying methods on button mushroom (*Agaricus bisporus* [JE Lange] Imbach) and oyster mushroom (*Pleurotus ostreatus* [Jacq.] Quélet). Pretreatments used were blanching, blanching followed by soaking in potassium metabisulfite, fermented whey, or curds. Drying methods were hot air cabinet dryer, fluidized bed dryer, vacuum dryer, and microwave oven.

Kotwaliwale and others (2007) studied the textural and optical changes of oyster mushroom during hot air drying at temperatures of 50, 55, 60, and 70°C. Also the effects of pre-drying treatments were evaluated. Drying temperature had an inverse effect on whiteness. Sulfination had a positive effect on whiteness, while blanching had a negative effect.

The volatile flavor composition of dry and fresh milky mushroom (*Calocybe indica* P&C) was analyzed by capillary gas chromatography (CGC) (Chandravadana et al. 2005). Drying reduced the concentration of 1-octen-3-ol, n-octaanol, and 3-octanone and increased the concentration of n-hexanal, 2,4-decadienol, 2,4-nonadienol, 2-octen—1-ol, 1-hexanol, decanol, and t-linalool oxide.

Valentao and others (2005) checked the influence of conservation procedure on phenolic compounds and organic acids in chanterelle (*Cantharellus cibarius* Fr.). Analyses were by HPLC-diode array detection (DAD) and HPLC-UV. Six phenolic compounds—3-, 4-, and 5-O-caffeoylquinic acid, caffeic acid, and rutin—and five organic acids—citric, ascorbic, malic, shikimic, and fumaric acids—were found.

REFERENCES

Adam E, Muhlbauer W, Esper A, Wolf W, Spiess W. 2000. Quality changes of onion (Allium cepa L.) as affected by the drying process. *Food/Nahrung* 44(1):32–7.

Ahmed J, Shivhare US, Singh G. 2001. Drying characteristics and product quality of coriander leaves. *Food Bioprod Process* 79(C2):103–6.

Alibas I. 2007. Microwave, air and combined microwave-air-drying parameters of pumpkin slices. *LWT—Food Sci Technol* 40(8):1445–51.

Al-Juamily KEJ, Khalifa AJN, Yassen TA. 2007. Testing of the performance of a fruit and vegetable solar drying system in Iraq. *Desalination* 209(1–3):163–70.

Ambrose DCP, Sreenarayanan VV. 1998. Studies on the dehydration of garlic. *J Food Sci Technol* 35(3):242–4.

Aragon MCV, Lozano M, De Espinosa VM. 2005. Evolution of the aromatic fraction in red pepper for paprika during La Vera region traditional drying process. *J Food Qual* 28(3): 211–21.

Arevalo-Pinedo A, Murr FEX. 2007. Influence of pre-treatments on the drying kinetics during vacuum drying of carrot and pumpkin. *J Food Eng* 80(1):152–6.

Balladin DA, Headley O. 1999. Evaluation of solar dried thyme (Thymus vulgaris Linne) herbs. *Renew Energy* 17(4):523–31.

Barbieri S, Elustondo M, Urbicain M. 2004. Retention of aroma compounds in basil dried with low pressure superheated steam. *J Food Eng* 65(1):109–15.

Baritaux O, Richard H, Touche J, Derbesy M. 1992. Effects of drying and storage of herbs and spices on the essential oil. I: Basil, Ocimum basilicum L. *Flavour Frag J* 7(5):267–71.

Bohm ME, Bade M, Kunz B. 2002. Quality stabilisation of fresh herbs using a combined vacuum-microwave drying process. *Adv Food Sci* 24(2):55–61.

Bondaruk J, Markowski M, Blaszczak W. 2007. Effect of drying conditions on the quality of vacuum-microwave dried potato cubes. *J Food Eng* 81(2):306–12.

Bowes KM, Zheljazkov VD. 2004. Factors affecting yields and essential oil quality of Ocimum sanctum L. and Ocimum basilicum L. cultivars. *J Am Soc Hortic Sci* 129(6):789–94.

Capecka E, Mareczek A, Leja M. 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chem* 93(2):223–6.

Chandra S. 2006. Dehydration and sensory quality evaluation of edible mushrooms: a critical review. *Journal of Food Sci Technol* 43(3):221–7.

Chandravadana MV, Vekateshwarlu G, Babu CSB, Roy TK, Shivashankara KS, Pandey M, Tewari RP, Selvaraj Y. 2005. Volatile flavour components of dry milky mushrooms (Calocybe indica). *Flavour Frag J* 20(6):715–17.

Chang CH, Lin HY, Chang CY, Liu YC. 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air dried tomatoes. *J Food Eng* 77(3):478–85.

Collins MD, Wasmund LM, Bosland PW. 1995. Improved method for quantifying capsaicinoids in Capsicum using high-performance liquid chromatography. *HortScience* 30(1): 13–40.

Daood HG, Kapitany J, Biacs P, Albrecht K. 2006. Drying temperature, endogenuous antioxidants and capsaicinoids affect carotenoid stability in paprika (red pepper slice), *J Sci Food Agric* 86(14):2450–7.

Davoodi MG, Vijayananad P, Kulkarni SG, Ramana KVR. 2007. Effect of different pretreatments and dehydration methods on quality characteristics and storage stability of tomato powder. *LWT—Food Sci Technol* 40(10):1832–40.

Di Cesare LF, Forni E, Viscardi D, Nani RC. 2003. Changes in the chemical composition of basil caused by different drying procedures. *J Agric Food Chem* 51(12):3575–81.

Di Cesare LF, Forni E, Viscardi D, Nani RC. 2004. Influence of drying techniques on the volatile phenolic compounds, chlorophyll and colour of oregano (Origanum vulgare L. ssp. prismaticum Gaudin). *Ital J Food Sci* 16(2):165–75.

Di Cesare LF, Nani R, Viscardi D, Fusari EL, Vitale R. 2001. The influence of blanching, drying and storage on the volatile fraction of the sage leaves. *Ind Aliment* 40(406):881–5.

Di Cesare LF, Proietti M, Maestrelli A, Giombelli R, Nani R, Ferrari V. 1999. Study of the volatile composition in the raw, dehydrofrozen and dried leek. *Ind Aliment* 38(385): 1113–16.

Diaz-Maroto MC, Palomo ES, Castro L, Vinas MAG, Perez-Coelio MS. 2004. Changes produced in the aroma compounds and structural integrity of basil (Ocimum basilcum L.) during drying. *J Sci Food Agric* 84(15):2070–6.

Diaz-Maroto MC, Pérez-Coello MS, Cabezudo MD. 2002. Effect of different drying methods on the volatile components of parsley (Petroselinum crispum L.). *Eur Food Res Technol* 215(3):227–30.

Diaz-Maroto MC, Pérez-Coello MS, Sánchez-Palomo E, González Viñas MA. 2007. Impact of drying and storage time on sensory characteristics of rosemary (Rosmarinus Officinalis L.). *J Sens Stud* 22(1):34–48.

Diaz-Maroto MC, Vinas MAG, Cabezudo MD. 2003. Evaluation of the effect of drying on aroma of parsley by free choice profiling. *Eur Food Res Technol* 216(3):227–32.

Doymaz I. 2007. Air drying characteristics of tomatoes. *J Food Eng* 78(4):1291–7.

Doymaz I, Tugrul N, Pala M. 2006. Drying characteristics of dill and parsley leaves. *J Food Eng* 77(3):559–65.

Durling NE, Catchpole OJ, Grey JB, Webby RF, Mitchell KA, Foo LY, Perry NB. 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. *Food Chem* 101(4):1417–24.

El-Sebaii AA, Aboul-Enein S, Ramadan MRI, El-Gohary HG. 2002. Experimental investigation of an indirect type natural convection solar dryer. *Energy Convers Manage* 43(16): 2251–66.

Ergunes G, Tarhan S. 2006. Color retention of red peppers by chemical pretreatments during greenhouse an open sun drying. *J Food Eng* 76(3):446–54.

Fathima A, Begum K, Rajalakshmi D. 2001. Microwave drying of selected greens and their sensory characteristics. *Plant Foods Hum Nutr* 56(4):303–11.

Fatouh M, Metwally MN, Helah AB, Shedid MH. 2006. Herbs drying using a heat pump dryer. *Energy Convers Manage* 47(15–16): 2629–43.

Gallali YM, Abujnah YS, Bannani FK. 2000. Preservation of fruits and vegetables using solar drier: A comparative study of natural and solar drying, III; chemical analysis and sensory evaluation data of the dries samples (grapes, figs, tomatoes and onions). *Renew Energy* 19(1–2):203–12.

Ghosh PK, Agrawai YC, Jayas DS, Kumbhar BK. 2006. Process development for osmo-hot air drying of carrot. *J Food Sci Technol* 43(1):65–8.

Giovanelli G, Zanoni B, Lavelli V, Nani R. 2002. Water sorption, drying and antioxidant properties of dried tomato products. *J Food Eng* 52(2):135–41.

Goula AM, Adamapoulos KG. 2005. Stability of lycopene during spray drying of tomato pulp. *LWT—Food Sci Technol* 38(5):479–87.

Hatamipour MS, Mowla D. 2003. Correlations for shrinkage, density and diffusivity for drying of maize and green peas in a fluidized bed with energy carrier. *J Food Eng* 59(2–3): 221–7.

Hatamipour MS, Mowla D. 2006. Drying behaviour of maize and green peas immersed in fluidized bed of inert energy carrier particles. *Food Biprod Process* 84(C3):220–6.

Heredia A, Barrera C, Andrés A. 2007. Drying of cherry tomato by a combination of different dehydration techniques. Comparison of kinetics and other related properties. *J Food Eng* 80(1):111–18.

Jambrak AR, Mason TJ, Paniwnyk L, Lelas V. 2007. Accelerated drying of button mushrooms. Brussels sprouts and cauliflower by applying power ultrasound and its rehydration properties. *J Food Eng* 81(1):88–97.

Janjai S, Tung P. 2005. Performance of a solar dryer using hot air from roof-integrated solar collectors for drying herbs and spices. *Renew Energy* 30(14):2085–95.

Jerkovic I, Mastelic J, Milos M. 2001a. The effect of air-drying on glycosidically bound volatiles from seasonally collected oregano (Origanum vulgare ssp hirtum) from Croata. *Food/Nahrung* 45(1):47–9.

Jerkovic I, Mastelic J, Milos M. 2001b. The impact of both the season of collection and drying on the volatile constituents of Origanum vulgare L. ssp hirtum grown wild in Croatia. *Int J Food Sci Technol* 36(6):649–54.

Joy CM, George PP, Jose KP. 2001. Solar tunnel drying of red chillis (Capsicum annuum L.). *J Food Sci Technol* 38(3):213–16.

Jun HR, Cho IH, Choi HK, Kim YS. 2005. Comparison of volatile components in fresh and dried red peppers (Capsicum annuum L.). *Food Sci Biotechnol* 14(3):392–8.

Kaur P, Kumar A, Arora S, Ghuman BS. 2006. Quality of dried coriander leaves as affected by pretreatments and method of drying. *Eur Food Res Technol* 223(2):189–94.

Kaymak-Ertekin F, Gedik A. 2005. Kinetic modelling of quality deterioration in onions during drying and storage. *J Food Eng* 68(4):443–53.

Kerkhofs NS, Lister CE, Savage GP. 2005. Change in colour and antioxidant content of tomato cultivars following forced-air drying. *Plant Foods Hum Nutr* 60(3):117–21.

Khraisheh MAM, McMinn WAM, Magee TRA. 2004. Quality and structural changes instarchy foods during microwave and convective drying. *Food Res Int* 37(5):497–503.

Kim S, Lee KW, Park J, Lee HJ, Hwang IK. 2006. Effect of drying in antioxidant activity and changes of ascorbic acid and colour by different drying and storage in Korean red pepper (Capsicum annuum, L.). *Int J Food Sci Technol* 41:90–5.

Kim S, Park J, Hwang IK. 2002. Changes in FA composition and antioxidative activity of pigment extracts from Korean red powder (Capsicum annuum L.) due to processing conditions. *J Am Oil Chem Soc* 79(12):1267–70.

Kim S, Park J, Hwang IK. 2004. Compsition of main carotenoids in Korean redpepper (Capsicum annuum L.) and changes of pigment stability during the drying and storage process. *J Food Sci* 69(1):C39–44.

Kotwaliwale N, Bakane P, Verma A. 2007. Changes in textural and optical properties of oyster mushroom during hot air drying. *J Food Eng* 78(4):1207–11.

Kozukue N, Han JS, Kozukue E, Lee SJ, Kim JA, Lee KR, Levin CE, Friedman M. 2005. Analysis of eight capsaicinoids n peppers and pepper-containg foods by high-performance liquid chromatography and liquid chromatography-mass spectrometry. *J Agric Food Chem* 53(23):9172–81.

Kumar DGP, Hebbar HU, Sukumar D, Ramesh MN. 2005. Infrared and hot-air drying of onions. *J Food Process Preserv* 29(2):132–50.

Latapi G, Barett DM. 2006a. Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part I. Use of steam blanching, boiling brine blanching, and dips in salt or sodium metabisulfite. *J Food Sci* 71(1):S24–31.

Latapi G, Barett DM. 2006b. Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part II. Effects of storage on nutritional and sensory quality of sun-dried tomatoes pretreated with sulphur, sodium metabisulfite, or salt. *J Food Sci* 71(1):S32–7.

Lewicki PP. 2006. Design of hot air drying for better foods. *Trends Food Sci Technol* 17(4): 153–63.

Lewicki PP, Le HV, Pomaranska-Lazuka W. 2002. Effect of pre-treatment onconvective drying of tomatoes. *J Food Eng* 54(2):141–6.

Li Y, Xu SY, Sun DW. 2007. Preparation of garlic powder with high allicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. *J Food Eng* 83(1):76–83.

Machewad GM, Kulkarni DN, Pawar VD, Surve VD. 2003. Studies on dehydration of carrot (Daucus carota L.). *J Food Sci Technol* 40(4):406–8.

Maeda EE, Salunkhe DK. 1981. Retention of Ascorbic acid and total carotene in solar dried vegetables. *J Food Sci* 46(4):1288–90.

Marabi A, Thieme U, Jacobson M, Saguy IS. 2006. Influence of drying method and rehydration time on sensory evaluation of rehydrated carrot particles. *J Food Eng* 72(3):211–17.

Markowski M, Stankiewicz I, Zapotoczny P, Borowska J. 2006. The effect of variety on drying characteristics and selected quality attributes of dried carrots. *Dry Technol* 24(8):1011–18.

Martinez S, Lopez M, Gonzalez-Raurich M, Alvarez AB. 2005. The effects of ripening stage and processing systems on vitamin C content in sweet peppers (Capsicum annuum L.). *Int J Food Sci Nutr* 56(1):45–51.

Maw BW, Butts CL, Purvis AC, Seebold K, Mullinix BG. 2004. High-temperature continuous-flow curing of sweet onions. *Appl Eng Agric* 20(5):657–63.

Mdziniso P, Hinds MJ, Bellmer DD, Brown B, Payton ME. 2006. Physical quality and carotene content of solar-dried green leafy and yellow succulent vegetables. *Plant Foods Hum Nutr* 61(1):13–21.

Negi PS, Roy SK. 2001. Effect of drying conditions on quality of green leaves during long term storage. *Food Res Int* 34(4):283–6.

Oberoi HS, Ku MA, Kaur J, Baboo B. 2005. Quality of red chili variety as affected by different drying methods. *J Food Sci Technol* 42(5):384–7.

Onayemi O, Badifu GIO. 1987. Effect of blanching and drying methods on the nutritional and sensory quality of leafy vegetables. *Plant Foods Hum Nutr* 37:291–8.

Paakkonen K. 2002. A combined infrared/heat pump drying technology applied to a rotary dryer. *Agric Food Sci Finland* 11(3):209–18.

Pande VK, Sonune AV, Philip SK. 2000. Solar drying of coriander and Methi leaves. *J Food Sci Technol* 37(6):592–5.

Park JH, Kim CS. 2007. The stability of color and antioxidant compounds in paprika (Capsicum annuum L) powder during the drying and storing process. *Food Sci Biotechnol* 16(2):187–92.

Parliament TH, Kolor MG, Maing IY. 1977. Identification of the major volatile components of cooked beets. *J Food Sci* 42(6):1592–3.

Perez-Galvez A, Hornero-Mendez D, Minguez-Mosquera MI. 2005. Dependence of carotenoid content and temperature-time regimes during the traditional slow drying of red pepper for paprika production at La Vera County. *Eur Food Res Technol* 221(5):645–52.

Pordesimo LO, Li H, Lee JH, Reddick BB. 2004. Effects of drying procedure, cultivar, and harvest number on capsaicin levels in dried jalapeno peppers. *Appl Eng Agric* 20(1):35–8.

Prakash S, Jha SK, Data N. 2004. Performance evaluation of blanched carrots dried by three different driers. *J Food Eng* 62(3):305–13.

Raghavan B, Rao LJ, Abraham KO. 1997. Effect of drying methods on the flavour quality of marjoram (Orgenaum marjorana L.). *Food/Nahrung* 41(3):159–61.

Rao LJ, Singh M, Raghavan B, Abraham KO. 1998. Rosemary (Rosmarinus officinalis L;): Impact of drying on its flavour quality. *J Food Qual* 21(2):107–15.

Ratti C, Araya-Farias M, Mendez-Lagunas L, Makhlouf J. 2007. Drying of garlic (Allium sativum) and its effect on allicin retention. *Dry Technol* 25(1–3):349–56.

Regier M, Mayer-Miebach E, Behsnillian D, Neff E, Schuchmann HP. 2005. Influences of drying and storage of lycopene-rich carrots on the carotenoid content. *Dry Technol* 23(4):989–98.

Sanjuan N, Hernando I, Lluch MA, Mulet A. 2005. Effects of low temperature blanching on texture, microstructure and rehydration capacity of carrots. *J Sci Food Agric* 85(12): 2071–6.

Schweiggert U, Schieber A, Carle R. 2006. Effects of blanching and storage on capsaicinoid stability ad peroxidase activity of hot chilli peppers (Capsicum frutescens L.). *Innov Food Sci Emerg Technol* 7(3):217–24.

Shanmugam V, Natarajan E. 2007. Experimental study of regenerative desiccant integrated solar dryer with and without reflective mirror. *Appl Therm Eng* 27(8–9): 1543–51.

Sharma GP, Prasad S. 2001. Drying of garlic (Allium sativum) cloves by microwave-hot air combination. *J Food Eng* 50(2):99–105.

Sigge GO, Hansmann CF, Joubert E. 1999. Optimizing the dehydration conditions of green bell peppers (Capsicum annuum L.): Quality criteria. *J Food Qual* 22(4):439–52.

Singh M, Raghavan B, Abraham KO. 1996. Processing of marjoram (Majorana hortensis Moench.) and rosemary (Rosmarinus officinalis L.). Effect of blanching methods on quality. *Food/Nahrung* 40(5):264–6.

Souza JS, Medeiros MFD, Magalhaães MMA, Rodrigues S, Fernandes FAN. 2007. Optimization of osmotic dehydration of tomatoes in a ternary system followed by air-drying. *J Food Eng* 83(4):501–9.

Soysal Y. 2004. Microwave drying characteristics of parsley. *Biosys Eng* 89(2):167–73.

Suman M, Kumari KK. 2002. A study on sensory evaluation, beta-carotene retention and shelf-life of dehydrated carrot products. *J Food Sci Technol* 39(6):677–81.

Tiwari A, Pandey JP. 2007. Mass transfer kinetics model of osmotic dehydration of sweet pepper. *J Food Sci Technol* 44(3):334–7.

Toor PK, Savage GP. 2006. Effect of semi-drying on the antioxidant components of tomatoes. *Food Chem* 94(1):90–7.

Topuz A, Ozdemir F. 2004. Influences of gamma irradiation and storage on the capsaicinoids of sun-dried and dehydrated paprika. *Food Chem* 86(4):509–15.

Uma D, Sharma GK. 2007. Effect op processing and storage on the physico-chemical properties of dry peas (Pisum sativum L.). *J Food Sci Technol*, 44(2):212–15.

Valentao P, Andrade PB, Rangel J, Ribeiro B, Silva BM, Baptista P, Seabra RM. 2005. Effect of the conservation procedure on the contents of phenolic compounds and organic acids in chanterelle (Cantharellus cibarius) mushroom. *J Agric Food Chem* 53(12):4925–31.

Van Ruth SM, Roozen JP, Posthumus MA. 1995. Instrumental and sensory evaluation of the flavour of dried French beans (Phaseolus vulgaris) influenced by storage conditions. *J Sci Food Agric* 69(3):393–401.

Venskutonis PR. 1997. Effect of drying on the volatile constituents of thyme (Thymus vulgaris L.) and sage (Salvia officinalis L.). *Food Chem* 59(2):219–27.

Walde SG, Velu V, Jyothirmayi T, Matg RG. 2006. Effects of pretreatments and drying methods on dehydration of mushroom. *J Food Eng* 74(1):108–15.

Wang J, Wang JS, Yu Y. 2007. Microwave drying characteristics and dried quality of pumpkin. *Int J Food Sci Technol* 42 (2):148–56.

Yaldyz O, Ertekyn C. 2001. Thin layer solar drying of some vegetables. *Dry Technol* 19(3–4):583–97.

Zhang M, Tang J, Mujumdar AS, Wang S. 2006. Trends in microwave-related drying of fruits and vegetables. *Trends Food Sci Technol* 17(10):524–34.

CHAPTER 51

Understanding Peanut Flavor: A Current Review

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INTRODUCTION

Peanuts are a significant commodity in the United States, with an average consumption of approximately 1 million tons per year (American Peanut Council 2007). In contrast with other countries where the end products are peanut oil, cake, and meal, the major market for U.S. peanuts is in edible products. Peanut butter accounts for about 50% of the market, while the other half is divided equally between snack nuts and confectionary products. Only about 15% of the U.S. crop serves as base for peanut oil production (American Peanut Council 2007).

The driving force for the consumption of peanuts is the unique and pleasant flavor that is developed during roasting (Sanders et al. 1997). Peanut flavor is influenced by genetic, environmental, physiological, and biochemical processes occurring in the seed, as well as handling, processing, and storage conditions (Sanders et al. 1995; Young et al. 1974). The numerous combinations of interactions of these factors affect the final chemical composition of peanuts, which in turn determine flavor quality. The flavor of roasted peanuts is determined by the composition and concentration of volatiles present, with over 200 aroma-active compounds identified to date (Schirack et al. 2006). Understanding the role of these aroma-active compounds may allow for better control of flavor of peanut products, which would benefit both consumers and the peanut industry. Although significant efforts have been made to identify the potent odorants in peanuts, the specific compound(s) or groups of compounds responsible for the roasted peanut flavor remain elusive.

This review presents a current summary of progress in peanut flavor research and the advances in understanding the chemical basis of peanut flavor by means of sensory and instrumental analysis. Pyrazines have long been considered as important components of roasted peanut flavor (Maga and Sizer 1973; Mason et al. 1966, 1967, 1969; Shibamoto and Bernhard 1976), but there is limited conclusive

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information showing that they are the basis for the roasted peanut flavor. This review provides an overview of current flavor chemistry techniques and common mechanisms for pyrazine formation in several model systems. In addition, numerous compounds that have been associated with typical roasted peanut flavors are reviewed. Finally, the major reactions and chemical changes associated with the most common off-flavors developed in roasted peanuts during processing and storage are discussed.

FLAVOR ISOLATION TECHNIQUES

Development of reliable, efficient methods to extract analytes from food systems has been a challenge to researchers. A proficient technique for the extraction of volatile compounds from foods should meet the following requirements: (1) extract the key compounds contributing to flavor, (2) do not destroy or alter the structure of relevant aroma compounds, (3) do not create new aroma compounds or artifacts, and (4) eliminate nonvolatile compounds that might interfere with gas chromatographic separation (Engel et al. 1999).

Several different analytic methods have been developed to study the volatile composition of foods including steam distillation, solvent extraction, simultaneous distillation–extraction (SDE), high vacuum distillation, solvent-assisted flavor evaporation (SAFE) method, and headspace analysis (Curioni and Bosset 2002; Sides et al. 2000). Numerous studies have shown that the composition of aroma extracts is highly dependent on the isolation technique employed (Fischer et al. 1995; Vandeweghe and Reineccius 1990). There is no single method that allows simultaneous recovery of the full range of aroma compounds present in a food system; thus, a combination of extraction techniques must be used to completely characterize the aroma-active volatile compounds (Drake et al. 2006).

Steam distillation is one of the oldest techniques for extracting volatile compounds from foods. Although the method is rapid and simple, the high temperatures utilized may lead to formation of artifacts in the samples. In addition, the volatiles are highly diluted in water after collection in cold traps (Sides et al. 2000).

Direct solvent extraction is a valuable technique for isolating higher-molecular-weight volatile compounds from nonvolatile food matrices (Wong and Park 1968). The extract is usually concentrated under nitrogen or in a rotatory evaporator. The shortcomings of this method are co-extraction of matrix components and extraction of relatively low amounts of high volatile compounds (Sides et al. 2000).

Currently, SDE is one of the most popular flavor isolation techniques. This method allows simultaneous extraction of steam distillates by solvents but has the limitation of forming thermally induced artifacts (Nickerson and Likens 1966). High vacuum transfer (HVT) is a technique that allows transferring of volatiles between two vessels based on an extreme temperature differential, thereby reducing the possibility of artifact formation (Schieberle and Grosch 1985; Weurman et al. 1970). SAFE is an improved and specialized version of the HVT technique (Engel et al. 1999). SAFE provides rapid isolation of volatiles from food suspensions, matrices with high fat content, and aqueous foods (Engel et al. 1999). SAFE has been widely employed in flavor chemistry research including analysis of volatile compounds in milk (Bendall and Olney 2001; Havemose et al. 2007), sweet cream butter (Lozano

et al. 2007), coffee beans (Scheidig et al. 2007), peanuts (Didzbalis et al. 2004; Schirack et al. 2006), and cheese (Suriyaphan et al. 2001; Whetstine et al. 2005).

Headspace analysis includes dynamic and static methods. Dynamic headspace analysis (DHA) or purge and trap is an effective method for the extraction of aroma compounds from foods. In this technique, an inert gas is continuously passed through the sample, and the volatile compounds are collected in a trap containing an adsorbent (Sides et al. 2000). This method has the advantages of being nondestructive, sensitive, and rapid. The main disadvantage is that water is collected with the volatile material. Static headspace is a technique based on the establishment of equilibrium between the food sample and the gas phase above it. Solid phase microextraction (SPME) is a variant of the static headspace method that has gained popularity in recent years. A fused silica fiber coated with an absorbent is immersed into the headspace above a liquid or solid sample, and analytes are extracted by diffusion onto the coating (Sides et al. 2000). SPME has been extensively used for quantitative analysis of flavor compounds because analytes are isolated without interferences from the matrix components (Steffen and Pawliszyn 1996). The main advantages of this method are simplicity, high speed, low cost, small sample volume, and high sensitivity (Kataoka et al. 2000). In general, extracts obtained by headspace analysis (dynamic and static) contain fewer compounds than those obtained by solvent extraction or distillation methods. In addition, headspace analysis is usually not suitable for isolating high-molecular-weight compounds, as well as tightly bound and encapsulated volatiles (Sides et al. 2000).

INSTRUMENTAL ANALYSIS

Separation of volatiles extracted from foods is generally performed using gas chromatography (GC). Following or during GC separation of compounds, identification may be accomplished using mass spectrometry (MS) and tentative identifications may be made using retention indices and aroma profiles from gas chromatography–olfactometry (GC-O) analysis. GC combined with MS (GC-MS) has been considered the ideal device for the identification of volatile compounds. In general, a mass spectrometer consists of an ion source, a mass-selective analyzer, and an ion detector. Atoms or molecules are ionized, and fragments of specific mass and charge are created. The mass-selective analyzer then separates the ions based on their mass-to-charge ratio (m/z). Molecules have distinctive fragmentation patterns, which can provide structural information, chemical formula, and molecular weight of the molecule, depending on the type of MS technique applied (Ravindranath 1989).

Although traditional GC analysis is a powerful tool for the identification and quantification of volatile compounds in foods, it does not provide information regarding the relative contribution of individual components to flavor. Usually, only a small percentage of the total compounds present are aroma active and thus contribute significantly to a particular flavor. Also, the total amount of a given compound is not necessarily related to its flavor impact, as the concentration present may still fall below sensory threshold (McGorrin 2002).

GC-O is a semiquantitative technique that provides links between GC data and odor-active compounds that potentially play a role in flavor (Fuller et al. 1964). In this technique, the GC has been modified with an olfactometer or sniffer port at the

detector end of the column, and a trained panelist (sniffer) describes the aroma of individual compounds as they elute from the GC column. Although GC-O is a valuable technique in identifying odor-active compounds, it may be difficult to determine the sensory relevance of these volatiles from a single chromatogram.

Dilution analysis such as aroma extract dilution analysis (AEDA) and CHARM Analysis™ are commonly used to determine the potential relative contribution of a compound to the flavor of the food (Acree et al. 1984; Grosch 1993). In dilution analysis, an extract is serially diluted and sniffed until no odorants are detected at the sniffer port. The lowest detectable amount eluting from the column is converted to a flavor dilution (FD) value in AEDA, or to a CHARM value in CHARM Analysis (Acree et al. 1984; Grosch 1993).

CORRELATING SENSORY AND INSTRUMENTAL ANALYSIS

Descriptive sensory analysis (DSA) is a powerful tool for flavor characterization of foods. DSA utilizes a lexicon of terms (descriptors) that define the various flavor characteristics of a food. A lexicon is developed in a moderated session of trained or experienced panelists who use words to describe the various flavor characteristics of a particular food. Upon completion, a lexicon is a defined and nonredundant list of flavor descriptors that describe the sensory characteristics found in a wide range of available samples of a particular food (Drake and Civille 2003). DSA makes use of a panel trained in the use of a lexicon that functions as an instrument to evaluate both quantitative (intensity) and qualitative (lexicon) components of a product (Meilgaard et al. 1991). Use of a highly trained panel results in reproducibility of results such that effective correlation of DSA with instrumental and/or consumer data can be achieved (Drake and Civille 2003). The most common DSA methods are the Flavour Profile Method™, Quantitative Descriptive Analysis™, Quantitative Flavour Profiling™, Free-Choice Profiling™, and Spectrum™ Method (Murray et al. 2001).

In general, relationships between sensory and instrumental analysis of foods can be determined by following three steps: (1) selection of flavor(s) of interest using DSA; (2) instrumental analysis of volatile extracts; and (3) confirmation of aroma-active compounds via quantitation, threshold testing, and DSA of model systems (Drake et al. 2006). Schirack et al. (2006) used this approach to show that phenylacetaldehyde, guaicol, and 2,6-dimethylpyrazine were responsible for the stale/floral and ashy off-flavor in high temperature microwave-blanched peanuts. Using a similar tactic, Didzbalis and others (2004) showed that fruit-like esters such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate along with short-chain organic acids (butanoic, 3-methlbutanoic, and hexanoic) were responsible for the fruity/fermented off-flavor developed in immature peanuts cured at high temperatures.

FORMATION OF VOLATILE COMPOUNDS

The predominant reactions involved in the formation of volatile compounds in roasted peanuts are Maillard reaction, Strecker degradation, thermal degradation

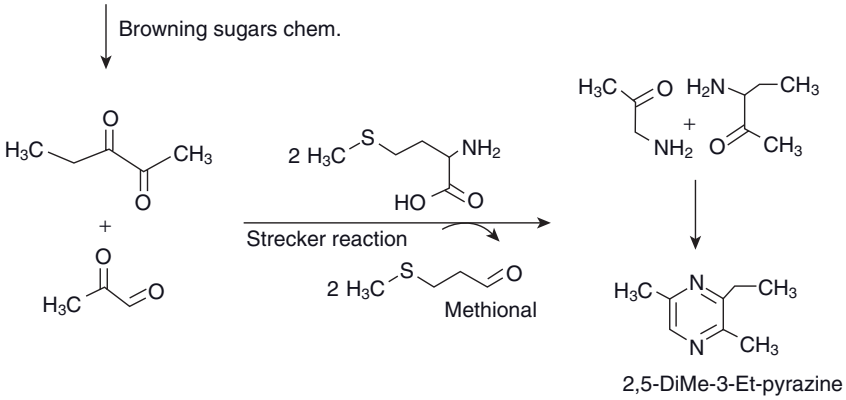

Figure 51.1. Formation of an alkylpyrazine through the Strecker degradation reaction.

of sugars (caramelization), and lipid oxidation (Cammarn et al. 1990; Coleman et al. 1994). The Maillard reaction involves the interaction of reducing sugars and amino compounds such as amines, amino acids, peptides, and proteins. The main products from this reaction are heterocyclic nitrogen compounds, such as furans, thiazoles, thiophenes, oxazoles, pyrroles, imidazoles, pyridines, and pyrazines (Hodge 1953; Hwang et al. 1995). The Strecker degradation involves the degradation of α-amino acids by reductones (α-dicarbonyls), which are usually derived from the Maillard reaction (Hodge 1967; Schonberg and Moubacher 1952). During the Strecker degradation, the α-dicarbonyls are converted into α-amino carbonyls, which eventually condense to form alkylpyrazines (Fennema 1996) (Fig. 51.1). Thermal degradation of sugars (caramelization) produces low-molecular-weight open-chain oxygen-containing compounds, as well as heterocyclic oxygen-containing species such as furan derivatives (Coleman et al. 1994). Lipid oxidation leads to the formation of oxygen-containing compounds such as aliphatic aldehydes, ketones, and alcohols (Coleman et al. 1994). In addition, free radicals, hydroperoxides, and secondary products formed during lipid oxidation may interact with nitrogen-containing compounds such as proteins and possibly pyrazines, modifying the precursors available for Maillard reactions as well as the flavor compounds already formed during roasting (Alzagtat and Alli 2002; Funes et al. 1982; Gardner 1979; St. Angelo and Graves 1986; Vercellotti et al. 1992; Williams et al. 2006). Lipids can be oxidized by both enzymatic and nonenzymatic mechanisms. In plant systems such as peanuts, lipoxygenase acts as a catalyst to oxidize polyunsaturated fatty acids to form hydroperoxides, which then break down to produce a number of secondary products (Fennema 1996). Although roasting of peanuts inactivates enzymes, lipid oxidation may still occur because lipoxygenase contains transition metals such as iron and copper that become catalysts in nonenzymatic mechanisms (Ory et al. 1992).

PEANUT COMPOSITION AND FLAVOR PRECURSORS

Peanut seeds consist of about 47% fat, 25% protein, 19% carbohydrates, and 7% water. The proximate composition of raw and roasted Virginia-type peanuts is

shown in Table 51.1 (Derise et al. 1974). Unsaturated fatty acid concentrations are high in peanut oil, which makes peanut products very susceptible to lipid oxidation (Ory et al. 1992; St. Angelo 1996). Oleic acid and linoleic acids are the most abundant fatty acids in the peanut seed (Jonnala et al. 2005). The main proteins are albumins and two globulins, arachin and conarachin. The carbohydrates include starch, pectin, cellulose, and sucrose (Hoffpauir 1953). Peanuts also contained a variety of minerals, as illustrated in Table 51.2 for the runner market type (Jonnala et al. 2005). Talcott and others (2005) found that peanuts are a good source of antioxidant polyphenolics such as *p*-coumaric acid. They reported that the amount of *p*-coumaric acid increased during roasting, probably as a result of hydrolytic reactions from the native esterified or bound forms.

A flavor precursor is usually a nonvolatile compound, which can be converted to a volatile compound under certain conditions such as thermal processing, fermentation, and storage (Newell et al. 1967). The unique roasted peanut flavor developed during the roasting of peanuts is influenced by a number of factors, such as genetics, seed size, maturity, curing, production, handling, and storage conditions (Mason et al. 1969; Oupadissakoon et al. 1980; Pattee et al. 1981; Vercellotti et al. 1994).

Sugars, proteins, and free amino acids have been considered the main precursors of roasted peanut flavor (Chiou et al. 1991; Koehler et al. 1969; Mason et al. 1969). Mason and Waller (1964) used particulate fractionation to identify the specific location of flavor precursors in peanuts. Using a density gradient centrifugation procedure, three fractions (upper, middle, and bottom layers) were obtained from resuspension of particulates in oil. The upper layer, which was rich in aleurone grains and protein bodies, produced the typical aroma of roasted peanuts when heated in oil, and thereby was considered the main source of flavor precursors. The critical minimum temperature for flavor formation was 132°C, which excluded enzymatically catalyzed reactions giving rise to flavor components.

Previous studies have reported the incidence of sucrose, fructose, and glucose in peanuts, with sucrose being the major sugar (Mason et al. 1969; Newell et al. 1967;

TABLE 51.1. Proximate Composition for Virginia-Type Raw and Roasted Peanuts

Constituent	Raw (%)	Roasted (%)
Moisture	6.7	2.5
Fiber	4.9	5.4
Fat	47	48.8
Nitrogen	4.53	4.76
Protein	24.7	26
Ash	2.54	3.39

Adapted from Derise and others (1974).

TABLE 51.2. Mineral Composition (mg/100 g) for Runner Market-Type Peanuts

P	Ca	K	Mg	Cu	Fe	Zn	Na
355.9	76.4	577.3	190.8	1.0	2.2	2.9	27.1

Adapted from Jonnala and others (2005).

Oupadissakoon and Young 1984). During roasting, sucrose is hydrolyzed into fructose and glucose by invertase, and these reducing sugars take part in browning reactions (Mason et al. 1969). Basha (1992) found that sucrose was the major soluble sugar constituent in peanuts, followed by glucosamine, stachyose, and raffinose, while the insoluble fraction contained glucosamide, arabinose, and trace levels of glucose and rhamnose.

Amino acids have been considered as the primary source of nitrogen for pyrazine structures formed during roasting (Koehler et al. 1969). Newell and others (1967) tracked the changes in the concentration of several amino acids during the roasting of mature and immature peanuts, and proposed that aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine were the main precursors of the typical peanut flavors, and threonine, tyrosine, and lysine the major precursors of the atypical or off-flavors. Arginine has also been considered the precursor of atypical bitter taste in roasted peanuts (Cobb and Johnson 1973; Woodroof 1983).

Flavor development is sensitive to peanut maturity (Sanders et al. 1982, 1989). The amount of carbohydrates and α-amino nitrogen in peanuts decreases with increasing maturity, possibly due to the utilization of these compounds in the seed for syntheses of starch, lipid, and protein during maturation (Oupadissakoon et al. 1980; Rodriguez et al. 1989; Vercellotti et al. 1994). Mature peanuts contain more of the amino acid precursors of typical roasted peanuts than immature peanuts (Pattee and Young 1987).

Moisture content is important in flavor development reactions. Raw peanuts containing 10.5% moisture had more soluble carbohydrates and glucose than peanuts containing 3.4% moisture (Chiou et al. 1991). This is in agreement with Pattee and others (1982), who found that peanuts stored at higher moisture contents (8.7–9.2%) contained more glucose, fructose, inositol, and raffinose than peanuts stored at lower moisture contents (6.2–6.3%). Higher-moisture peanuts had lower concentrations of pyrazines and roasted peanut flavor, as well as higher sensory scores for undesirable flavors associated with lipid oxidation, such as painty and cardboardy (Abegaz et al. 2004).

Understanding the changes in flavor precursor content during roasting is also essential for peanut flavor optimization. Chiou and others (1991) noticed that the amount of precursor amino acids in peanuts increased in the early stages of roasting, most likely due to hydrolysis. However, a decrease in the concentration of these compounds was observed as roasting proceeded, probably due to their involvement in further chemical reactions. Thus, the original content of flavor precursors in raw peanuts may not be a final indicator of flavor quality. Basha and Young (1985) observed a decrease in methionine-rich proteins during roasting and suggested that they might be involved in the formation of pyrazine compounds.

VOLATILE COMPOSITION OF ROASTED PEANUTS

During roasting, flavor precursors in peanuts undergo Maillard or nonenzymatic browning reactions, producing numerous heterocyclic compounds that give rise to roasted peanut flavor. Heat-treated aqueous extracts of roasted peanuts contained several volatile compounds that were consistent with Maillard reactions such as pyrazines (Coleman et al. 1994).

Early studies into peanut flavor chemistry focused on isolating and identifying thermal products of roasting, but their importance and balance for the overall flavor were not discussed. In a study conducted by Walradt and others (1971), steam volatiles from roasted Spanish peanuts were fractionated using preparative GC, and the individual fractions were analyzed by GC-MS. A total of 187 compounds were identified, including phenols, carbonyls, alcohols, aromatic hydrocarbons, esters, terpenes, and pyrazines. Several alkyl- and alkenylpyrazines were reported for the first time in roasted peanuts. Johnson and others (1971b) isolated several volatile compounds of roasted peanuts by vacuum degassing of the pressed oil, followed by fractionation of the condensate. A total of 24 new compounds were identified in the neutral fraction, including seven furans, six pyrroles, three 2-phenyl-2-alkenals, and two thiophenes. Ho and others (1981) isolated flavor compounds from the Florunner variety roasted peanuts by removal of volatiles from the headspace and subsequent condensation. The isolated compounds were fractionated using preparative GC, and the fractions were identified by a combination of infrared and MS. A total of 131 compounds were identified (70 for the first time) in roasted peanuts, including lactones, pyrazines, pyrroles, pyridines, sulfides, thiazoles, thiophenes, furanoids, oxazoles, oxazolines, and sulfides. Using a similar extraction method, Lee and others (1981) identified several new volatile compounds in Florunner roasted peanuts, including eight thiazoles, seven oxazoles, and three oxazolines. Buckholz and others (1980) identified the following compounds using a polymer adsorption method followed by MS: isobutyraldehyde, 2-methylbutanal, 1-methylpyrrole, 2-methylpyrazine, and 2-5-dimethylpyrazine.

Brown and others (1972) found that roasted peanuts contained higher amounts of total carbonyl (compounds containing the functional group C=O) than raw peanuts, and concluded that this was probably due to the acceleration of lipid oxidation at high temperatures, and to a lesser extent to Maillard reactions and Strecker degradation. In the same study, 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal were present in roasted peanuts, whereas they were absent in raw peanuts. These aldehydes are produced by Strecker degradation of the corresponding amino acids valine, leucine, and isoleucine, and are thought to be associated with the harsh aroma of freshly roasted peanuts (Mason et al. 1967).

Although early studies characterized the volatile compounds in roasted peanuts, limited information was made available regarding odor quality and intensity. In addition, the flavor isolation and separation techniques used in these studies may have led to the formation of artifacts and loss of volatiles.

ACTIVE AROMA COMPOUNDS AND SENSORY PERCEPTION

The human sensory perception of peanut flavor is determined by a combination of the gustatory, olfactory, and trigeminal systems. The gustatory system detects basic tastes such as sweet, bitter, salty, sour, and umami; the trigeminal system relates to perception of chemical feeling factors such as astringency, pungency, and acridness, and the olfactory system detects volatile compounds (Lawless and Heymann 1998). Volatile compounds are the key element in the flavor profile of roasted peanuts, and they can be perceived orthonasally or retronasally.

In the early 1980s, a descriptive lexicon containing several terms and definitions was developed for sensory evaluation of roasted peanuts (Oupadissakoon and

Young 1984). Among the attributes were astringent, bite, burnt, chemical, earthy, green, nutty, oil, rancid, roasted peanut, sour, stale, bitter, and sweet. A few years later, Johnsen and others (1988) developed a more complete language, which included terms that described the degree of roast, such as raw bean and dark roast, as well as the flavors generated at different stages of oxidation, such as cardboardy and painty (Table 51.3). Later on, the terminology was modified, and the new

TABLE 51.3. Lexicon of Peanut Flavors Developed by Johnsen and Others (1988)

	Descriptor	Definition
Aromatics	Roasted peanutty	The aromatic associated with medium-roast peanuts (about 3–4 USDA color chips) and having fragrant character such as methylpyrazine
	Raw beany	The aromatic associated with light-roast peanuts (about 1–2 on USDA color chips) and having legume-like character (specify beans or pea if possible)
	Dark roasted peanut	The aromatic associated with dark roasted peanuts (4+ on USDA color chips) and having very browned or toasted character
	Sweet aromatic	The aromatics associated with sweet material such as caramel, vanilla, molasses, fruit (specify type)
	Woody/hulls/ skins	The aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins
	Cardboardy	The aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard
	Painty	The aromatic associated with linseed oil, or oil-based paint
	Burnt	The aromatic associated with very dark roast, burnt starches, and carbohydrates (burnt toast or espresso coffee)
	Green	The aromatic associated with uncooked vegetables/ grass twigs, *cis*-3-hexanal
	Earthy	The aromatic associated with wet dirt and mulch
	Grainy	The aromatic associated with raw grain (bran, starch, corn, sorghum)
	Fishy	The aromatic associated with trimethylamine, cod-liver oil, or old fish
	Chemical/plastic	The aromatic associated with plastic and burnt plastics
	Skunky/ mercaptan	The aromatic associated with sulfur compounds, such as mercaptan, which exhibit skunk-like character
Tastes	Sweet	The taste on the tongue associated with sugars
	Sour	The taste on the tongue associated with acids
	Salty	The taste on the tongue associated with sodium ions
	Bitter	The taste on the tongue associated with bitter agents such as caffeine or quinine
Chemical feelings	Astringent	The chemical feeling factor on the tongue, described as puckering/dry and associated with tannins or alum
	Metallic	The chemical feeling factor on the tongue described as flat, metallic and associated with iron and copper

USDA, United States Department of Agriculture.

descriptor "fruity," which is an off-flavor associated with high temperature curing, was added (Sanders et al. 1989).

The chemical basis for peanut flavor quality has been extensively investigated over the past several years. Bett and others (1994) compared the flavor of roasted peanuts from different origins and declared that U.S.-grown peanuts had more intense peanut flavor and less intense fruity/fermented flavor than peanuts from Argentina and China. Peanuts from Argentina had the highest levels of off-flavor-related compounds, while Chinese-grown peanuts were characterized as cardboardy. Similarly, Young and others (2005) used DSA and consumer testing to compare peanuts from different origins. Argentina peanuts were described as musty and sweet, Chinese peanuts exhibited woody/hull/skins flavors as well as bitter and sour tastes, and U.S.-grown peanuts were characterized by sweet aromatic, roasted peanut, and dark roast. Consumer testing showed that U.S.-grown had the highest overall liking scores, followed by China- and Argentina-grown peanuts, respectively.

Many studies have identified and described the odor characteristics of volatile compounds (Table 51.4), but their odor potency and actual contribution to flavor was not elucidated. Although GC-O is not a new technology (Fuller et al. 1964), application to peanut flavor volatiles is relatively new. GC-O was not applied in most, if any, of the early studies dealing with aroma description of volatile compounds isolated from roasted peanuts. Ho and others (1981) identified several compounds in the aroma of roasted peanuts and suggested that a few of them might contribute to the flavor profile of roasted peanuts. For example,

TABLE 51.4. Chemical Compounds Isolated from Roasted Peanuts

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
2-Isopropyl-4,5-dimethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho and others (1981)
2-Propyl-4,5-diethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho and others (1981)
2-Crotolactone	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho and others (1981)
3-Methyl-2-crotolactone	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho and others (1981)
N-methylpyrrole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Sweet/woody	Ho and others 1981

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
2-Isopropyl-4,5-dimethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Lee and others (1981)
2-Propyl-4,5-diethylpyrazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Lee and others (1981)
2-Acetyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/popcorn	Lee and others (1981)
2,4,5-Trimethyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/sweet/green	Lee and others (1981)
2,4-Diethyl-5-propyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/sweet/green	Lee and others (1981)
4,5-Dimethyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Green/sweet/vegetable	Lee and others (1981)
5-Butyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Green/ sweet/vegetable	Lee and others (1981)
2,4,5-Trimethyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Woody/green	Lee and others (1981)
2-Methyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/sweet	Lee and others (1981)
2,4-Dimethyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/sweet	Lee and others (1981)
Pentanal	Purge and trap	N/A	Solventy/green	Buckholz and others (1980)
3-Methylpyridine	Dynamic headspace	GC-O	Roasted peanut butter	Braddock and others (1995)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
Benzeneacetaldehyde	Dynamic headspace	GC-O	Floral/sweet/ caramel	Braddock and others (1995
2-3-Dihydrobenzofuran	Dynamic headspace	GC-O	Rubbery/ harsh	Braddock and others (1995)
Benzothiazone	Dynamic headspace	GC-O	Harsh/burnt/ rubbery	Braddock and others (1995)
Hexanal	Dynamic headspace	GC-O	Green, grassy	Braddock and others (1995)
Nonanal	Dynamic headspace	GC-O	Floral	Braddock and others (1995)
Heptan-2-ol	Dynamic headspace	GC-O	Pungent/green	Braddock and others (1995)
Acetic acid	Dynamic headspace	GC-O	Bread dough/ yeasty	Braddock and others (1995)
Phenylacetaldehyde	SAFE (NB fraction)	AEDA	Rosy/green	Schirack and others (2006)
Guaiacol	SAFE (NB fraction)	AEDA	Burnt	Schirack and others (2006)
2-Methylbutanal	SAFE (NB fraction)	AEDA	Chocolate/ malty	Schirack and others (2006)
Toluene	SAFE (NB fraction)	AEDA	Sweet/ chemical	Schirack and others (2006)
2,3-Butanediol	SAFE (NB fraction)	AEDA	Fruity	Schirack and others (2006)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
Furfural	SAFE (A fraction)	AEDA	Sweet	Schirack and others (2006)
(*E*)-2-hexenal	SAFE (A fraction)	AEDA	Fruity	Schirack and others (2006)
Ethyl valerate	SAFE (A fraction)	AEDA	Fruity	Schirack and others (2006)
Heptanal	SAFE (NB fraction)	AEDA	Fatty	Schirack and others (2006)
(*E*,*Z*)-2,4-heptadienal	SAFE (NB fraction)	AEDA	Fatty	Schirack and others (2006)
Methyl hexanoate	SAFE (A fraction)	AEDA	Sweet	Schirack and others (2006)
Furaneol™ (2,5-dimethyl-4-hydroxy-3(2H)-furanone)	SAFE (A fraction)	AEDA	Burnt sugar	Schirack and others (2006)
Acetophenone	SAFE (NB fraction)	AEDA	Fruity/sweet	Schirack and others (2006)
Maltol (3-hydroxyl-2-methyl-4H-pyran-4-one)	SAFE (A fraction)	AEDA	Cotton candy	Schirack and others (2006)
Nonanal	SAFE (NB fraction)	AEDA	Green/floral	Schirack and others (2006)
4-Ethylbenzaldehyde	SAFE (A fraction)	AEDA	Burnt sugar	Schirack and others (2006)
3-Ethylphenol	SAFE (NB fraction)	AEDA	Old books/ musty	Schirack and others (2006)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
Decanal	SAFE (NB fraction)	AEDA	Fried	Schirack and others (2006)
(*E*,*E*)-2,4-decadienal	SAFE (NB fraction)	AEDA	Fried/oxidized	Schirack and others (2006)
Decanoic acid	SAFE (NB fraction)	AEDA	Oxidized	Schirack and others (2006)
Delta-elemene	SAFE (NB fraction)	AEDA	Wood	Schirack and others (2006)
4-Acetoxy-2,5-dimethyl-3(2H)-furanone	SAFE (A fraction)	AEDA	Burnt sugar	Schirack and others (2006)
δ-Decalactone	SAFE (A fraction)	AEDA	Sweet/fruity	Schirack and others (2006)
Geranyl butyrate	SAFE (NB fraction)	AEDA	Rosy	Schirack and others (2006)
Tetradecanal	SAFE (NB fraction)	AEDA	Honey/hay	Schirack and others (2006)
(*E*)-2-hexenoic acid	SAFE (NB fraction)	AEDA	Fatty	Schirack and others (2006)
Pantolactone	SAFE (A fraction)	AEDA	Burnt sugar	Schirack and others (2006)
Benzaldehyde	SAFE (A fraction)	AEDA	Sweet/malty	Schirack and others (2006)
Methyl cinnamate	SAFE (A fraction)	AEDA	Strawberry	Schirack and others (2006)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
Ethyl 2-methylpropanoate		GC-O	Fruity-fermented	Didzbalis and others (2004)
2,3-Butanedione	SAFE	GC-O	Buttery	Didzbalis and others (2004)
2,3-Pentanedione	SAFE	GC-O	Buttery	Didzbalis and others (2004)
Ethyl 2-methylbutanoate	SAFE	GC-O	Green/fruity	Didzbalis and others (2004)
Ethyl 3-methylbutanoate	SAFE	GC-O	Fruity/apple like	Didzbalis and others (2004)
Hexanal	SAFE	GC-O	Grassy	Didzbalis and others (2004)
Octanal	SAFE	GC-O	Citrus like	Didzbalis and others (2004)
2-Furanmethanethiol	SAFE	GC-O	Coffee like	Didzbalis and others (2004)
Methional	SAFE	GC-O	Potato like	Didzbalis and others (2004)
Butanoic acid	SAFE	GC-O	Sharp/sour	Didzbalis and others (2004)
Phenylacetaldehyde	SAFE	GC-O	Honey like	Didzbalis and others (2004)
3-Methylbutanoic acid	SAFE	GC-O	Rancid/cheese	Didzbalis and others (2004)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
Hexanoic acid	SAFE	GC-O	Cheesy/fatty	Didzbalis and others (2004)
2-Methoxyphenol	SAFE	GC-O	Sweet/smoky	Didzbalis and others (2004)
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	SAFE	GC-O	Strawberry like	Didzbalis and others (2004)
Acetoin/2,3-butadione	SAFE (NB fraction)	AEDA	Buttery/ butterscotch	Greene (2007)
3-Methylbutanal	SAFE (NB fraction)	AEDA	Malty/ chocolate	Greene (2007)
Hexanal	SAFE (NB fraction)	AEDA	Green/grassy	Greene (2007)
Methional	SAFE (NB fraction)	AEDA	Potato	Greene (2007)
2-Acetyl-1-pyrroline	SAFE (NB fraction)	AEDA	Popcorn	Greene (2007)
1-Octen-3-one	SAFE (NB fraction)	AEDA	Metallic/ mushroom	Greene (2007)
2-Ethyl-6-methylpyrazine	SAFE (NB fraction)	AEDA	Sweet	Greene (2007)
Trimethylpyrazine	SAFE (NB fraction)	AEDA	Earthy/soil/ dirt	Greene (2007)
Phenylacetaldehyde	SAFE (NB fraction)	AEDA	Rosy/floral	Greene (2007)
2-Ethyl-3,5-dimethylpyrazine	SAFE (NB fraction)	AEDA	Earthy/soil/ dirt	Greene (2007)
2,3-Diethyl-5-methylpyrazine	SAFE (NB fraction)	AEDA	Earthy/soil/ dirt	Greene (2007)
2-Methoxy-4-vinylphenol	SAFE (NB fraction)	AEDA	Licorice/ sweet	Greene (2007)
2-Ethyl-3,5-dimethylpyrazine	SAFE (A fraction)	AEDA	Earthy/soil/ dirt	Greene (2007)
Acetic acid	SAFE (A fraction)	AEDA	Vinegar/ acetic acid	Greene (2007)
Methional	SAFE (A fraction)	AEDA	Potato	Greene (2007)
Butanoic acid	SAFE (A fraction)	AEDA	Sweaty/musty/ cheesy	Greene (2007)
Ethyl-2-methylbutanoate	Simultaneous distillation–extraction (N fraction)	AEDA	Fruity	Matsui and others (1998)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
(*Z*)-2-nonenal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty/green	Matsui and others (1998)
(*E*,*E*)-2,4-decadienal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty, deep-fried	Matsui and others (1998)
(*E*)-β-damascenone	Simultaneous distillation–extraction (N fraction)	AEDA	Boiled apple like	Matsui and others (1998)
Ethyl isobutyrate	Simultaneous distillation–extraction (N fraction)	AEDA	fruity	Matsui and others (1998)
3-Mercapto-2-butanone	Simultaneous distillation–extraction (N fraction)	AEDA	Cooked meat like	Matsui and others (1998)
2-Acetyl-1-pyrroline	Simultaneous distillation–extraction (B fraction)	AEDA	Roasty/sweet	Matsui and others (1998)
Dimethyl trisulfide	Simultaneous distillation–extraction (N fraction)	AEDA	Sulfurous	Matsui and others (1998)
2-Propionyl-1-pyrroline	Simultaneous distillation–extraction (B fraction)	AEDA	Roasty	Matsui and others (1998)
2-Furfurylthiol	Simultaneous distillation–extraction (N fraction)	AEDA	Sweet/smoky	Matsui and others (1998)
(*E*,*Z*)-2,4-nonadienal	Simultaneous distillation–extraction (N fraction)	AEDA	Green	Matsui and others (1998)
(*E*,*Z*)-2,4-decadienal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty/green	Matsui and others (1998)
2-Methoxyphenol (guaiacol)	Simultaneous distillation–extraction (N fraction)	AEDA	Burnt	Matsui and others (1998)

TABLE 51.4. ***Continued***

Compounds	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
2-Phenyl-2-butenal	Simultaneous distillation–extraction (N fraction)	AEDA	Green/phenolic	Matsui and others (1998)
3-Methoxy-4-hydroxybenzaldehyde (vanillin)	Simultaneous distillation–extraction (A fraction)	AEDA	Vanilla like	Matsui and others (1998)
3-Methylbutanal	Simultaneous distillation–extraction (N fraction)	AEDA	Malty	Matsui and others (1998)
D-limonene	Simultaneous distillation–extraction (N fraction)	AEDA	Lemon like	Matsui and others (1998)
Octanal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty	Matsui and others (1998)
(*E*)-2-nonenal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty/green	Matsui and others (1998)
Phenylacetaldehyde	Simultaneous distillation–extraction (N fraction)	AEDA	Sweet/honey like	Matsui and others (1998)
(*E*,*E*)-2,4-nonadienal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty, deep-fried	Matsui and others (1998)
(*E*)-2-undecenal	Simultaneous distillation–extraction (N fraction)	AEDA	Fatty	Matsui and others (1998)
Hexanoic acid	Simultaneous distillation–extraction (A fraction)	AEDA	Sweaty	Matsui and others (1998)
2-Methoxy-4-vinylphenol (4-vinylguaicol)	Simultaneous distillation–extraction (N fraction)	AEDA	Spicy/phenolic	Matsui and others (1998)
δ-Dodecalactone	Simultaneous distillation–extraction (N fraction)	AEDA	Sweet	Matsui and others (1998)

[a]Fraction in which the compound appeared.
A, acidic; B, basic; N, neutral; N/A, not available.

2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylthiazole had a pleasant nutty odor. 2-Crotolactone and 3-methyl-2-crotolactone were also described as nutty. *N*-methylpyrrole was characterized as having a sweet, woody odor. Most of the oxazoles identified in their study were described as having a green nutty aroma. In general, the pyrazines were described as producing pleasant roasted nut-like notes.

Similarly, Lee and others (1981) proposed that numerous compounds might play a role in the aroma of roasted peanuts. For example, they reported that 2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylpyrazole had a pleasant nutty flavor. 2-Acetyloxazole was described as having nutty and popcorn-like character, while 2,4,5-trimethyloxazole and 2,4-diethyl-5-propyloxazole were described as nutty, sweet, and green. The authors also suggested that 4,5-dimethyloxazole and 5-butyloxazole might impart a green, sweet, and vegetable note to the aroma of roasted peanuts. Finally, 2,4,5-trimethyl-3-oxazoline had woody and green odors, and 2-methyl-3-oxazoline and 2,4-dimethyl-3-oxazoline were characterized as nutty and sweet. Brown and others (1973) reported that hexanal and octanal and possibly nonanal and 2-nonenal were associated with beany flavor. Buckholz and Daun (1981) suggested that 2,4-dimethyl-3-thiazoline was associated with the nut skin attribute in the flavor of roasted peanuts.

Low-molecular-weight aldehydes have been associated with the harsh green notes from fresh roasted peanuts (Johnson et al. 1971a,b; Mason et al. 1966). Pentanal has been described as having a solvent-like note and was negatively related to sensory preference of roasted peanuts (Buckholz et al. 1980). Brown and others (1972) suggested that 2-heptanal, 2-octenal, 2-nonenal, and 2,4-decadienal were associated with fatty and deep-fried notes of cold-pressed oil from freshly roasted peanuts. The sweet aroma from roasted peanuts has been attributed to phenylacetaldehyde (Mason et al. 1967).

More recent peanut flavor research has benefited from DSA as well as GC-O analytic methods that facilitate the identification of key odor-active compounds (Braddock et al. 1995; Didzbalis et al. 2004; Greene et al. 2008; Matsui et al. 1998; Schirack et al. 2006). Further, several techniques such as AEDA and CHARM Analysis have been used in flavor chemistry research to determine the relative strength of odor components and thus identify the most potent odorants contributing to a flavor (Braddock et al. 1995; Grosch 1993; Matsui et al. 1998; Schirack et al. 2006).

Braddock and others (1995) used GC-O to evaluate the odor characteristics of several compounds isolated from roasted peanuts. 3-Methylpyridine had an intense roasted peanut odor, benzeneacetaldyde was described as flowery and sweet, and 2-3-dihydrobenzofuran provided a harsh, sulfur-like aroma. Benzothiazone smelled burnt at the sniffer port, and hexanal provided a green, grassy off-note. Nonanal was described as floral, while heptan-2-ol and 1-pentanol had slightly pungent and green aromas. The most potent odorants in the volatiles extracted from the headspace of freshly roasted peanuts were 2,5-dimethylpyrazine, methylpyrazine, and 2-ethyl-3-methylpyrazine, as determined by AEDA. These compounds were described as malty/chocolate, grilled chicken/savory, and roasted, respectively.

Schirack and others (2006) used GC-O to characterize the aroma properties of solvent extracts from roasted peanuts, and subsequently used AEDA to determine those with the highest impact to the overall flavor. They identified over 200 aroma-active compounds, and 38 were reported as the main flavor-active volatiles

of roasted peanuts. Among the pyrazines were 2,6-dimethylpyrazine ($FD_3 = 6$); 2-ethyl-5-methylpyrazine ($FD_3 = 4$); 2,5-dimethyl-3-ethylpyrazine ($FD_3 = 7$); 2-ethyl-3,5-dimethylpyrazine ($FD_3 = 8$); 2,3-diethyl-2-methylpyrazine ($FD_3 = 6$); and 3-methoxy-2,5-dimethylpyrazine ($FD_3 = 4$) (Table 51.5). Several other classes of compounds were reported to contribute to the overall flavor of roasted peanuts, as shown in Table 51.4.

Basha and Young (1996) separated peanut seed proteins into 10 fractions by gel filtration and found that one of these fractions produced several volatile compounds associated with off-flavors in roasted peanuts. In a subsequent study, they concluded that the protein fraction that was capable of producing off-flavor volatiles was lipoprotein in nature and rich in oleic acid (Basha et al. 1998). Some of the compounds produced by this protein fraction were *N*-methylpyrrole pentane, acetone, dimethyl sulfide, 2-methylpropanol, pentanal, and hexanal. These compounds have been associated with a variety of off-flavors such as musty, fruity, tongue burn, and beany (Young and Hovis 1990). It was suggested that in addition to lipid oxidation, thermal degradation of proteins might be a source of the off-flavor compounds in roasted peanuts.

Ethyl acetate was associated with fruity off-flavors developed at high curing temperatures and was considered an indicator of deterioration of aroma and flavor (Singleton et al. 1971). Similarly, Pattee and others (1965) reported that ethyl acetate and acetaldehyde contributed to off-flavors developed in high-temperature-cured peanuts. Sanders and others (1989) reported that immature peanuts cured at higher temperatures had lower intensities of sweet aromatic and roasted peanut and higher intensities of fruity/fermented, painty, sour, and bitter flavor attributes. Some reductones such as hydroxyfuranones and furaneols have been associated with the fruity/fermented off-flavors found in improperly dried or freeze-damaged peanuts (Vercellotti et al. 1994).

In a recent study, Didzbalis and others (2004) used GC-O and SAFE to identify the compounds responsible for fruity/fermented off-flavor developed in immature peanuts artificially subjected to constant high-temperature (40°C) curing. Fruity esters, such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate, and high levels of short-chain organic acids, such as butanoic, 3-methylbutanoic, and hexanoic, were associated with the fruity fermented off-note. Model system studies showed that the short-chain organic acids were responsible for the cheesy/fermented aroma, while the esters contributed to the fruity, apple-like aromas. Greene (2007) and Greene and others (2008) used instrumental and DSA to compare roasted peanuts containing naturally occurring and artificially created fruity fermented off-flavors. The esters identified by Didzbalis and others (2004) were not identified in samples having naturally occurring fruity fermented off-flavor. In addition, methylpropanoate was absent in both natural and artificially created fruity fermented samples. Immature peanuts cured at high temperature (40°C) (artificially created off-flavors) contained ethyl-2-methylbutanoate and ethyl 3-methylbutanoate, which was in agreement with Didzbalis and others (2004). Sensory differences between natural and artificially created fruity fermented were also reported. The former was usually described as having an overripe fruit note, whereas the latter was described as rotten garbage/soured off-flavor. Greene (2007) suggested that differences between natural and artificially created samples were responsible for the observed differences.

TABLE 51.5. Pyrazine Compounds Isolated from Roasted Peanuts

Compound	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
2,5-Dimethyl-3-ethylpyrazine	SAFE (AC fraction)	GC-O	Brothy	Schirack and others (2006)
2,6-Dimethyl-3-ethylpyrazine	SAFE (NB fraction)	GC-O	Nutty/earthy	Schirack and others (2006)
2,6-Dimethylpyrazine	SAFE (NB fraction)	GC-O	Nutty/earthy	Schirack and others (2006)
2,3-Diethyl-5-methylpyrazine	SAFE (NB fraction)	GC-O	Roasted	Schirack and others (2006)
3,5-Diethyl-2-methylpyrazine	SAFE (NB fraction)	GC-O	Roasted	Schirack and others (2006)
2-Ethyl-5-methylpyrazine	SAFE (AC fraction)	GC-O	Fruity/sweet	Schirack and others (2006)
2-Ethyl-5-methylpyrazine	Dynamic headspace	GC-O	Nutty/roasted	Braddock and others (1995)
Methylpyrazine	Dynamic headspace	GC/O	Grilled chicken, savory	Braddock and others (1995)
2,5-Dimethyl-pyrazine	Dynamic headspace	GC-O	Chocolaty/malty	Braddock and others (1995)
Ethylpyrazine	Dynamic headspace	GC-O	Dark roasted/toasted	Braddock and others (1995)
2,3-Dimethylpyrazine	Dynamic headspace	GC-O	Roasted	Braddock and others (1995)
2-Ethyl-3-methylpyrazine	Dynamic headspace	GC-O	Roasted	Braddock and others (1995)
3-Ethyl-2,5-dimethylpyrazine	Dynamic headspace	GC-O	Roasted/slightly sweet	Braddock and others (1995)
2-Ethylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas–liquid chromatography)	Roasted/nutty	Koehler and others (1971)
2-Methylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas–liquid chromatography)	Roasted/nutty	Koehler and others (1971)
Methylethylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas–liquid chromatography)	Roasted/nutty	Koehler and others (1971)
2-Ethyl-3,5-dimethylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Roast	Matsui and others (1998)

TABLE 51.4. ***Continued***

Compound	Isolation Method (and Fraction[a])	Odor Detection Method	Odor Quality	Reference
2,3-Diethyl-5-methylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Roasty	Matsui and others (1998)
3-Ethyl-2,5-dimethylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Roasty	Matsui and others (1998)
2-Ethenyl-3,5-dimethylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Roasty	Matsui and others (1998)
2,5-(or 2,6)-Diethylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Sweet	Matsui and others (1998)
2-Ethyl-5-methylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Sweet	Matsui and others (1998)
2-Ethyl-3-methylpyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Sweet	Matsui and others (1998)
2-Methoxy-5-((*E*)-1-propenyl) pyrazine	Simultaneous distillation–extraction (B fraction)	GC-O	Sweet/earthy	Matsui and others (1998)
2,6-Dimethylpyrazine	SAFE	GC-O	Nutty	Didzbalis and others (2004)
2-Ethylpyrazine	SAFE	GC-O	Nutty	Didzbalis and others (2004)
2,3-Dimethylpyrazine	SAFE	GC-O	Nutty	Didzbalis and others (2004)
2-Ethyl-5-methylpyrazine	SAFE	GC-O	Sweet/nutty	Didzbalis and others (2004)
Trimethylpyrazine	SAFE	GC-O	Nutty	Didzbalis and others (2004)
2-Ethyl-3,6-dimethylpyrazine	SAFE	GC-O	Roasty	Didzbalis and others (2004)
2-Ethyl-3,5-dimethylpyrazine	SAFE	GC-O	Roasty	Didzbalis and others (2004)

[a]Fraction in which the compound appeared.
A, acidic; B, basic; N, neutral.

PYRAZINES AND ROASTED PEANUT FLAVOR

Investigation of roasted peanut flavor has been an ongoing effort for over 40 years (Brown et al. 1968; Mason and Waller 1964; Mason et al. 1966; Newell et al. 1967; Waller 1969). Pyrazines, which are heterocyclic compounds containing two nitrogen atoms, have been traditionally suggested to be the key element for the typical aroma of roasted peanuts (Mason et al. 1966, 1967, 1969; Maga and Sizer 1973; Shibamoto and Bernhard 1976). Table 51.5 shows the major pyrazine compounds that have been identified in roasted peanuts, along with the aroma descriptors associated with them.

Mason and others (1966) reported that the following pyrazines produced typical nutty flavors: methylpyrazine; 2,5-dimethylpyrazine; trimethylpyrazine, methylethylpyrazine; and dimethylethylpyrazine. Johnson and others (1971a) analyzed the basic fraction volatiles of roasted peanuts, which were isolated by vacuum degassing of the pressed oil. Several alkylpyrazines were reported for the first time, and as a group, they contributed to the "nut-like" character of typical roasted peanuts. Buckholz and others (1980) correlated sensory evaluation with instrumental analysis of peanuts roasted for various lengths of time and found that a decrease in carbonyls and subsequent increase in pyrazines were indications of good quality peanut flavor. For instance, a positive correlation was noticed between the concentration of 2-ethyl-6-methyl pyrazine, which has roasted, nutty notes, and sensory preference. Crippen and others (1992) reported that pyrazines, methylbutanal, methylpropanal, and sulfur compounds (e.g., methanethiol, carbon disulfide, and dimethyl sulfide) were associated with dark roasted flavors. In a study conducted by Leunissen and others (1996), methylpyrazine was directly related to perception of roasted flavor at low concentrations.

Tsantili-Kakoulidou and Kier (1992) developed a mathematical model to predict the odor strength of pyrazines as a function of their topology, shape, and electronic features. They found that as the alkyl substituents became longer and more branched in the molecule, odor strength of pyrazines increased (lower threshold values). Ho and others (1983) reported that, in general, alkylpyrazines have nutty, green, and vegetable-like aromas. Monosubstituted pyrazines and substituted methylpyrazines have been described as nutty and roasted peanutty (Masuda and Mihara 1988).

Using GC-O, Braddock and others (1995) identified several aroma-active compounds from freshly roasted peanuts. 2,5-Dimethylpyrazine, methylpyrazine, and 2-ethyl-3-methylpyrazine were described as malty/chocolate, grilled chicken/savory, and roasted, respectively. In addition, 2,5-dimethylpyrazine was characterized as chocolaty/malty, and ethylpyrazine as dark/roasted. Baker and others (2003) examined four peanut genotypes and found that among the pyrazines tested, 2,5-dimethylpyrazine correlated the most with roasted peanut flavor and aroma obtained from sensory data (flavor: $R^2 > 0.83$; aroma: $R^2 > 0.87$). They concluded that this compound could be used as a sensory predictor of roasting in peanut products. Roasted peanuts from the Fall crop in Taiwan were found to contain higher levels of total pyrazines than peanuts from the Spring crop, which was consistent with the general consumer concept that the former is richer in peanut flavor (Ku et al. 1998).

In an early study by Koehler and others (1971), the threshold concentrations of several pyrazines in water and oil were determined. Although none of the compounds alone had the typical aroma of roasted peanuts, several alkylpyrazines,

especially 2-ethylpyrazine, 2-methylpyrazine, and methylethylpyrazine, produced "roasted" and "nutty" responses. However, the concentration of 2-methylpyrazine in roasted peanuts was below the odor threshold level in both water and oil.

THEORIES FOR PYRAZINE FORMATION

Roasting conditions directly influence the formation of pyrazines and perception of roasted peanut flavor. In a study by Leunissen and others (1996), the amount of pyrazines increased as roasting conditions changed from mild (low temperature/short time) to severe (high temperature/long time). The authors also reported that methyl and other pyrazines were desirable at low concentrations, but they became increasingly bitter at high concentrations as determined by DSA. In another study, the optimum roasting condition tested for four peanut genotypes was 175°C/15 min, where the highest level of pyrazines and sensory scores for roasted peanut flavor were achieved (Baker et al. 2003).

The effect of reaction temperature on the formation of pyrazines has also been investigated. Hwang and others (1994) found that the total amount of pyrazines increased with reaction temperature in a model system containing glucose and lysine. In addition, long-chain alkylpyrazines had higher activation energy; thus, they required higher temperatures for formation. Koehler and Odell (1970) studied the effect of temperature for pyrazine synthesis in a sugar–amino acid model system and found that few pyrazines were formed below temperatures of 100°C. They also reported that the yield of pyrazines increased as the temperature increased up to 150°C. Coleman and Steichen (2006) noticed that reaction temperature not only influenced the total yield but also affected the distribution of pyrazines on microwave heat-treated systems containing rhamnose, ammonium hydroxide, leucine, and valine. In a model system where glucose and asparagine were heated at 120°C for 24 h, the major compound formed in the early stages of the reaction was methylpyrazine. However, as the reaction proceeded, the amount of dimethylpyrazine increased until its ratio to methylpyrazine became constant at about 3 (Koehler and Odell 1970). Shibamoto and Bernhard (1976) found that reactant ratio, as well as reaction time and temperature, influenced total yield but not the distribution of pyrazines formed in ammonia–glucose model systems.

Several mechanisms for pyrazine formation have been proposed. In general, sugars and sugar degradation products are considered as the primary carbon sources for pyrazine carbon structures formed during roasting, while amino acids are considered to be the nitrogen supply (Dawes and Edwards 1966; Koehler et al. 1969). The most currently accepted pathway for pyrazine formation involves the reaction of amino acids with α-dicarbonyls compounds (intermediate products in the Maillard reaction) through Strecker degradation, producing α-amino carbonyls, which then condense to give rise to alkylpyrazines (Fig. 51.1) (Fennema 1996).

Shibamoto and Bernhard (1977a,b) proposed 10 α-amino carbonyl fragments (Fig. 51.2) that can produce the following upon condensation: pyrazine; 2-methylpyrazine; 2,5- and 2,6-dimethlypyrazine; trimethylpyrazine; 2-ethylpyrazine; 2-ethyl-5-methyl- and 2-ethyl-6-methylpyrazine; and 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine. For example, Figure 51.3 illustrates the synthesis of methylpyrazine from condensation of fragments I and V.

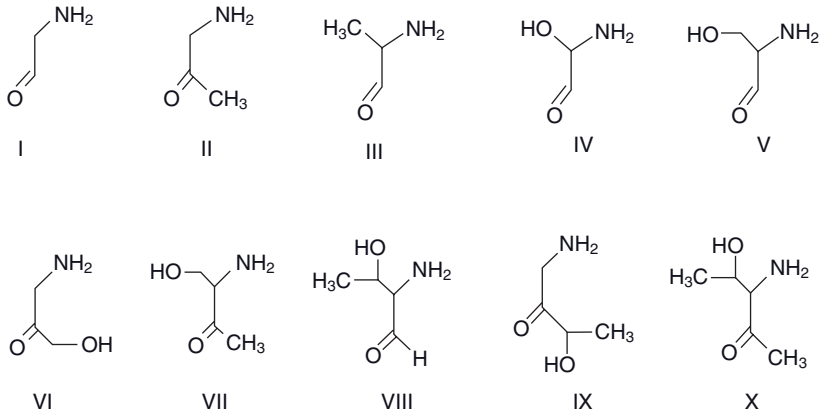

Figure 51.2. α-Amino carbonyl intermediates proposed by Shibamoto and Bernhard (1977a,b).

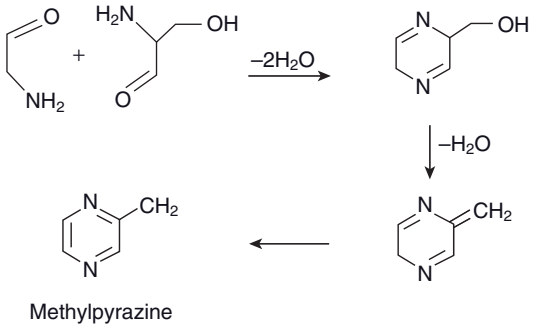

Figure 51.3. Mechanism for the formation of methylpyrazine from fragments proposed by Shibamoto and Bernhard (1977a,b).

Another well-accepted pathway assumes that ammonia is released from amino acids during pyrolysis and serves as the intermediate for pyrazine formation. A proposed mechanism of deamination from α- and β-amino acids is shown in Figure 51.4 (Shu 1998). It is well-known that glutamine releases ammonia easier than other amino acids through the process of deamidation and deamination during thermal degradation (Sohn and Ho 1995). Thus, if ammonia served as the intermediate during pyrazine formation, higher yield of pyrazine compounds would be expected in reaction systems involving glutamine as compared with other amino acids. In fact, Chen and Ho (1999) investigated the formation of pyrazines in ribose/glucose/fructose–glutamine/serine/threonine model systems, and found that the glutamine-containing systems generated more total pyrazines than serine- and threonine-containing systems. Furthermore, if ammonia functions as an intermediate in the formation of pyrazines, a similar distribution pattern of these compounds would be expected regardless of the precursor amino acid. Van Praag and others (1968) reported that a similar series of pyrazines was formed when fructose reacted with different amino acids such as glycine, serine, leucine, isoleucine, valine, and alanine.

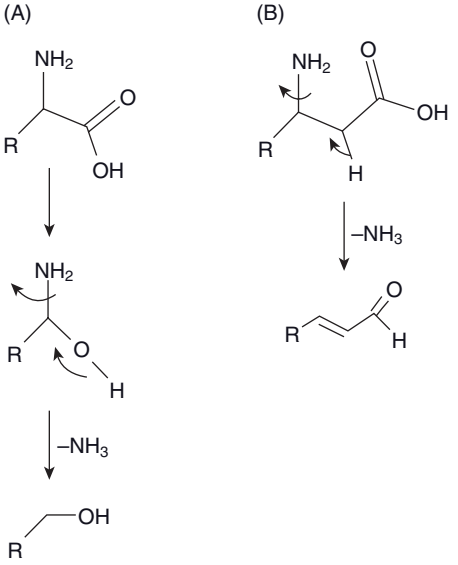

Figure 51.4. Mechanism of deamination from α-amino acid (A) and β-amino acid (B) proposed by Shu (1998).

Similarly, Newell and others (1967) indicated that essentially, the same pyrazines were formed in sugar amino acid systems, regardless of the nitrogen source employed. Shu (1998) obtained tetramethylpyrazine from the reaction between acyloins (α-hydroxy ketones) and amino acids. They concluded that ammonia was released from the amino acids and served as the intermediate because acyloins are not involved with Strecker degradation, and are known to produce pyrazines when reacted with ammonia.

Conversely, Koehler and others (1969) reported that the distribution of pyrazines formed in glucose–amino acid and glucose–ammonium chloride systems was dependent on the nitrogen source. Ammonium chloride yielded mostly pyrazine and only traces of alkylated pyrazines, while amino acids gave mostly alkylated pyrazines with very small amounts of pyrazine. They concluded that nitrogen was still bound to the amino acid upon condensation with the sugar. Similarly, Wong and Bernhard (1988) reported that the types and amounts of pyrazines were dependent on the nitrogen source in model systems containing glucose mixed with ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate. Hwang and others (1995) compared the total yield of pyrazines for several amino acid–glucose model systems and reported that lysine and glycine were among the most and least reactive amino acids in the synthesis of pyrazines, respectively. Arnoldi and others (1988) reacted fructose with eight different amino acids in deodorized cocoa–butter–water model systems and concluded that the synthesis of some pyrazines was aspecific, that is, it occurred with every amino acid, but that others were specific, that is, it occurred only with certain amino acids. Pyrazines formed from specific amino acids were generally high-molecular-weight compounds, having long, branched, or oxygenated substituents, which probably originated from the precursor amino acid.

Koehler and others (1969) proposed that condensation of nitrogen with two- or three-carbon fragments from hexoses led to the formation of alkalized pyrazines. For example, methylpyrazine would be formed from condensation of nitrogen with one two-carbon fragment and one three-carbon fragment, dimethylpyrazine from two two-carbon fragments, and so on. These sugar fragments could be formed by retro-aldol condensation, which is catalyzed by amines (Shibamoto and Russell 1977). Amino groups of lysine have been shown to catalyze the fragmentation of sugar molecules during Maillard reaction (Hwang et al. 1994), which is consistent with the observation of Whistler and BeMiller (1958) that the higher the pH, the faster the rate of sugar fragmentation. Shibamoto and Bernhard (1976) also reported that adding sodium hydroxide or ammonia to glucose–ammonia model systems increased total pyrazine yield in a similar way. They concluded that besides serving as reactant for the synthesis of pyrazines, ammonia functions as a basic catalyst for sugar fragmentation. Carbon source has been shown to affect the yield of pyrazines, probably due to differences in fragmentation. Fructose produced the highest yields of alkylpyrazines as well as dimethylpyrazine-to-methylpyrazine ratio among glucose, sucrose, and arabinose (Koehler and Odell 1970). In another model system study, rhamnose was shown to yield more alkyl-substituted pyrazines such as ethyl-dimethylpyrazines than glucose when reacting with ammonia (Shibamoto and Bernhard 1978; Shibamoto and Russell 1977).

An alternate pathway for pyrazine formation involves pyrolysis of amino acids and does not require the presence of sugar. Several pyrazines were obtained from heating individual nitrogenous organic compounds under airflow (Wang and Odell 1973). Alkylpyrazines were also obtained without a carbohydrate source from the pyrolysis of α-hydroxy amino acids (Kato et al. 1970). Similarly, Shu (1999) obtained several pyrazines by heating serine or threonine as well as a combination of these at different temperatures. They suggested that conversion of α-hydroxy carbonyls into α-amino carbonyls by decarbonylation followed by dehydration gave rise to alkylpyrazines.

LIPID OXIDATION AND OFF-FLAVORS

Peanuts contain approximately 50% fat, of which about 80% is unsaturated. Because of the high level of unsaturated fatty acids, peanuts are susceptible to lipid oxidation, which produces monohydroperoxides that may become precursors for volatile aldehydes such as nonanal, octanal, decanal, and hexanal (Min 1988; Min et al. 1989; Nawar 1985; Warner et al. 1996). Several studies have shown that off-notes such as cardboardy, painty, and oxidized are associated with these compounds (Braddock et al. 1995; Reed et al. 2002). Figure 51.5 shows the mechanism of lipid oxidation and formation of primary and secondary degradation products (Shahidi 2000).

Oleic acid and linoleic acids represent about 50% and 30% of the total fatty acid composition of normal peanuts, respectively (Cobb and Johnson 1973; Mercer et al. 1990). The University of Florida developed a high-oleic peanut line containing >80% oleic acid and <3% linoleic acid (Gorbet and Knauft 1997; Knauft et al. 2000). Because oxidation of linoleic acid occurs about 10 times faster than the oxidation of oleic acid (Nawar 1985), high-oleic acid peanut varieties have higher stability against lipid oxidation and thereby longer shelf life than normal oleic lines (Braddock

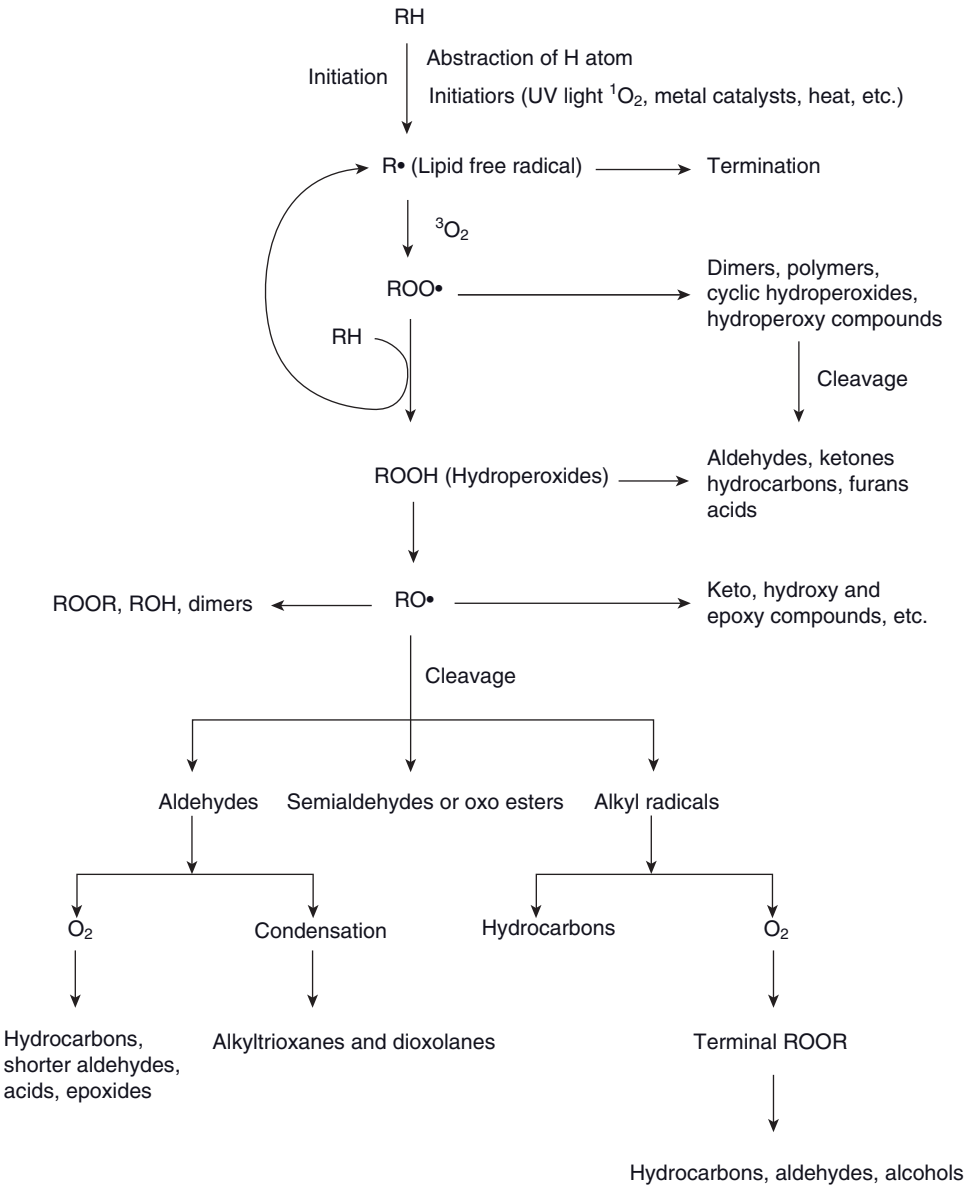

Figure 51.5. Mechanism of lipid oxidation adapted from Shahidi (2000).

et al. 1995; Mugendi et al. 1998; Nepote et al. 2006a; O'Keefe et al. 1993; Reed et al. 2002).

Isleib and others (2006) compared the sensory profile and chemical composition of normal- and high-oleic peanuts with two databases. From the first one, they found no differences in off-flavors, but small differences in roasted peanut, astringency, over-roasted, and nutty attributes between these two varieties. From the second database, they observed that the high oleic line had slightly lower intensities of cardboardy and painty notes. However, they concluded that the sensory differences

between freshly roasted normal and high-oleic peanuts were too small in magnitude for an average consumer to detect. Indeed, Nepote and others (2006a) found no difference between normal- and high-oleic freshly roasted peanuts in a consumer acceptance test. Talcott and others (2005) reported that high-oleic roasted peanuts had higher burnt peanut flavor and aroma than normal oleic lines, but declared that no differences existed for roasted peanut flavor and aroma, sweetness, and bitterness.

PEANUT STORAGE AND FLAVOR FADE

Flavor fade is defined as the loss of positive attributes associated with fresh-roasted peanuts such as "roasted peanut flavor" accompanied by the development of off-flavors during storage (Abegaz et al. 2004). Understanding the sources of flavor fade in peanuts is beneficial for both processors and consumers. It requires knowledge of lipid oxidation and carbonyl-amine reactions, and flavor entrapment between proteins and lipid hydroperoxides, as well as degradation of heterocyclic nitrogen compounds by lipid radicals and hydroperoxides (Alzagtat and Alli 2002; Warner et al. 1996; Williams et al. 2006).

Lipid oxidation leads to the formation of numerous undesirable compounds such as hexanal, heptanal, octanal, and nonanal, which are associated with painty, cardboardy, and oxidized flavors. In addition, subproducts of lipid oxidation may interact with roasted peanut flavor compounds such as pyrazines, ultimately leading to flavor fade (Warner et al. 1996; Williams et al. 2006). Flavor fade may also be associated with the masking of roasted peanut flavor compounds, such as pyrazines, by large quantities of low-molecular-weight aldehydes formed during lipid oxidation (Dimick 1994).

Braddock and others (1995) compared the flavor stability of normal and high-oleic roasted peanuts stored at 25°C for 74 days, and concluded that pyrazines and peanut flavor were more stable in the high-oleic variety than in normal peanuts. Normal oleic variety peanuts had significantly lower pyrazine FD values than high-oleic peanuts. Hexanal content as well as painty and cardboardy attributes were lower for the high-oleic variety. After 74 days of storage, the FD value for hexanal in the normal oleic variety was twice as much as the high-oleic variety. Similar results were reported by Nepote and others (2006a), who found that the high-oleic variety developed less cardboardy flavor and had a slower rate of peanut flavor loss than normal-oleic peanuts stored at 23 and 40°C. They predicted the shelf life of high-oleic roasted peanuts to be 25 and 10 times longer than normal roasted peanuts when stored at 23 and 40°C, respectively.

Williams and others (2006) conducted short-term storage studies with fresh roasted peanuts to evaluate changes in the concentrations of hexanal and pyrazines over time. An increase in the concentration of hexanal with parallel increases in painty and cardboardy flavors was reported. In addition, decreases in the concentrations of several pyrazines such as 2,3-diethylpyrazine, 2-3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine were accompanied by a decrease in roasted peanut flavor.

Similarly, Bett and Boylston (1992) found a decrease in the concentration of alkylpyrazines in roasted peanuts stored at 37°C for 12 weeks, especially during the

initial weeks. Reed and others (2002) also showed that pyrazines and peanut flavor decreased during storage of high- and normal-oleic peanuts, and that the concentration of pyrazines for the high oleic were higher than the normal oleic during storage. This is in agreement with Vercellotti and others (1992) who reported that compounds responsible for fresh roasted peanut flavor disappeared in rancid peanuts. The decrease in the content of pyrazines in these studies might be explained by flavor entrapment or degradation of pyrazines by free radicals or hydroperoxides from lipid oxidation (Funes et al. 1982; Gardner 1979; St. Angelo and Graves 1986; Vercellotti et al. 1992; Williams et al. 2006).

In contrast, Warner and others (1996) found no change trends in the concentration of several pyrazines such as 2,6-dimethylpyrazine, 2-methylpyrazine, 2-ethyl-5-methylpyrazine, and 6-methylpyrazine, but a decrease in the roasted peanut flavor over 65 days of storage at 65°C. They did notice an increase in the concentration of hexanal, heptanal, octanal, and nonanal during storage, which was accompanied by an increase of oxidative rancid flavor. They suggested that peanut roasted flavor was masked by the low-molecular-weight aldehydes produced during lipid oxidation during storage. The authors suggested that the high storage temperature at which their study was conducted (65°C) might have influenced the differences observed between their results and previous reports dealing with pyrazine changes over time. However, several studies have shown that pyrazines are not usually formed below 70°C (Koehler and Odell 1970; Maga 1982; Shibamoto and Bernhard 1976).

Pattee and others (1999) conducted low-temperature long-term stability studies in roasted peanut paste and found that lipid oxidation occurred even at temperature as low as –23°C. At this condition, the intensity of stale flavor, as well as fruity/fermented flavor increased over time up to 13 months. Peanut flavor, however, seemed to be stable. This is in agreement with the observation of Pattee and others (2002) who reported that roasted peanut flavor was stable in normal- and high-oleic roasted peanuts stored at –20°C for 2 months.

Pattee and others (1971) stored unshelled peanuts under simulated warehouse conditions and shelled peanuts under controlled environmental conditions, and found that total volatiles increased with storage time. Pentane, acetaldehyde, and methanol represented the majority of these compounds, which were probably formed due to enzyme activity. In a recent study, Nepote and others (2006b) tested the effect of adding a honey coating to roasted peanuts to prevent lipid oxidation during storage. They found that the intensity of cardboardy and oxidized notes increased, while peanut flavor decreased during storage at 23 and 40°C for both the coated and control roasted peanuts. However, the degree of change for the honey-coated samples was less pronounced, and thus, they were more resistant to lipid oxidation.

The role of moisture in flavor fade of peanut products is not clearly understood. Reed and others (2002) studied the effect of water activity in the flavor stability of roasted peanuts and found that lowering a_w augmented the formation of lipid oxidation products as well as loss of pyrazine compounds. Mate and others (1996) also found that low relative humidity (~20%) increased the rate of lipid oxidation of peanuts compared with high relative humidity (~60%). In contrast, Felland and Koehler (1997) found that adding 2.5% and 5% moisture to peanut butter decreased perceived roasted aroma and flavor and increased development of off-flavors during 29 days of storage at 25°C. Abegaz and others (2004) also reported that peanut

butter containing 2% and 5% added moisture had lower roasted peanut flavor intensity and lower concentrations of pyrazines than samples without added moisture during storage at 21°C for 52 weeks. Baker and others (2002) found that intermediate water activities (0.33 and 0.44) were the best to control lipid oxidation in high-oleic peanuts, as measured by peroxide value.

CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

The unique flavor developed during the roasting of peanuts is one of the major factors influencing consumer choice and acceptance. Investigation of peanut flavor quality has been an ongoing challenge, and although pyrazines have been considered the source of roasted peanut flavor for over 40 years, there is limited information that unquestionably proves it to be true. The fact that many pyrazines have been identified in the volatile composition of roasted peanuts does not necessarily mean they play a major role in peanut flavor. Compounds may be present in a food while having little contribution to flavor if their concentrations are below sensory threshold (McGorrin 2002). In addition, the fact that many studies have reported that pyrazines produce nutty/roasted notes does not specifically imply they are the basis for roasted peanut flavor. The aroma of individual compounds may not be directly related to their actual role in flavor due to interactions with the matrix and other chemical compounds present in the food (Drake and Civille 2003).

Most of the studies that have investigated the flavor chemistry of peanuts were conducted several decades ago. The isolation and separation techniques of flavor components used in these studies may have led to the formation of several artifacts and loss of volatiles. DSA using references and a defined language was not conducted nor were model system studies carried out to specifically determine the role of pyrazine compounds in the flavor profile of roasted peanuts. Odor activity value (OAV), which is the ratio of concentration to the sensory odor threshold, may indicate the importance of a given compound to flavor (Drake 2007). To the best of our knowledge, OAVs of the compounds isolated in early studies were not reported. The relative importance and balance of these compounds for the overall flavor of roasted peanuts had not been elucidated by any other means.

More recent peanut flavor research has benefited from analytic methods that facilitate the identification of key odor-active compounds such as GC-O and AEDA. Schirack and others (2006) found that 38 compounds were the main contributors for the aroma of roasted peanuts. Only seven of these compounds were pyrazines. It is plausible that several other compounds other than pyrazines might be associated with the roasted peanut flavor.

Although GC-O is a valuable technique to screen compounds that fall into threshold ranges, it does not necessarily indicate that they are essential for the aroma of a food. Accurate links cannot be established due to possible interactions of these individual compounds with the matrix and other compounds present in the food (Drake and Civille 2003). Consequently, confirmation of the key aroma-contributing compounds by means of model systems that mimic the food matrix is needed. Further research into peanut flavor quality demands the use of model systems to undoubtedly pinpoint the compounds contributing to roasted peanut flavor.

REFERENCES

Abegaz EG, Kerr WL, Koehler PE. 2004. The role of moisture in flavor changes of model peanut confections during storage. *Lebensm Wiss Technol* 37(2):215–25.

Acree TE, Barnard J, Cunningham DG. 1984. A procedure for the sensory analysis of gas-chromatographic effluents. *Food Chem* 14(4):273–86.

Alzagtat AA, Alli I. 2002. Protein-lipid interactions in food systems: A review. *Int J Food Sci Nutr* 53(3):249–60.

American Peanut Council. 2007. Peanut types and production. http://www.peanutsusa.com/USA.

Arnoldi A, Arnoldi C, Baldi O, Griffini A. 1988. Flavor components in the Maillard reaction of different amino acids with fructose in cocoa butter water—Qualitative and quantitative-analysis of pyrazines. *J Agric Food Chem* 36(5):988–92.

Baker GL, Cornell JA, Gorbet DW, O'Keefe SF, Sims CA, Talcott ST. 2003. Determination of pyrazine and flavor variations in peanut genotypes during roasting. *J Food Sci* 68(1): 394–400.

Baker GL, Sims CA, Gorbet DA, Sanders TH, O'Keefe SF. 2002. Storage water activity effect on oxidation and sensory properties of high-oleic peanuts. *J Food Sci* 67(5):1600–3.

Basha SM. 1992. Soluble sugar composition of peanut seed. *J Agric Food Chem* 40(5): 780–3.

Basha SM, Ying M, Vives MR, Young CT, Boyd LC. 1998. Fractionation and characterization of a protein fraction producing off-flavor volatiles in peanut seed. *J Agric Food Chem* 46(6):2130–5.

Basha SM, Young CT. 1985. Changes in the polypeptide composition of peanut (*Arachis hypogaea* L.) seed during oil roasting. *J Agric Food Chem* 33(3):350–4.

Basha SM, Young CT. 1996. Protein fraction producing off-flavor headspace volatiles in peanut seed. *J Agric Food Chem* 44(10):3070–4.

Bendall JG, Olney SD. 2001. Hept-cis-4-enal: Analysis and flavour contribution to fresh milk. *Int Dairy J* 11(11-12):855–64.

Bett K, Boylston TD. 1992. Effect of storage on roasted peanut quality. In St. Angelo AJ (ed.), *Lipid Oxidation in Food. ACS Symposium Series 500*. Washington, DC: American Chemical Society, pp. 322–43.

Bett KL, Vercellotti JR, Lovegren NV, Sanders TH, Hinsch RT, Rasmussen GK. 1994. A comparison of the flavor and compositional quality of peanuts from several origins. *Food Chem* 51(1):21–7.

Braddock JC, Sims CA, O'Keefe SF. 1995. Flavor and oxidative stability of roasted high oleic-acid peanuts. *J Food Sci* 60(3):489–93.

Brown BA, Konigsba KS, Ellison FE, Mann GE. 1968. Separating and isolating aroma and flavor constituents of roasted peanuts. *J Food Sci* 33(6):595–8.

Brown DF, Senn VJ, Dollear FG. 1973. Comparison of carbonyl-compounds in roasted and nonroasted Spanish and runner peanuts. *J Agric Food Chem* 21(3):463–7.

Brown DF, Stanley JB, Senn VJ, Dollear FG. 1972. Comparison of carbonyl-compounds in raw and roasted runner peanuts. 1. Major qualitative and some quantitative differences. *J Agric Food Chem* 20(3):700–6.

Buckholz LL, Daun H. 1981. Instrumental and sensory characteristics of roasted peanut flavor volatiles. In Teranishi R, Barrera-Benitez H (eds.), *Quality of Selected Fruits and Vegetables of North America. ACS Symposium Series 170*. Washington, DC: American Chemical Society, pp. 163–81.

Buckholz LL, Daun H, Stier E, Trout R. 1980. Influence of roasting time on sensory attributes of fresh roasted peanuts. *J Food Sci* 45(3):547–54.

Cammarn SR, Lange TJ, Beckett GD. 1990. Continuous fluidized-bed roasting. *Chem Eng Prog* 86(6):40–6.

Chen JH, Ho CT. 1999. Comparison of volatile generation in serine/threonine/glutamine-ribose/glucose/fructose model systems. *J Agric Food Chem* 47(2):643–7.

Chiou RYY, Chang YS, Tsai TT, Ho S. 1991. Variation of flavor-related characteristics of peanuts during roasting as affected by initial moisture contents. *J Agric Food Chem* 39(6):1155–8.

Cobb WY, Johnson BR. 1973. Physicochemical properties of peanut. In Wilson CT (ed.), *Peanuts: Culture and Uses*. Roanoke, VA: Stone Printing, pp. 209–63.

Coleman WM, Steichen TJ. 2006. Sugar and selected amino acid influences on the structure of pyrazines in microwave heat-treated formulations. *J Sci Food Agric* 86(3):380–91.

Coleman WM, White JL, Perfetti TA. 1994. Characteristics of heat-treated aqueous extracts of peanuts and cashews. *J Agric Food Chem* 42(1):190–4.

Crippen KL, Vercellotti JR, Lovegren NV, Sanders TH. 1992. Defining roasted peanut flavor quality. Part 2. Correlation of GC volatiles and sensory flavor attributes. In Charalambous G (ed.), *Food Science and Human Nutrition,* Vol. 29. Amsterdam: Elsevier Science Publishers BV, pp. 211–27.

Curioni PMG, Bosset JO. 2002. Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int Dairy J* 12(12):959–84.

Dawes IW, Edwards RA. 1966. Methyl substituted pyrazines as volatile reaction products of heated aqueous aldose amino-acid mixtures. *Chem Ind* 53:2203.

Derise NL, Lau HA, Ritchey SJ, Murphy EW. 1974. Yield, proximate composition and mineral element content of 3 cultivars of raw and roasted peanuts. *J Food Sci* 39(2):264–6.

Didzbalis J, Ritter KA, Trail AC, Plog FJ. 2004. Identification of fruity/fermented odorants in high-temperature-cured roasted peanuts. *J Agric Food Chem* 52(15):4828–33.

Dimick PS. 1994. Peanut flavor-fade research report. *Manuf Confect* 74(1):45–8.

Drake MA. 2007. Defining the flavor of dairy products. *J Dairy Sci* 90:184.

Drake MA, Civille GV. 2003. Flavor Lexicons. *Comp Rev Food Sci* 2:33–40.

Drake MA, Miracle RE, Caudle AD, Cadwallader KR. 2006. Relating sensory and instrumental analyses. In Marsili R (ed.), *Sensory-Directed Flavor Analysis*. Boca Raton, FL: CRC Press, Taylor and Francis Publishing, pp. 23–55.

Engel W, Bahr W, Schieberle P. 1999. Solvent assisted flavour evaporation—A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur Food Res Technol* 209(3-4):237–41.

Felland SL, Koehler PE. 1997. Sensory, chemical, and physical changes in increased water activity peanut butter products. *J Food Qual* 20(2):145–56.

Fennema OR. 1996. *Food Chemistry*, 3rd Ed. New York: Marcel Dekker, pp. 756–9.

Fischer N, Hammerschmidt F, Brunke E. 1995. Analytical investigation of the flavor of Cupuacu. In Rouseff RL, Leahy MM (eds.), *Fruity Flavors: Biogenesis, Characterization and Authentication. ACS Symposium Series 596*. Washington, DC: American Chemical Society, pp. 8–20.

Fuller GH, Steltenkamp GA, Tisserand GA. 1964. The gas chromatograph with human sensor: Perfumer model. *Ann N Y Acad Sci* 116:711–24.

Funes JA, Weiss U, Karel M. 1982. Effects of reaction conditions and reactant concentrations on polymerization of lysozyme reacted with peroxidizing lipids. *J Agric Food Chem* 30(6): 1204–8.

Gardner HW. 1979. Lipid hydroperoxide reactivity with proteins and amino acids—Review. *J Agric Food Chem* 27(2):220–9.

Gorbet DW, Knauft DA. 1997. SunOleic 97R peanut. University of Florida, IFAS Circular S-400.

Greene LG. 2007. Characterization of naturally occurring fruity fermented off-flavor in peanuts using descriptive sensory, consumer, and instrumental analysis. PhD thesis, North Carolina State University, Raleigh, NC.

Greene JL, Sanders TH, Drake MA. 2008. Characterization of volatile compounds contributing to naturally occurring fruity fermented flavor in peanuts. *J Agric Food Chem* 56(17):8096–102.

Grosch W. 1993. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci Technol* 4(3):68–73.

Havemose MS, Justesen P, Bredie WLP, Nielsen JH. 2007. Measurement of volatile oxidation products from milk using solvent-assisted flavour evaporation and solid phase microextraction. *Int Dairy J* 17(7):746–52.

Ho CT, Jin QZ, Lee MH, Chang SS. 1983. Positive identification of new alkyloxazoles, alkylthiazoles, and piperidine in roasted peanut flavor. *J Agric Food Chem* 31(6):1384–6.

Ho CT, Lee MH, Chang SS. 1981. Isolation and identification of volatile compounds from roasted peanuts. *J Food Sci* 47(1):127–33.

Hodge JE. 1953. Dehydrated foods—Chemistry of browning reactions in model systems. *J Agric Food Chem* 1(15):928–43.

Hodge JE. 1967. Origin of flavor in foods, nonenzymatic browning reactions. In Schultz HW, Day EA, Libbey LM (eds.), *Chemistry and Physiology of Flavors*. Westport, CT: AVI Publishing, pp. 465–91.

Hoffpauir CL. 1953. Peanut composition: Relation to processing and utilization. *Agric Food Chem* 1(10):668–71.

Hwang HI, Hartman TG, Ho CT. 1995. Relative reactivities of amino acids in pyrazine formation. *J Agric Food Chem* 43(1):179–84.

Hwang HI, Hartman TG, Rosen RT, Lech J, Ho CT. 1994. Formation of pyrazines from the Maillard reaction of glucose and lysine-alpha-amine-N-15. *J Agric Food Chem* 42(4): 1000–4.

Isleib TG, Pattee HE, Sanders TH, Hendrix KW, Dean LO. 2006. Compositional and sensory comparisons between normal- and high-oleic peanuts. *J Agric Food Chem* 54(5): 1759–63.

Johnsen PB, Civille GV, Vercellotti JR, Sanders TH. 1988. Development of a lexicon for the description of peanut flavor. *J Sens Stud* 3:9–17.

Johnson BR, Waller GR, Burlinga AL. 1971a. Volatile components of roasted peanuts: Basic fraction. *J Agric Food Chem* 19(5):1020–4.

Johnson BR, Waller GR, Foltz RL. 1971b. Volatile components of roasted peanuts: Neutral fraction. *J Agric Food Chem* 19(5):1025–7.

Jonnala RS, Dunford NT, Chenault K. 2005. Nutritional composition of genetically modified peanut varieties. *J Food Sci* 70(4):S254–6.

Kataoka H, Lord HL, Pawliszyn J. 2000. Applications of solid-phase microextraction in food analysis. *J Chromatogr* 880(1-2):35–62.

Kato S, Kurata T, Ishitsuka R, Fujimaki M. 1970. Pyrolysis of β-hydroxy amino acids, especially L-serine. *Agric Biol Chem* 34:1826–32.

Knauft DA, Gorbet DW, Norden AJ. 2000. Enhanced peanut products and plant lines. U.S. Patent 6,121,472.

Koehler PE, Mason ME, Newell JA. 1969. Formation of pyrazine compounds in sugar-amino acid model systems. *J Agric Food Chem* 17(2):393–6.

Koehler PE, Mason ME, Odell GV. 1971. Odor threshold levels of pyrazine compounds and assessment of their role in flavor of roasted foods. *J Food Sci* 36(5):816–18.

Koehler PE, Odell GV. 1970. Factors affecting formation of pyrazine compounds in sugar-amine reactions. *J Agric Food Chem* 18(5):895–8.

Ku KL, Lee RS, Young CT, Chiou RYY. 1998. R7oasted peanut flavor and related compositional characteristics of peanut kernels of spring and fall crops grown in Taiwan. *J Agric Food Chem* 46(8):3220–4.

Lawless HT, Heymann H. 1998. *Sensory Evaluation of Food*. New York: Chapman & Hall, pp. 29–82.

Lee MH, Ho CT, Chang SS. 1981. Thiazoles, oxazoles, and oxazolines identified in the volatile flavor of roasted peanuts. *J Agric Food Chem* 29(3):684–6.

Leunissen M, Davidson VJ, Kakuda Y. 1996. Analysis of volatile flavor components in roasted peanuts using supercritical fluid extraction and gas chromatography mass spectrometry. *J Agric Food Chem* 44(9):2694–9.

Lozano PR, Miracle ER, Krause AJ, Drake M, Cadwallader KR. 2007. Effect of cold storage and packaging material on the major aroma components of sweet cream butter. *J Agric Food Chem* 55(19):7840–6.

Maga JA. 1982. Pyrazines in flavor. In Morton ID, Macleod AJ (eds.), *Food Flavors: Part A. Introduction*. New York: Elsevier Scientific Publishing, pp. 283–323.

Maga JA, Sizer CE. 1973. Pyrazines in foods—Review. *J Agric Food Chem* 21(1):22–30.

Mason ME, Johnson B, Hamming M. 1966. Flavor components of roasted peanuts. Some low molecular weight pyrazines and a pyrrole. *J Agric Food Chem* 14(5):454–60.

Mason ME, Johnson B, Hamming MC. 1967. Volatile components of roasted peanuts. Major monocarbonyls and some noncarbonyl components. *J Agric Food Chem* 15(1):66–73.

Mason ME, Newell JA, Johnson BR, Koehler PE, Waller GR. 1969. Nonvolatile flavor components of peanuts. *J Agric Food Chem* 17(4):728–32.

Mason ME, Waller GR. 1964. Isolation and localization of precursors of roasted peanut flavor. *J Agric Food Chem* 12(3):274–8.

Masuda H, Mihara S. 1988. Olfactive properties of alkylpyrazines and 3-substituted 2-alkylpyrazines. *J Agric Food Chem* 36(3):584–7.

Mate JI, Salveit ME, Krochta JM. 1996. Peanut and walnut rancidity: Effects of oxygen, concentration and relative humidity. *J Food Sci* 61:465–72.

Matsui T, Guth H, Grosch W. 1998. A comparative study of potent odorants in peanut, hazelnut, and pumpkin seed oils on the basis of aroma extract dilution analysis (AEDA) and gas chromatography olfactometry of headspace samples (GCOH). *Fett/Lipid* 100(2): 51–6.

McGorrin RJ. 2002. Character impact compounds: flavors and off flavors in foods. In Marsili R (ed.), *Flavor, Fragrance, and Odor Analysis*. New York: Marcel Dekker, pp. 375–413.

Meilgaard MC, Civille GV, Carr BT. 1991. *Sensory Evaluation Techniques*, 2nd Ed. Boca Raton, FL: CRC Press.

Mercer LC, Wynne JC, Young CT. 1990. Inheritance of fatty acid content in peanut oil. *Peanut Sci* 17:17–21.

Min DB. 1988. Singlet oxygen oxidation in vegetable-oils. *J Am Oil Chem Soc* 65(4):481–2.

Min DB, Lee SH, Lee EC. 1989. Singlet oxygen oxidation of vegetable oils. In Min DB, Smouse TH (eds.), *Flavor Chemistry of Lipid Foods*. Champaign, IL: AOCS, pp. 57–97.

Mugendi JB, Sims CA, Gorbet DW, O'Keefe SF. 1998. Flavor stability of high oleic peanuts stored at low humidity. *J Am Oil Chem Soc* 75(1):21–5.

Murray JM, Delahunty CM, Baxter IA. 2001. Descriptive sensory analysis: Past, present and future. *Food Res Int* 34(6):461–71.

Nawar W. 1985. Lipids. In Fennema O (ed.), *Food Chemistry*, 2nd Ed. New York: Marcel Dekker, pp. 139–244.

Nepote V, Mestrallet MG, Accietto RH, Galizzi M, Grosso NR. 2006a. Chemical and sensory stability of roasted high-oleic peanuts from Argentina. *J Sci Food Agric* 86(6):944–52.

Nepote V, Mestrallet MG, Ryan L, Conci S, Grosso NR. 2006b. Sensorial and chemical changes in honey roasted peanuts and roasted peanuts stored under different temperatures. *J Sci Food Agric* 86(7):1057–63.

Newell JA, Mason ME, Matlock RS. 1967. Precursors of typical and atypical roasted peanut flavor. *J Agric Food Chem* 15(5):767–72.

Nickerson GB, Likens ST. 1966. Gas chromatographic evidence for the occurrence of hop oil components in beer. *J Chromatogr* 21:1–5.

O'Keefe SF, Wiley VA, Knauft DA. 1993. Comparison of oxidative stability of high-oleic and normal-oleic peanut oils. *J Am Oil Chem Soc* 70(5):489–92.

Ory RL, Crippen KL, Lovegren NV. 1992. Off-flavors in peanuts and peanut products. In Charalambous G (ed.), *Developments in Food Science v. 29: Off-Flavors in Foods and Beverages*. Amsterdam: Elsevier Science Publishers, pp. 57–75.

Oupadissakoon C, Young CT. 1984. Modeling of roasted peanut flavor for some Virginia-type peanuts from amino acid and sugar contents. *J Food Sci* 49(1):52–8.

Oupadissakoon C, Young CT, Giesbrecht FG, Perry A. 1980. Effect of location and time of harvest on free amino acid and free sugar contents of Florigiant peanuts. *Peanut Sci* 7:61–5.

Pattee HE, Beasley EO, Singleto, JA. 1965. Isolation and identification of volatile components from high-temperature-cured off-flavor peanuts. *J Food Sci* 30(3):388–92.

Pattee HE, Giesbrecht FG, Isleib TG. 1999. Sensory attribute variation in low-temperature-stored roasted peanut paste. *J Agric Food Chem* 47(6):2415–20.

Pattee HE, Isleib TG, Giesbrecht FG, Cui Z. 2002. Prediction of parental genetic compatibility to enhance flavor attributes of peanuts. In Rajasekaran K, Jacks TJ, Finley JW (eds.), *Crop Biotechnology*. Washington: American Chemical Society, pp. 217–30.

Pattee HE, Singleto JA, Johns EB. 1971. Effects of storage time and conditions on peanut volatiles. *J Agric Food Chem* 19(1):134–7.

Pattee HE, Young CT. 1987. Peanut quality: Effects of amino acid and carbohydrate composition on roasted flavor. *Fla Agric Exp Stn Bull* 874.

Pattee HE, Young CT, Giesbrecht FG. 1981. Seed size and storage effects on carbohydrates of peanuts. *J Agric Food Chem* 29(4):800–2.

Pattee HE, Young CT, Pearson JL, Singleton JA, Giesbrecht FG. 1982. Storage and moisture effects on peanut composition and roasted flavor. *Peanut Sci* 9:98–101.

Ravindranath B. 1989. *Principles and Practice of Chromatography*. New York: Halstead Press, pp. 179–85.

Reed KA, Sims CA, Gorbet DW, O'Keefe SF. 2002. Storage water activity affects flavor fade in high and normal oleic peanuts. *Food Res Int* 35(8):769–74.

Rodriguez MM, Basha SM, Sanders TH. 1989. Maturity and roasting of peanuts as related to precursors of roasted flavor. *J Agric Food Chem* 37(3):760–5.

Sanders TH, Lansden JA, Greene RL, Stanley J, Drexler, Williams EJ. 1982. Oil characteristics of peanut fruit separated by a nondestructive maturity classification method. *Peanut Sci* 9:20–3.

Sanders TH, Pattee HE, Vercellotti JR, Bett KL. 1995. Advances in peanut flavor quality. In Pattee HE, Stalker HT (eds.), *Advances in Peanut Science*. Stillwater, OK: American Peanut Research and Education Society, pp. 528–53.

Sanders TH, Vercellotti JR, Bett KL, Greene RL. 1997. The role of maturation in quality of stacpole-cured peanuts. *Peanut Sci* 24:25–31.

Sanders TH, Vercellotti JR, Crippen KL, Civille GV. 1989. Effect of maturity on roast color and descriptive flavor of peanuts. *J Food Sci* 54(2):475–7.

Scheidig C, Czerny M, Schieberle P. 2007. Changes in key odorants of raw coffee beans during storage under defined conditions. *J Agric Food Chem* 55(14):5768–75.

Schieberle P, Grosch W. 1985. Identification of the volatile flavour compounds of wheat bread crust-comparison with rye bread crust. *Z Lebensm Unters Forsch* 180:474–8.

Schirack AV, Drake MA, Sanders TH, Sandeep KP. 2006. Characterization of aroma-active compounds in microwave blanched peanuts. *J Food Sci* 71(9):C513–20.

Schonberg A, Moubacher R. 1952. The Strecker degradation of alpha-amino acids. *Chem Rev* 50(2):261–77.

Shahidi F. 2000. Lipids in flavor formation. In Risch SJ, Ho CT (eds.), *Flavor Chemistry. Industrial and Academic Research. ACS Symposium Series 756*. Washington, DC: American Chemical Society, pp. 24–43.

Shibamoto T, Bernhard RA. 1976. Effect of time, temperature, and reactant ratio on pyrazine formation in model systems. *J Agric Food Chem* 24(4):847–52.

Shibamoto T, Bernhard RA. 1977a. Investigation of pyrazine formation pathways in sugar-ammonia model systems. *J Agric Food Chem* 25(3):609–14.

Shibamoto T, Bernhard RA. 1977b. Investigation of pyrazine formation pathways in glucose-ammonia model systems. *Agric Biol Chem* 41(1):143–53.

Shibamoto T, Bernhard RA. 1978. Formation of heterocyclic-compounds from reaction of L-rhamnose with ammonia. *J Agric Food Chem* 26(1):183–7.

Shibamoto T, Russell GF. 1977. A study of volatiles isolated from a D-glucose hydrogen sulfide ammonia model system. *J Agric Food Chem* 25(1):109–12.

Shu CK. 1998. Pyrazine formation from amino acids and reducing sugars, a pathway other than Strecker degradation. *J Agric Food Chem* 46(4):1515–17.

Shu CK. 1999. Pyrazine formation from serine and threonine. *J Agric Food Chem* 47(10): 4332–5.

Sides A, Robards K, Helliwell S. 2000. Developments in extraction techniques and their application to analysis of volatiles in foods. *Trends Anal Chem* 19(5):322–9.

Singleton JA, Pattee HE, Johns EB. 1971. Influence of curing temperature on volatile components of peanuts. *J Agric Food Chem* 19(1):130–3.

Sohn M, Ho CT. 1995. Ammonia generation during thermal degradation of amino acids. *J Agric Food Chem* 43(12):3001–3.

St. Angelo AJ. 1996. Lipid oxidation in foods. *Crit Rev Food Sci Nutr* 36(3):175–224.

St. Angelo AJ, Graves EE. 1986. Studies of lipid protein-interaction in stored raw peanuts and peanut flours. *J Agric Food Chem* 34(4):643–6.

Steffen A, Pawliszyn J. 1996. Analysis of flavor volatiles using headspace solid-phase microextraction. *J Agric Food Chem* 44(8):2187–93.

Suriyaphan O, Drake M, Chen XQ, Cadwallader KR. 2001. Characteristic aroma components of British farmhouse cheddar cheese. *J Agric Food Chem* 49(3):1382–7.

Talcott ST, Passeretti S, Duncan CE, Gorbet DW. 2005. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. *Food Chem* 90(3):379–88.

Tsantili-Kakoulidou A, Kier LB. 1992. A quantitative structure activity relationship (QSAR) study of alkylpyrazine odor modalities. *Pharm Res* 9(10):1321–3.

Vandeweghe P, Reineccius GA. 1990. Comparison of flavor isolation techniques applied to cheddar cheese. *J Agric Food Chem* 38(7):1549–52.

Van Praag M, Stein HS, Tibbetts MS. 1968. Steam volatile aroma constituents of roasted cocoa beans. *J Agric Food Chem* 16(6):1005–8.

Vercellotti JR, Mills OE, Bett KL, Sullen DL. 1992. Gas chromatographic analyses of lipid oxidation volatiles in foods. In St. Angelo A (ed.), *Lipid Oxidation in Food*. Washington, DC: American Chemical Society, pp. 232–63.

Vercellotti JR, Munchausen LL, Sanders TH, Garegg PJ, Seffers P. 1994. Confirmation of sugars and reductones in complex peanut flavor precursor extracts by ion chromatography and methylation analysis. *Food Chem* 50(3):221–30.

Waller GR. 1969. Nonvolatile flavor components of peanuts. *J Agric Food Chem* 17:728–32.

Walradt JP, Pittet AO, Kinlin TE, Muralidh R, Sanderso A. 1971. Volatile components of roasted peanuts. *J Agric Food Chem* 19(5):972–9.

Wang PS, Odell GV. 1973. Formation of pyrazines from thermal treatment of some amino-hydroxy compounds. *J Agric Food Chem* 21(5):868–70.

Warner KJH, Dimick PS, Ziegler GR, Mumma RO, Hollender R. 1996. "Flavor-fade" and off-flavors in ground roasted peanuts as related to selected pyrazines and aldehydes. *J Food Sci* 61(2):469–72.

Weurman C, Groenen PJ, Van Gemert J. 1970. Experiments on "high vacuum transfer" in food odour research. *Nahrung* 14:607–16.

Whetstine MEC, Cadwallader KR, Drake MA. 2005. Characterization of aroma compounds responsible for the rosy/floral flavor in cheddar cheese. *J Agric Food Chem* 53(8): 3126–32.

Whistler RL, BeMiller JN. 1958. Alkaline degradation of polysaccharides. *Adv Carbohydr Chem* 13:289–329.

Williams JP, Duncan SE, Williams RC, Mallikarjunan K, Eigel WN, O'Keefe SF. 2006. Flavor fade in peanuts during short-term storage. *J Food Sci* 71(3):S265–9.

Wong JM, Bernhard RA. 1988. Effect of nitrogen-source on pyrazine formation. *J Agric Food Chem* 36(1):123–9.

Wong NP, Park OW. 1968. Simple technique for extracting flavor compounds from fatty foods. *J Dairy Sci* 11:1768–9.

Woodroof JG. 1983. *Peanuts: Production, Processing, Products*. Westport, CT: AVI Publishing.

Young CT, Hovis AR. 1990. A method for the rapid analysis of headspace volatiles of raw and roasted peanuts. *J Food Sci* 55(1):279–80.

Young CT, Waller GR, Matlock RS, Morrison RD, Hammons RO. 1974. Some environmental factors affecting free amino-acid composition in 6 varieties of peanuts. *J Am Oil Chem Soc* 51(6):265–8.

Young ND, Sanders TH, Drake MA, Osborne J, Civille GV. 2005. Descriptive analysis and US consumer acceptability of peanuts from different origins. *Food Qual Prefer* 16(1):37–43.

CHAPTER 52

Spices, Seasonings, and Essential Oils

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INTRODUCTION

Essential oils (also called volatile or ethereal oils) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). They are formed mainly by hydrocarbon, oxygenated terpenes, and oxygenated sesquiterpenes. The components of essential oils are important because their qualitative and quantitative composition determine the characteristics of the oil, which, in turn, could have an effect on its antimicrobial potential (Fisher and Phillips 2008). Essential oils can be obtained by expression, fermentation, crystallization, filtration, enfleurage, or extraction, but steam distillation is the most commonly used method in commercial production, although elevated temperatures for long extraction periods can cause chemical modifications in the oil components and often a loss of the most volatile molecules (Tigrine-Kordjani et al. 2006). Wilkes and others (2000) also mentioned that distillation, combined distillation–extraction, and solvent extraction are three of the isolation procedures for extracting essential oils from spices. The proportion of different essential oils extracted by steam distillation is 93%, and the remaining 7% is extracted by the other methods (Masango 2005).

Phenolic compounds, which are always present in essential oils, have been associated with the health benefits derived from consuming large amounts of fruits and vegetables (Balasundram et al. 2006). Although phenolic compounds are present in almost all foods of plant origin, fruits, vegetables, and beverages are the major sources of these compounds in the human diet.

An estimated 3000 essential oils are known, of which about 300 are commercially important, destined chiefly for the flavors and fragrances market (Van de Braak and Leijten 1999 in Burt 2004).

Supercritical fluid extraction (SFE) is a unit operation that uses a variety of fluids (typically CO_2), high pressures (2000–4000 psi), and elevated temperatures (40–150°C). SFE generally produces clean extracts with so little residual organic solvent.

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

Application of SFE in the food industry has been centered mainly on triglyceride extraction (oil recovery), deodorization of animal fats and brewer's yeast, and the decaffeination of tea and coffee. More specific applications include the isolation of α- and β-tocopherols from wheat germ, the analysis of free fatty acids (FFA) in fresh and rancid milk products, and the analysis of aromas and fragrances in aromatic herbs (Morales et al. 1998).

Microwave-assisted extraction can be used for analysis of food flavor compounds from plant or animal tissue, in a microwave-transparent solvent. Another technique is pressurized fluid extraction (PFE), which is performed at near-SFE conditions. The results are comparable to Soxhlet extraction, but PFE takes only 5% of the time and consumes only 10% of the organic solvent. Soxhlet extraction is one of the oldest ways of solid sample treatment, commonly known as solid–liquid extraction. Luque de Castro and García-Ayuso (1998) presented an overview of the evolution of Soxhlet extraction of solid materials and compared its performance with that of other conventional and new extraction techniques. A comparison among traditional and modern extraction methods can be seen in Table 52.1, taking into account the range of sample size, solvent volume, extraction time, degree of automation, and cost.

In Mendes and others (2007), the extractive methods are extensively described and compared.

The greatest use of essential oils is in food (as flavorings), perfumes (fragrances and aftershaves), and pharmaceuticals (for their functional properties). Individual components of the essential oils are also used as food flavorings, either extracted from plant material or synthetically manufactured.

Steam distillation is the most commonly used method for producing essential oils on a commercial basis. Extraction by means of liquid carbon dioxide under low temperatures and high pressures produces a more natural organoleptic profile but is much more expensive (Moyler 1998 in Burt 2004). The difference in organoleptic profile indicates a difference in the composition of oils obtained by solvent extraction as opposed to distillation, and this may also influence antimicrobial properties.

Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Recently, there has been a considerable

TABLE 52.1. Comparison of Selected Extraction Methods for Solid Sample Preparation (Wilkes et al. 2000)

Extraction Method	Sample Size (g)	Solvent Volume (ml)	Time (h)	Degree of Automation	Cost
Sonication	20–50	100–300	0.5–1.0	None	Low
Traditional Soxhlet	10–20	200–500	12–24	None	Very low
Modern Soxhlet	10–20	50–100	1–4	Mostly	Moderate
SFE	5–10	10–20	0.5–1.0	Fully	High
Pressurized-fluid	1–30	10–45	0.2–0.3	Fully	High
Closed vessel-microwave	2–5	30	0.1–0.2	Mostly	Moderate
Open-vessel-microwave	2–10	20–30	0.1–0.2	Mostly	Moderate

Note: Very low, less than US$1000; low, less than US$10,000; moderate, US$10,000–20,000; high, more than US$20,000.

interest in extracts and essential oils from common culinary herbs, spices, and aromatic plants characterized by a notable antimicrobial activity. Raw and/or processed foods are open to contamination during their production, sale, and distribution. Thus, at present, it is necessary to use preservatives in the food industry to prevent the growth of food spoilage microbes. Although a small number of food preservatives containing essential oils are commercially available, until the early 1990s very few studies of the activity of essential oils in foods had been published. Generally, the susceptibility of bacteria to the antimicrobial effect of essential oils also appears to increase with a decrease in the pH of the food, the storage temperature, and the amount of oxygen within the packaging. The physical structure of a food may limit the antibacterial activity of essential oil. Moreover, it has been established that certain essential oils stand out as better antibacterial agents than the commonly used preservatives for meat applications (Hayouni et al. 2008).

Tigrine-Kordjani and others (2006) proposed the use of microwave "dry" distillation or microwave-accelerated distillation (MAD) as a method of extracting edible essential oils extensively used in the fragrance, flavor, and pharmaceutical industries as well as in aromatherapy. It is a combination of microwave heating and dry distillation, performed at atmospheric pressure without adding any solvent or water. Isolation and concentration of volatile compounds is performed by a single stage.

Essential oils are volatile and therefore need to be stored in air-light containers in the dark in order to prevent compositional changes. Natural antioxidants, produced by SFE, are also of interest for the food industry because they do not alter the aroma, flavor, and color of the foodstuffs. Compositional analysis is carried out by gas chromatography and mass spectrometry or its headspace. Natural oxidants can contain more than 60 components; major components constitute more than 85% of the essential oil, whereas other components are present only in trace amounts. Some components show antibacterial activity, as in the case of sage (Marino et al. 2001), Thymus (Paster et al. 1995; Marino et al. 1999), and oregano (Paster et al. 1995).

According to Burt (2004), in vegetable dishes, just as for meat products, the antimicrobial activity of essential oils is benefited by a decrease in storage temperature and/or a decrease in the pH of the food. Vegetables generally have a low-fat content, which may contribute to the successful results obtained with essential oils. Carvacrol and cinnamaldehyde were very effective at reducing the viable count of the natural flora on kiwifruit, but less effective on honeydew melon. It is possible that this difference is dependent on the difference in pH between the fruits; the pH of kiwifruit was 3.2–3.6 and that of the melon was 5.4–5.5.

If essential oils were to be more widely applied as antibacterials in foods, the organoleptic impact would be important. Foods generally associated with herbs, spices, or seasonings would be the least affected by this phenomenon, and information on the flavor impact of oregano essential oil in meat and fish supports this (Burt 2004).

Omobuwajo (2007) says that the use of flavorings in processed foods is determined by a number of factors. These factors are not limited to, but generally include a demonstrable need for the flavor; compliance with legal requirements; compatibility with other ingredients; and ability to withstand processing, packaging, storage, distribution, and merchandizing and acceptability conditions. Omobuwajo also

detailed that 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.

A number of essential oils have been registered by the European Commission for use as flavors in foodstuffs.

GENERAL COMMENTS

In the literature, there are tons of research work related to essential oil extraction from medicinal plants, but those that relate to spices, vegetable, and fruits are scarce. Many studies involve the extraction of flavor and off-flavor components that are present in essential oils, but they do not investigate the method of essential oil extraction. Thus, new and expressive studies about the importance of choosing the best technique for extracting essential oils were selected. These studies also discussed the future applications of the obtained essential oils.

A combined process of supercritical extraction and reverses osmosis membrane was used in the extraction of nutmeg essential oil. Nutmeg (*Myristica fragrans* Houttuyn) is a seed rich in essential oils composed mainly of monoterpenes, oxygenated monoterpenes, aromatic compounds, and sesquiterpenes, which are compounds with molecular weights ranging from 134 to 208 g/gmol.

Under the pressure and temperatures tested, more than 90% of the oil was retained with the maintenance of the CO_2 fluxes (Spricigo et al. 2001).

Benkeblia (2004) studied the essential oils of onions and garlic extracted by steam distillation. Essential oil extracts have been considered natural preservatives or food additives and can further be used for controlling pathogens. Onions and garlic are composed mainly of water (85–90 g/100 g and 60–70 g/100 g fresh weight, respectively), and the most significant components, medicinally, are the organosulfur-containing compounds.

A new process design and operation for steam distillation of essential oils that increases oil yield and reduces loss of polar compounds was developed. A packed bed of the raw materials, as opposed to hydrodistillation, was used. The essential oil of Artemisia was extracted and found to have about 100 components, with camphor as the major component of the oil (Masango 2005).

Volatiles that are present in essential oils of spieces can be obtained using the solid phase microextraction (SPME), a technique that uses a stationary phase coated on a fused-silica fiber, placed in the headspace of a sample vial. After equilibration of volatiles in the headspace and on the solid surface of the coated fiber, the analytes are thermally desorbed by insertion into a gas chromatography port. Different raw materials have been used, such as fruit juices (pear, orange, apple, grapefruit) and spices (paprika, ground pepper, cinnamon, onion flakes, nutmeg) (Wilkes et al. 2000).

Bergamot peel oil, the most valuable essential oil because of its unique fragrance and freshness, was extracted by pervaporation, which allows the recovery of the aroma compounds at low temperatures, preserving their molecular integrity and high selectivity and respecting the environment (no solvent use; Figoli et al. 2006).

Volatiles from blackcurrant were extracted using steam distillation with pentane, and the fractionation produced three fractions: neutral, acidic, and basic fractions. The essential oil of blackcurrant buds gives off a strong terpenic flavor, over-

whelmed by a catty note. The components were analyzed by gas chromatography, and the most polar volatile components possess a typical blackcurrant odor and contribute to the overall pleasant fruity aroma (Piry et al. 1995).

Vegetable oil from seeds is traditionally produced by hexane extraction from ground seeds. The process is very efficient, but its major problem is represented by hexane elimination after extraction. Therefore, several studies have proposed the substitution of the traditional processes by SFE-CO_2 of oil from seeds. Reverchon and De Marco (2006) presented a table containing an analysis of the influence of some process parameters, such as pressure, temperature, extraction time, percentages of co-solvents, and solvent flow rates. The raw materials investigated are presented in Table 52.2.

Raventós and others (2008) reported on natural products that were processed by SFE for use in the food processing industry related to vegetables and fruits. The products were carrot, freeze-dried carrot, sweet potatoes, tomato paste waste, tomato skin, red grape skin, grape seeds, soya bean, rice bran oil, dried orange peel, juice from citrus fruit, and oil from bitter orange peel. Almost of all them used carbon dioxide as solvent and sometimes ethanol as co-solvent. The temperatures varied from 40 to 140°C and pressures from 138 to 700 bar. In general, the extraction yields were good for the majority of the products and comparable to those obtained from conventional methods.

The essential oils of coriander, ginger, and wheat germ were extracted using supercritical fluid. CO_2 was used at 45°C and 177 bar in the case of coriander and provides extracts with high antioxidant activity and yields. Co-solvents (ethanol and isopropyl alcohol) were applied with the CO_2 for the ginger process at 25–35°C and 200–250 bar. The wheat germ was used to extract vitamin E at 40°C and 275 bar for 90 min. The amount of total vitamin E extracted was higher than those obtained using traditional extraction methods (Herrero et al. 2006).

TABLE 52.2. SFE of Oleoresins (OR), Essential (EO), Volatile (VO), and Seed (SO) Oils

Raw Material	Extract	Raw Material	Extract
Anise seeds	EO	Lemon eucalyptus	EO
Bacuri fruit shells	EO	Lemongrass leaves	EO
Basil leaves	EO	Lovage leaves and roots	EO
Borage seeds	SO	Marjoram leaves	EO
Cashew	VO	Mint leaves	EO
Celery roots	SO	Oregano	EO
Chamomile flowers	EO and OR	Palm kernel oil	SO
Clove bud	EO	Pennyroyal	EO
Coriander seeds	SO	Pepper, black	EO
Eucalyptus leaves	EO	Pepper, red	OR
Fennel seeds	SO	Rye bran	Alkylresorcinols
Grape seeds	SO	Sage leaves	EO
Hiprose seeds	SO	Spiked thyme	EO
Juniper fruits	VO	Star anise	EO
Laurel leaves	EO	Thyme	EO
Lemon balm	EO	Tuberose concrete	EO
Lemon bergamot	EO	Vermonia seeds	SO

Balasundram and others (2006) studied many fruits and vegetables to verify the presence of phenolic compounds in the essential oils. They concluded that there are wide variations between the total phenolic contents of different fruits or vegetables, or even the same fruits or vegetables reported by different authors. These differences may be due to the complexity of these groups of compounds, and the methods of extraction and analysis. For example, phenolic compounds present in fruits are found in both free and bound forms (mainly as b-glycosides), but as the latter are often excluded from analyses, the total phenolic contents of fruits are often underestimated. In addition, the phenolic content of plant foods depends on a number of intrinsic (genus, species, and cultivars) and extrinsic (agronomic, environmental, handling, and storage) factors. The phenolic content of some fruits, that is, banana, lychee, mango, and persimmon, is considerably lower than that of berries and grapes. Organically grown strawberries were found to have a higher phenolic content than conventionally grown crops. Processing and storage may have varying impacts on different phenolic compounds, as seen in berry processing. where myricetin and kaempferol were found to be more prone to losses than quercetin. Table 52.3 shows the fruits that have been studied with respect to the presence of phenolic compounds.

TABLE 52.3. Phenolics Content of Selected Fruits (Balasundram et al. 2006)

Fruit Total Phenolics	Content
Apple	296.3 ± 6.4[a]
Banana	90.4 ± 3.2[a]
Black plum	143.5 ± 40.6[b]
Blackberry	417–555[a]
Blackberry (*Rubus* species)	26.7–452.7[a]
Blueberry (*Vaccinium* species)	171–961[a]
Cherry	105.4 ± 27.0[b]
Cranberry	527.2 ± 21.5[a]
Guava (pink)	126.4 ± 6.0[a]
Guava (white)	247.3 ± 4.5[a]
Litchi (lichee)	28.8 ± 1.7[a]
Mango	56.0 ± 2.1[a]
Peach	84.6 ± 0.7[a]
Papaya	57.6 ± 4.1[a]
Persimmon	1.45c
Pineapple	94.3 ± 1.5[a]
Plums	174–375[a]
Prunes (pitted)	184.0 ± 85.5[a]
Raisins	399.4 ± 57.6[b]
Rambutan	1.64 ± 0.04[c]
Raspberry	114–178[a]
Red grape	201.0 ± 2.9[a]
Starfruit (sweet)	209.9 ± 10.4[a]
Strawberry	160 ± 1.2[a]

[a]Gallic acid equivalents/100 g fresh weight.
[b]Catechin equivalents/100 g fresh weight.
[c]Chlorogenic acid equivalents/100 g fresh weight.

Another technology, molecular distillation, was studied to better refine the grape seed oil. The objective was to obtain commercially acceptable values of FFA content (less than 0.1%), while preserving the minor valuable compounds such as tocopherols. Mollecular distillation is ideal because FFA are more volatile than tocopherols. Therefore, it is possible to separate these two components of the volatile fraction, promoting the total evaporation of the FFA while retaining the tocopherols in the residue fraction (Martinello et al. 2007).

A comparison of the efficiencies of the supercritical fluid and organic solvents (normal stirring, Soxhlet, microwave-assisted irradiation, ultrasonic irradiation) was carried out for the pomegranate essential oil extraction. Pomegranate is the oldest edible fruit and belongs to the Punicacea family. Petroleum benzene and hexane were used as organic solvents. CO_2 was used as supercritical fluid, with water, ethanol, and hexane as co-solvents, varying the temperature, pressure, and volume of co-solvent. Significant differences were observed in the yield between the methods studied, although the composition of fatty acids did not differ (Abbasi et al. 2008).

Fisher and Phillips (2008) related the importance of the antimicrobial potential and organoleptic properties of citrus oils as additives in various types of food. Citrus oils contain 85–99% of volatile components and 1–15% of nonvolatile components. The combination of antimicrobial properties and the aromas and flavors of essential oils that lend themselves to their use in food has also led to research into the uses of essential oils as potential food preservatives, although considering their potential, studies involving citrus oils themselves have not been well documented. For essential oils to have an antimicrobial application in food they must not only be safe for consumption but also able to reduce the initial microbial load during production to extend the shelf life of foodstuffs.

Virgin oil was extensively studied by Morales and others (1998) and Jiménez and others (2007). In these two works, new strategies were performed to better recover virgin oil with most of its volatile compounds, such as tocopherols and carotenoids. The technologies employed involved supercritical fluid extraction and high-power ultrasound. Off-flavor components were not detected in the extract.

Different dried fruits of *Zanthoxylum rhesta* were used in India with the objective of extracting the essential oil present in the pericarp of the fruit. The dried fruits are used as condiments and have spice value, especially for fish preparations. The fruits are digestive and appetizing. The essential oil obtained is called Mullilam oil and has been investigated by several other authors. The processes employed were subcritical $-CO_2$, modified methanol–subcritical CO_2, hydrodistillation, and traditional solvent extraction. The yield varied from 1.8% to 6.4% of the essential oil extracted. Twenty-eight components were found in the essential oil using methanol–diethyl ether. The better results were obtained using subcritical CO_2 and hexane as solvents with the presence of 50 components in the essential oil (Rout et al. 2007).

Glisic and others (2007) evaluated the performance of supercritical fluid in carrot fruit essential oil extraction. The chemical composition and antimicrobial activity of carrot fruit essential oil were also investigated. Carrot fruit essential oil is widely used as a flavor ingredient in most major food categories, and as a fragrance component in perfumes, cosmetics, and soaps. It is the source of sesquiterpenic alcohols, carotol and daucol, and the sesquiterpene β-caryophyllene. The conventional method of carrot essential oil isolation is steam distillation of dried fruits. The best yield was obtained at 40°C and 10 MPa. The main component of the essential oil

was carotol. The supercritical extract was characterized by the presence of heavier molecular weight compounds, while some lighter compounds, such as pinenes, were not detected.

Hayouni and others (2008) extracted the essential oils of *Schinus molle* L. and *Salvia officinalis* L. to evaluate the *in vitro* antimicrobial activity of the studied essential oils. They concluded that although the antibacterial activity of both essential oils in minced beef meat was clearly evident, the addition of these oils had notable effects on the flavor and taste of the meat at concentrations more than 2% for *S. molle* and 1.5% for *S. officinalis*. One solution to the above-mentioned problem may be the use of combinations of different food preservation systems. In this context, each of the essential oils was used with low water activity (addition of NaCl) and low refrigeration temperatures. Results about *Salmonella* growth showed that some combinations can be recommended for eliminating germs from minced raw beef. By using this method, a stable and—from a microbiological point of view—safe meat can be produced without substantial loss in sensory quality. Results obtained by Hayouni and others (2008) suggest that the essential oils of *S. officinalis* and *S. molle* possess antimicrobial activity, and therefore they can be used in the biotechnological fields as natural preservative ingredients in the food and pharmaceutical industries.

Although a few articles involved the presence of the interest raw materials, the investigations were so distinct in relation to the technologies applied, providing a comparison between them.

FINAL REMARKS

According to Wilkes and others (2000), the relationship among sample preparation methods, separation and detection systems, and the integrity of quantitative results may be summarized in a common modern aphorism: "Cheap, fast, good … choose any two." Conventional Soxhlet extraction has been the most used extraction method worldwide for a number of decades. A series of minor changes has allowed conventional Soxhlet to be used in specific applications prohibited to the unchanged device. The design and use of new fast techniques such as supercritical fluid extraction, accelerated solvent extraction, and microwave-assisted solvent extraction had relegated Soxhlet to being an old and time-consuming technique. As a result, the majority of related studies investigated new technologies and tested them in the essential oil extraction of different raw materials involving seeds, vegetable, and fruits. Moreover, research interest is not only in the extraction process but also in the quality of the essential oil. This fact is important because many essential oils have been used as additives in many types of foods, with different organoleptic, antimicrobial, and stability effects.

Although the literature is rich in research investigating essential oils and the different technologies for extracting them, studies of essential oils of fruits are scarce and most of them examine the characteristics of the oils and their activity. Some articles have investigated the essential oils of spices (Diaz-Maroto et al. 2002; Nielsen and Rios 2000; Shekarforoush et al. 2007; Valle et al. 2005).

Burt (2004) says that the most interesting area of application of essential oils is in the inhibition of growth and reduction in numbers of the more serious food pathogens. It may be possible to use essential oils in foods that had not been previ-

ously associated with herby or spicy flavor. Individual essential oil components, many of them approved food flavorings, can also impart certain flavors to foods. They can produce undesirable organoleptic effects that can limit the use of essential oil to the type of food. Some authors believe that synergistic effects can be exploited to maximize the antibacterial activity of essential oils and minimize the concentrations required to achieve a particular antibacterial effect.

Although many studies treated different raw materials to extract their essential oils for future use, interactions between essential oils and their components, and other food ingredients and food additives need to be investigated. Another factor is the stability of essential oils during food processing, a factor that also needs to be studied.

Concerning maximum recovery of volatile and flavor components present in essential oils, supercritical fluid extraction and microwave-assisted distillation/trapping/extraction are particularly promising systems. Microwave distillation is quicker, more effective, and environmentally friendly.

REFERENCES

Abbasi H, Rezaei K, Rashidi L. 2008. Extraction of essential oils from the seeds of pomegranate using different organic solvents and supercritical CO_2. *J Am Oil Chem Soc* 85:83–9.

Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem* 99:191–203.

Benkeblia N. 2004. Antomicrobial activity of essential extracts of various onions (Allium cepa) and garlic (Allium sativum). *Lebenson Wiss Technol* 37:263–8.

Burt S. 2004. Essential oils: Their antibacterial properties and potential applications in foods––A review. *Int J Food Microbiol* 94:223–53.

Diaz-Maroto MC, Perez-Coelho MS, Cabezudo MD. 2002. Supercritical carbon dioxide extraction of volatiles from spices: Comparison with simultaneous distillation–extraction. *J Chromatogr A* 947:23–9.

Figoli A, Donato L, Carnevale R, Tundis R, Statti GA, Menichini F, Drioli E. 2006. Bergamot essential oil extraction by pervaporation. *Desalination* 193:160–5.

Fisher K, Phillips C. 2008.Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends Food Sci Technol* 19:156–64.

Glisic SB, Misic DR, Stamenic MD, Zizovic IT. 2007. Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity. *Food Chem* 105:346–52.

Hayouni WA, Chraief I, Abedrabba M, Bouix M, Leveau JY, Mohammed H, Hamdi M. 2008. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. *Int J Food Microbiol* 125:242–51.

Herrero M, Cifuentes A, Ibañez E. 2006. Sub and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae. *Food Chem* 98:136–48.

Luque de Castro MD, García-Ayuso LE. 1998. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Anal Chim Acta* 369:1–10.

Jiménez A, Beltrán G, Uceda M. 2007. High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin oil characteristics. *Ultrasonics Sonochemistry* 14:725–31.

Marino M, Bersani C, Comi G. 1999. Antimicrobial activity of the essential oils of *Thymus vulgari* L. measured using a bio-impedometric method. *J Food Prot* 62(9):1017–23.

Marino M, Bersani C, Comi G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int J Food Microbiol* 67:187–95.

Martinello M, Hecker G, Pramparo MDC. 2007. Grape seed oil deacidification by molecular distillation: Analysis of operative variables influence using the response surface methodology. *J Food Eng* 81:60–4.

Masango P, 2005. Cleaner production of essential oils by steam distillation. *J Cleaner Prod* 13:833–9.

Mendes MF, Pessoa FLP, Melo SAB, Queiroz EM. 2007. Extraction modes. In Hui YH (ed.), *Handbook of Food Products Manufacturing*, 1st Ed. New York: John Wiley, pp. 147–56.

Morales MT, Berry AJ, McIntyre PS, Aparício R. 1998. Tentative analysis of virgin oil aroma by supercritical fluid extraction-high resolution gas chromatography-mass spectrometry. *J Chromatogr A* 819:267–75.

Moyler D. 1998. CO_2 extraction and other new technologies: An update on commercial adoption. International Federation of Essential Oils and Aroma Trades, 21st International Conference on Essential Oils and Aromas. IFEAT, London, pp. 33–9.

Omobuwajo TO. 2007. Food flavorings. In Hui YH (ed.), *Principles of Applications in Handbook of Food Products Manufacturing*. New York: John Wiley & Sons, pp. 119–30.

Nielsen PV, Rios R. 2000. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *Int J Food Microbiol* 60:219–29.

Paster N, Menasherov M, Ravid U, Juven B. 1995. Antifungal activity of orégano and thyme essential oils applied as fumigants against fungi attacking stored grain. *J Food Prot* 58(1): 81–5.

Piry J, Príbela A, Durcacanská J, Farkas P. 1995. Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. *Food Chem* 54:73–7.

Raventós M, Duarte S, Alarcon R. 2008. Application and possibilities of supercritical CO_2 extraction in food processing industry: An overview. *Food Sci Technol Int* 8:269–84.

Reverchon E, De Marco I. 2006. Supercritical fluid extraction and fractionation of natural matter. *J Supercrit Fluids* 38:146–66.

Rout PK, Naik SN, Rao YR, Jadeja G, Maheshwari RC. 2007. Extraction and composition of volatiles from *Zanthoxylum rhesta*: Comparison of subcritical CO_2 and traditional processes. *J Supercrit Fluids* 42:334–41.

Shekarforoush SS, Nazer AHK, Firouzi R, Rostami M. 2007. Effects of storage temperatures and essential oils of oregano and nutmeg on the growth and survival of *Escherichia coli* O157:H7 in barbecued chicken used in Iran. *Food Control* 18:1428–33.

Spricigo CB, Bolzan A, Machado RAF, Carlson LHC, Petrus JCC. 2001. Separation of nutmeg essential oil and dense CO_2 with a cellulose acetate reverse osmosis membrane. *J Memb Sci* 188:173–9.

Tigrine-Kordjani N, Meklati BY, Chemat F. 2006. Microwave "dry" distillation as a useful tool for extraction of edible essential oils. *Int J Aromather* 16:141–7.

Valle JM, del Fuente JC, la Cardarelli DA. 2005. Contributions to supercritical extraction of vegetable substrates in Latin America. *J Food Eng* 67:35–57.

Van de Braak SAAJ, Leitjen GCJJ. 1999. *Essential Oils and Oleoresins: A Survey in The Netherlands and Other Major Markets in the European Union*. Rotterdam: CBI, Centre of the Promotion of Imports from Developing Countries.

Wilkes JG, Conte ED, Kim Y, Holcomb M, Sutherland JB, Miller DW. 2000. Sample preparation for the analysis of flavors and off-flavors in foods. *J Chromatogr A* 880–3-33.

CHAPTER 53

Flavor of Canola Oil

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INTRODUCTION

Flavor, appearance, odor, texture, and sound are sensory characteristics of foods that drive consumers' acceptance of a product. The first set of attributes that influence consumers' attitude toward a food product are color, shape, and general appearance. Subsequently, a combination of odor, flavor, texture, and sound is responsible for the overall acceptance of the product.

Flavor consists of the combination of taste (i.e., sweet, sour, salty, bitter, and umami) and odor as perceived through the posterior nares (retronasal olfaction). Odor perception depends on the detection of volatile compounds present in the material—in this case, foods. This perception can occur via inhalation (orthonasal olfaction) or via the posterior nares through the nasopharyngeal passage (retronasal olfaction). Although the mechanism involved in odor perception is the same (i.e., interaction between a volatile compound and the olfactory epithelium), the types of molecules involved in this process are different. During the chewing process, the application of shear in the food matrix and temperature increases in the oral cavity promote the release of molecules with lower vapor pressures, which are then detected in the olfactory epithelium via the posterior nares (Bett and Dionigi 1997; Dodd et al. 1992).

A constant and reproducible flavor profile is expected by consumers in the foods they eat. An important component is lipids, which impart specific characteristics of flavor and mouthfeel to foods. Manufacturing a food product with good organoleptic properties and maintaining these good flavor characteristics during transportation and storage can be a challenge, especially in foods with a high lipid content because lipids tend to oxidize when put in contact with air and light. The result of this oxidative reaction is the generation of off-flavors usually described as rancidity. Some of the factors that affect the oxidation rate in lipids are the percentage of polyunsaturated fatty acids, the surface area, the presence of pro-oxidants and antioxidants, oxygen, light, and temperature. Lipid distribution in foods also affects the oxidation rate (Fritscher 1994; Paradis 1993). Foods with a continuous lipid phase

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

have a rate of oxidation similar to that of the bulk lipid. However, low-fat foods, where lipids are generally dispersed in the polar components, have rates of oxidation that are affected by water activity, the ratio of free to bound lipids, and pH. The oxidation rate in food lipids can be controlled by changing the processing conditions (Dimakou et al. 2007; Faraji et al. 2004; Mancuso et al. 1999; Okuda et al. 2005), adding different antioxidants (Fomuso et al. 2002; Mei et al. 1999; Silvestre et al. 2000; Tong et al. 2000) or emulsifiers (Hu et al. 2003), mixing with other oils (Let et al. 2005), and choosing the appropriate packaging materials and design. In the case of fried snack foods, opaque, low-permeability, and gas-flushable package materials are used. In this case, prior to sealing the package, the packaged product is generally flushed with nitrogen (Moreira et al. 1999).

To ensure consistent flavor profiles, the flavor quality of foods can be evaluated using analytical and sensory techniques. Analytical techniques assess food quality in terms of their physicochemical stability (Coppin and Pike 2001; Frankel 1998; Rousseau 2004). Sensory techniques, on the other hand, address the organoleptic quality of foods, providing information most closely related to consumers' perception (AOCS 1997). Flavor defects are usually perceived by trained panelists before they are detected by instrumental techniques. For example, flavors that have been described as rancid, metallic, soapy, fishy, and beany can occur at very low levels of oxidation, detected only by sensory techniques. Some of the molecules that are responsible for these off-flavors can be determined using instrumental techniques, such as gas chromatography; however, the relationship with the sensory profile of the product is not always straightforward. Therefore, a combination of instrumental and sensory techniques is the best approach for determining the flavor quality of a product.

The degree and rate of oxidation and the formation of rancid off-flavors depend on the lipid chemical composition, among other factors. The bland flavor of canola oil and partially hydrogenated canola oil make them versatile vegetable oils that are widely used in salad oil, margarine, the baking industry, confectionery, food formulations, and as a frying shortening.

Rapeseed, and therefore canola, refers to crops from the species *Brassica napus* L., *Brassica rapa* L. (formerly *Brassica campestris* L.), and *Brassica juncea* L. (Bengstsson et al. 1972). Rapeseed is characterized by the presence of fatty acids with chain lengths longer than C_{18}, the principal fatty acid being erucic acid (cis-22:1[*n*-9]). Traditional rapeseed oils had erucic acids levels ranging from 20% for *B. rapa* cultivars to about 40% for *B. napus* types. In addition, these oils had high levels of glucosinolates. The negative health implications of erucic fatty acids and glucosinolates challenged rapeseed breeders to develop a cultivar that will have lower levels of these compounds suitable for food consumption (Daun 1984; Daun and Adolphe 1997; Sauer and Kramer 1983). This new rapeseed oil with low erucic acid and low glucosinolates levels was called canola. Canola oil is grown in northern climates, mainly in Canada, China, and northern Europe. The particular chemical composition of canola oil makes it desirable from a nutritional point of view. High levels of oleic acid, usually close to 60%, and significant amounts of linoleic and alpha-linolenic acids (Table 53.1), with very low levels of saturated fatty acids (6–7%), are some of the characteristics that make canola attractive to producers and consumers (Holmes and Bennet 1979; Jonsson 1975; Przybylski and Mag 2002; Ratnayake and Daun 2004).

The refined, bleached, and deodorized (RBD) canola oil is light in color and has a bland flavor (McDonald 2004). The objective of this chapter is to review the flavor and stability of canola oil by providing the reader with scientific references related to this topic. Factors that influence the flavor profile of vegetable oils, in particular canola oil, will be reviewed in this chapter. A brief discussion of mechanisms involved in flavor development in oils and oil-containing foods is also included.

CHEMICAL COMPOSITION OF CANOLA OIL AND OTHER RELATED OILS

Off-flavors generated as a consequence of oxidation can mask the desired bland flavor of canola oil. The chemical composition of an oil determines its flavor profile and organoleptic stability. Oils with high contents of polyunsaturated fatty acids oxidize faster than oils with high contents of saturated or monounsaturated fatty acids.

Table 53.1 shows the chemical composition of canola oil and related oils. Canola oil is characterized by a very low content of saturated fatty acids (~6%), a high content of monounsaturated fatty acids (~62%), and about 30% of polyunsaturated fatty acids. The high content of polyunsaturated fatty acids affects the oil's oxidative stability. Plant breeders have developed canola oil variants with a reduced content (~2%) of linolenic fatty acids (LLCAN) (Scarth et al. 1988), which are significantly more stable toward oxidation than the regular canola oil (Przybylski et al. 1993; Petukhov et al. 1999; Warner and Mounts 1993). However, reducing the amount of linolenic acid is not an ideal situation for improving the quality of oil. At least 2% of linolenic acid is needed in frying oils to develop the desired flavors obtained

TABLE 53.1. Fatty Acid Chemical Composition of Canola Oil and Some Varieties

Fatty Acid	Canola	HEAR	LLCAN	HOCAN	LTCAN
10:0	—	—	—	—	0.1
12:0	—	—	—	—	38.8
14:0	0.1	—	0.1	0.1	4.1
16:0	3.6	4.0	3.9	3.4	2.7
18:0	1.5	1.0	1.3	2.5	1.6
20:0	0.6	1.0	0.6	0.9	0.4
22:0	0.3	0.8	0.4	0.5	0.2
24:0	0.2	0.3	0.3	0.3	0.2
16:1	0.2	0.3	0.2	0.2	0.2
18:1	61.6	14.8	61.4	77.8	32.8
20:1	1.4	10.0	1.5	1.6	0.8
22:1	0.2	45.1	0.1	0.1	0.5
18:2n–6	21.7	14.1	28.1	9.8	11.3
18:3n–3	9.6	9.1	2.1	2.6	6.3
18:3n–6	—	1.0	—	—	—
TOTAL	101	101.5	100	99.8	100

HEAR, high-erucic acid rapeseed; LLCAN, low-linolenic acid canola oil; HOCAN, high-oleic canola oil; LTCAN, high-lauric canola oil.

Source: Przybylski and Mag 2002. Published with permission of Blackwell Publishing.

during exposure at high temperatures (Warner and Mounts 1993) since typical fried food flavors result from controlled oxidation of linolenic acid. To further improve frying stability, canola oil with a higher content (60–85%) of oleic acid (HOCAN) was obtained through breeding techniques (Petukhov et al. 1999). An additional variety of canola oil with a higher content (~40%) of lauric acid (LTCAN) was also developed through breeding, and it can be used as a semisolid fat in confectionery coatings, coffee whiteners, whipped toppings, and center filling fats (Przybylski and Mag 2002).

In summary, different varieties of canola oil contribute to the desirable flavors of foods. For example, if a frying oil is needed, then a canola oil with a low linolenic acid content (~2%) can be used to delay oxidation while promoting the desired frying flavors. On the other hand, oils high in oleic acid (chemical composition similar to olive oil) would be ideal for salad dressings or frying oils. Finally, canola oils high in lauric fatty acids can be used in confections to obtain the desired mouth-feel and texture in fillings and coatings.

FLAVOR STABILITY OF CANOLA OIL

Deodorized, bleached canola oil is an ideal ingredient for salad dressings and mayonnaise because it has a bland flavor; it is light in color and remains free-running at refrigeration temperature (5°C). Fully RBD canola oil has a bland, slightly nutty, and buttery flavor when fresh. When it oxidizes, the volatile compounds formed from the breakdown of fatty acids, particularly those that are highly unsaturated, such as linolenic acid, develop a painty odor (Warner 1994). The characterization of canola oil flavor and its stability has become an important area of research. Analyzing the factors that contribute to the flavor deterioration of canola oil helps in the production of high-quality oils that meet consumers' expectations. The oxidative stability and flavor quality of canola oil have been studied under different conditions. A summary of the results is presented in this section.

The degree and rate of lipid oxidation can significantly affect food quality. Off-flavors generated during the oxidation process negatively affect the flavor profile of foods. The degree and rate of oxidation in a food or lipid needs to be quantified as a quality control tool to ensure good flavor quality. Both analytical and sensory techniques can be used for this purpose. These techniques usually measure the concentration of the oxidation reaction products, which are related, directly or indirectly, to the flavor of the product (White 2000). Some of the most common methods of evaluation are described below. Before briefly describing these methods, it is wise to review the basic mechanisms involved during the oxidation process.

Lipid Oxidation Mechanisms

Lipid oxidation, or autoxidation, occurs when a triacylglyceride or a free fatty acid is in contact with air and light. Light is usually the initiator of a self-propagating, radical forming reaction (Eq. 1). The autoxidation reaction can be divided in three steps as describe below (Eqs. 1–6). During the first step (*initiation*), an initiator, usually photons from light, triggers the generation of a free radical by subtracting a hydrogen (H) from an unsaturated fat (RH). The dissociation energy for the H at

the carbon adjacent to a double bond is fairly low (Ohloff et al. 1987) and, therefore, this H is released with the generation of free radicals (R•, H•). Once a free radical (R•) is formed, it reacts with O_2 to create a peroxy free radical (ROO•), which, in turn, slowly subtracts more H from other RH molecules. These two steps are called *propagation* since the reaction self-propagates until no more O_2 is available (Eqs. 2 and 3). Nonradical products are formed during the *termination* step (Eqs. 4–6) as a consequence of the reaction between free radicals (White 2000). Hydroperoxides generated during the propagation step decompose further to yield aldehydes, ketones, acids, and alcohols, which cause the typical undesirable off-flavors in fats and oils. Although these reactions seem to happen in a stepwise manner, they occur simultaneously and competitively.

Initiation

$$RH \xrightarrow{\text{initiator}} R\bullet + H\bullet \quad (1)$$

Propagation

$$R\bullet + O_2 \longrightarrow ROO\bullet \quad (2)$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \quad (3)$$

Termination

$$R\bullet + R\bullet \rightarrow 2R \quad (4)$$

$$R\bullet + ROO\bullet \rightarrow ROOR \quad (5)$$

$$ROO + ROO\bullet \rightarrow ROOR + O_2 \quad (6)$$

Analytical Methods to Measure Lipid Oxidation

Assessing the extent of lipid oxidation is a common practice to determine the flavor quality of a food. Several methods exist to measure lipid oxidation. Sensory techniques are also very useful; their use and specific applications will be described later in this chapter. For detailed information about the different techniques to measure oxidation, including their advantages and disadvantages, please refer to Nawar (1996). The analytical methods used to measure oxidation can be summarized as follows:

- *Physicochemical*
  - Peroxide value (PV)
  - TBA test
  - Carbonyl value
  - Anisidine value
  - Kreis test
  - Ultraviolet spectrophotometry

- Fluorescence
- Gas chromatography

- *Accelerated tests*
  - Schaal oven test
  - Active oxygen method
  - Rancimat method
  - Oxygen absorption tests
- *Kinetic methods*
  - Differential scanning calorimetry

Physicochemical Methods These types of methods are based on the quantification of the products from the oxidation reaction, usually secondary products such as aldehydes and ketones. These methods include PV, thiobarbituric acid (TBA) test, carbonyl value, anisidine value, and Kreis test. The PV measures the amount of peroxides (ROOH) that are generated during the oxidation by titration with iodine (iodimetry). The result is expressed in milliequivalents of oxygen per kilogram of fat. The TBA test consists of measuring the amount of malonaldehyde, a secondary product from the oxidation reaction, by the formation of a chromagen via a condensation reaction with TBA. The chromagen concentration is determined by absorbance measurement at 532 nm. Methods for determining total carbonyl compounds are usually based on measurements of hydrazones that arise from the reaction of aldehydes or ketones (oxidation products) with 2,4-dinitrophenylhydrazine. The anisidine value measures aldehyde concentration. In the presence of acetic acid, aldehydes react with *p*-anisidine, forming a yellow product with a maximum absorption at 350 nm. Finally, the Kreis test measures the red color obtained from the reaction between epoxy aldehydes or their acetals with the Kreis reagent, phloroglucinol (Gray 1978).

Ultraviolet spectrophotometry is used to measure the presence of conjugated dienes (absorbance at 234 nm) and conjugated trienes (absorbance at 268 nm). Fluorescence methods provide a relatively sensitive measurement of fluorescent compounds that may develop from the interaction of carbonyl compounds with constituents possessing free amino groups. Chromatographic techniques can be used to separate and quantify products of lipid oxidation. Volatile, polar, or small molecular compounds can be separated, identified, and quantified using chromatography with UV detection.

Accelerated Tests In some cases, the oxidation reaction can be accelerated by placing the sample at high temperatures in the presence of light. These tests are very useful for predicting oxidation stability. In the Schaal oven test, the sample is stored at about 65°C and periodically tested until oxidative rancidity is detected. Rancidity is measured either by organoleptic techniques or by PV. During the active oxygen method, the sample is kept at 98°C while air is continuously bubbled through it at a constant rate. The time needed to obtain a specific PV is then determined. The rancimat method measures the conductivity generated as a consequence of the formation of oxidation products. The oxidation process is accelerated by bubbling oxygen into the sample, which is kept at 100°C. Finally, the oxygen absorption method consists of measuring the amount of oxygen consumed by the sample.

Kinetic Methods These refer to a particular type of accelerated methods. A differential scanning calorimeter is used to measure the heat released during the oxidation process. For more information about this technique and applications, please refer to Litwinienko (2005) and Thurgood and others (2007).

Any of the methods described above can be used to measure lipid oxidation and, therefore, flavor quality. Each one of them has advantages and disadvantages. Usually, a combination of methods is needed to quantify the oxidation in food samples and to have a good representation of the sample's organoleptic quality.

Oxidative Stability of Canola Oil

The flavor profile of an oil and its stability greatly depends on its chemical composition; the higher the amount of polyunsaturated fatty acids, the lower the oxidative stability and the faster the flavor deterioration. A myriad of scientific publications have been published that evaluate the oxidative and flavor stability of vegetable oils, in particular canola oil. Malcolmson and others (1994) studied the oxidation stability of canola and sunflower oils stored using the Schaal oven test. They found a greater stability of canola and sunflower oils compared with soybean oils as evidenced by the induction period for off-flavor generation. When stored in the presence of light, canola oil had a greater stability than cottonseed and soybean oils but had a lower stability than sunflower oil. In addition, canola oil was less stable than cottonseed oil during storage at 40°C when used as a frying oil for potato chips. In contrast, when canola oil was stored in glass bottles in the absence of light at 24°C for 16 weeks, Hawrysh and others (1989) did not find any changes in the flavor of canola oils, and reported low PV. From this observation, Malcolmson and others (1994) related accelerated tests measurements to real-time measurements by speculating that a sensory induction period of 2–4 days at 60–65°C is a good predictor of good flavor and odor quality maintenance for at least 16 weeks at room temperature (Malcolmson and Vaisey-Genser 2008).

Raghavan and others (1994) evaluated the flavor of canola oil using dynamic headspace gas chromatography. Typical oxidation volatile products such as pentanal, hexanal, octanal, nonanal, and decadienal were quantified. This study identified three major products of canola oil oxidation: *cis*, *trans*-, and *trans*, *trans*-2,4-heptadienals, which are responsible for the generation of oily, fatty, and putty odors. Correlations between the volatile oxidation products and sensory scores were performed, offering an effective and reliable manner of determining the flavor quality of canola oil. Results from this study were confirmed and supported by Richards and others (2005), who presented a good correlation between the hexanal and heptadienal concentrations and the PV obtained in canola oils.

As already mentioned, to improve the oxidative stability of canola oil, oils with lower contents of linolenic acid (LLCAN) are developed through breeding techniques. Warner and Mounts (1993) studied the frying stability of soybean and canola oils with low contents of linolenic fatty acids. They found that after heating at 190°C, oils with lower contents of linolenic fatty acids had less odor intensity than the standard oils for fishy, burnt, rubbery, smoky, and acrid odors. The same tendency for increased flavor quality of LLCAN was found in fried products, where fishy flavors were detected in products fried in standard canola oil. These results were supported by Przybylski and others (1993), who showed that LLCAN had greater

stability during a Schaal oven test with no significant changes in PV, TBA, and hydroperoxide values. In addition, Przybylski's group did not find any significant changes in the overall odor intensity or odor pleasantness for the LLCAN during storage but found significant changes for regular canola oil. This enhanced storage stability of LLCAN was confirmed by measuring volatile compounds such as dieneals and total volatile carbonyls. Similarly, Eskin and others (1989) reported increased oxidation stability as measured by analytical methods, and improved room odor development for LLCAN when heated to frying temperatures. A study by Prevot and others (1990) also reported a significant decrease in odor formation in heated LLCAN. It reported an increased fruity odor in the LLCAN oils and a lower intensity of fishy and painty odors when compared with regular Canadian and French canola oils.

The oxidative stability of canola oil can be further improved by mixing with vegetable oils low in linolenic acid such as sunflower, cottonseed, or palm. In 1988, Durance-Tod and collaborators studied the flavor and oxidative stability of canola/sunflower and canola/cottonseed oil blends using the Schaal oven test and on light exposure (Durance-Tod et al. 1988). They measured lipid oxidation with several analytical techniques, including PV, hydroperoxide value, TBA number, total volatile carbonyls, dienals, and volatiles by gas chromatography and sensory techniques (odor intensity and overall acceptability). They found that blending canola oil with sunflower or cottonseed oils improved its stability, with a lightstruck flavor formation in the canola/cottonseed oil blends exposed to light (Durance-Tod et al. 1988). Later, Frankel and Huang (1994) showed improved oxidation stability for soybean, canola, and corn oils when blended with different proportions of high oleic sunflower oil. These researchers found that mixtures of canola oil and high oleic sunflower oil had the same or better oxidative stability than the hydrogenated canola oil.

An additional strategy to improve the stability of canola oil is the addition of antioxidants. Önal and Ergin (2002) found that the addition of 200 ppm α-tocopherol and 200 ppm ascorbyl palmitate increased the oxidative stability of canola oil. Other antioxidants that can be used to delay canola oil oxidation are tertiary butylhydroquinone (TBHQ; Hawrysh et al. 1988), propyl gallate, and ascorbic acid (Hawrysh et al. 1992). These have all shown to effectively delay oxidation of canola oil stored at 60°C in the absence of light. However, the most promising approach to extending the shelf life of canola oil appears to be the reduction of linolenic acid through plant breeding (Malcolmson and Vaisey-Genser 2008).

THE ROLE OF CANOLA OIL DURING FRYING EVENTS

During frying, lipids act as the heating medium but also impart flavor to the food either by penetrating into the material's pores or by generating particular flavors through specific chemical reactions. During deep fat frying, hydrolytic, oxidative, and pyrolytic reactions occur. The relative reaction rates of these processes are related to the type of frying oil, fried material, frying equipment, and conditions such as time, temperature, and the presence of minor, non-lipidic substances. Oxidation reactions have the greatest effect on fried flavors. Fats and oils are slowly oxidized even at ambient temperature, and when they reach frying temperatures,

oxidation accelerates (Pokorny 1989). This oxidation mechanism is similar to the autoxidation mechanism of lipids observed at room temperatures, which was described in the previous section.

Generation of typical fried flavors is not instantaneous; it requires some time to develop. During the first stages of frying, the flavor improves, remaining constant for a certain amount of time, after which the flavor decreases, generating bitter side notes, with pungent and irritating sensations. Frying conditions should be controlled to obtain optimum sensory quality for the maximum frying time (Pokorny 1989). Air and metal traces influence the flavor stability of frying oils by increasing the oxidation rate and therefore decreasing the oxidative stability, especially at high frying temperatures. The flavor deterioration caused by the presence of air can be inhibited by protecting the frying oils against air.

The flavor of commercial frying oils is usually bland. As early as 1978, Dobbs and others (1978) reported flavor deterioration, as evidenced by sulfur, fishy, and painty odors, in canola oils heated to frying temperatures of 190°C The oxidative stability of canola oil during frying can be improved by adding antioxidants. Tocopherols are the most commonly present natural antioxidants in oils. However, at high frying temperatures, antioxidant concentration slowly decreases, especially when frying oils are highly unsaturated (Carlson and Tabacchi 1986).

Canola oil is not ideal for frying because of its high linolenic acid content (Table 53.1) and low oxidative stability as compared with other oils. The linolenic acid content of canola oil is 9.6% as compared with soybean, sunflower, and olive oil, which have 8.4%, 0.2%, and 0.7% of linolenic acid, respectively. The ideal frying oil should contain a low proportion of unsaturated fatty acids to increase its oxidative stability and have approximately 2% linolenic acid to ensure the generation of desired fried flavors. Using partially hydrogenated canola oil, mixing with linolenic-rich oils, adding antioxidants, or using low-linolenic varieties are the best strategies for obtaining desirable flavor profiles in fried foods without oxidation notes (Pokorny 1989).

FLAVOR OF CANOLA OIL: SENSORY TECHNIQUES

Food flavors can be measured by instrumental and sensory techniques. Instrumental techniques involve the quantification of specific molecules related to flavor generation or deterioration, providing an indirect measurement of consumers' perception. Depending on the extraction and measuring technique used during instrumental analysis, molecules measured by instrumental techniques might not represent consumers' perception. For this reason, sensory evaluation is, in general, more accurate and sensitive to flavor detection and quantification since they provide a direct measurement of the foods' flavor profile as perceived by consumers.

In 1996, Warner and Nelsen conducted a study in collaboration with the American Oil Chemists' Society (Warner and Nelsen 1996) to determine the effectiveness of sensory analysis in the evaluation of vegetable oil flavor. They compared sensory evaluation techniques and gas chromatographic analyses of volatile compounds in vegetable oils with different levels of oxidation. They found that both sensory evaluation techniques and instrumental analysis can be used to determine the flavor quality of oils. While the accuracy of determination with both methods was higher

for soybean, canola, and sunflower oils than for corn oil, the variation in sensory determinations was lower for the nonaged oil, and as storage time increased, differences among panelists became greater.

The flavor quality of vegetable oils can be assessed by quantifying the presence (or absence) of specific flavor attributes. Terms used to describe the flavor profile of fresh oils (nonaged) with no off-flavors notes are nutty, buttery, corny, and beany, among others. Terms such as hydrogenated, burned, weedy, grassy, painty, and fishy are used to describe off-notes in oils. These attributes, positive and negative, contribute to the flavor profile of the oil. A complete list of these attributes can be found in Warner (1994). Standardized procedures to evaluate the flavor attributes of vegetable oils can be found in the Official Methods of the American Oil Chemists' Society (AOCS Cg 2-83 1999) and the American Society for Testing and Materials (ASTM E 1627-94 2004, E 1346-90 2000).

Different sensory techniques can be used to evaluate the flavor of oils, and consumer and descriptive tests are the most common. Information about frequent sensory evaluation techniques can be found in Meilgaard and others (2007a,b). Warner (1989, 1994) also provides an excellent review of the different sensory evaluation techniques available and their application to the evaluation of flavor quality of fats, oils, and lipid-containing foods. Canola oil flavors evaluated using some of these techniques are described below.

Descriptive Analysis

In descriptive analysis, panelists are instructed to detect and identify odors, flavor, appearance, and texture attributes in the samples and to rate the intensity of each one of them. When using sensory evaluation to determine the quality of an oil, flavor and odor are the main attributes quantified. Descriptive studies performed on canola oils show that crude or refined bleached canola oil is characterized by a cabbage, sulfur, and grassy/green flavor. Descriptors found in deodorized fresh canola oil include nutty and buttery, while rancid, painty, fishy, grassy, and metallic are commonly detected in slightly oxidized canola oil (Warner 1994).

In 1996, Leveaux and Resurreccion provided a detailed description of canola oil flavor together with the descriptors for other vegetable oils (Leveaux and Resurreccion 1996). Using a descriptive panel, these authors evaluated the presence and intensities of color, odor, flavor, and texture of canola, cottonseed, peanut, and soybean oils. Panelists were trained on the identification and quantification of appearance, odor–flavor and texture attributes using a 150-mm scale. Panelists used this scale to quantify the intensity of each attribute. The stronger the perception of a specific attribute, the higher the rating on the 150-mm scale. For example, a rating of 130 mm for a specific attribute indicates a stronger intensity than a rating of 90 mm for that same attribute. Tables 53.2 and 53.3 present the attributes found and their intensities. As observed in Table 53.2, canola oil had the second rating in color after cottonseed oil. Canola oil was characterized by a hydrogenated odor, followed by a fishy and nutty odor. Similarly to the odor, the most intense flavor characteristics of canola oil were hydrogenated, followed by nutty and fishy. Finally, canola oil had the highest viscosity among the oils and the second highest rating for mouthcoat, after cottonseed oil.

TABLE 53.2. Mean and Standard Deviation of Descriptive Analysis (Appearance and Odor) Attributes Scores of Canola, Cottonseed, Peanut, and Soybean Oils

	Oils							
	Canola		Cottonseed		Peanut		Soybean	
Attributes	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Appearance								
Color	38.8_{b}	16.6	55.5_{a}	11.2	26.4_{c}	8.9	20.0_{d}	9.0
Odor								
Hydrogenated	15.3_{b}	11.7	24.9_{b}	13.8	14.5_{b}	7.6	12.5_{b}	11.6
Nutty	8.6_{a}	12.5	10.7_{a}	14.2	7.3_{a}	11.7	7.5_{a}	9.5
Fishy	9.3_{a}	12.6	5.1_{b}	8.3	1.7_{b}	4.2	5.2_{b}	4.4
Corny	5.8_{a}	9.9	5.1_{a}	8.7	4.0_{a}	8.1	3.9_{a}	5.8
Buttery	4.0_{b}	7.5	5.1_{b}	7.8	9.3_{a}	9.9	5.1_{b}	6.6
Weedy	0.6_{a}	2.7	0.9_{a}	3.6	0.6_{a}	2.4	0.0_{a}	0.0
Beany	3.3_{a}	6.7	3.6_{a}	7.2	0.0_{b}	0.0	1.2_{ab}	4.2
Cardboard	2.7_{ab}	6.0	4.1_{a}	8.4	0.6_{b}	2.4	1.7_{ab}	5.2
Fruity	4.7_{ab}	7.9	6.8_{ab}	11.1	7.6_{a}	9.6	3.1_{b}	5.9
Hully	1.1_{ab}	4.6	3.5_{a}	8.0	0.3_{b}	1.7	0.3_{b}	1.7
Painty	3.6_{a}	7.9	4.8_{a}	10.4	2.0_{a}	6.3	3.8_{a}	10.6
Waxy	1.6_{a}	4.7	2.6_{a}	5.9	1.5_{a}	4.7	2.2_{a}	4.9
Musty	0.9_{ab}	3.9	0.8_{ab}	3.2	0.0_{b}	0.0	1.8_{a}	5.0

Means in each row with different letters are significantly different ($p < 0.05$).
Source: Leveaux and Resurreccion 1996. Reproduced under permission of Blackwell Publishing.

TABLE 53.3. Mean and Standard Deviation of Descriptive Analysis (Flavor and Texture) Attributes Scores of Canola, Cottonseed, Peanut and Soybean Oils

	Oils							
	Canola		Cottonseed		Peanut		Soybean	
Attributes	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Flavor								
Hydrogenated	19.5_{b}	12.2	31.1_{a}	15.9	19.1_{b}	8.8	23.3_{b}	19.2
Nutty	16.4_{a}	16.0	16.9_{a}	18.2	15.0_{a}	18.2	13.0_{a}	13.6
Fishy	13.6_{a}	18.3	6.2_{b}	8.7	5.8_{b}	13.5	4.3_{b}	7.0
Corny	6.5_{a}	10.1	9.3_{a}	10.1	6.6_{a}	9.3	6.6_{a}	6.8
Buttery	5.1_{b}	8.0	11.1_{a}	14.6	10.5_{ab}	11.7	7.1_{ab}	7.6
Weedy	2.2_{a}	5.7	2.2_{a}	5.1	1.6_{a}	4.5	1.0_{a}	3.6
Beany	3.3_{ab}	6.2	4.0_{a}	8.0	0.07_{b}	2.5	1.5_{ab}	4.2
Cardboard	5.8_{a}	11.6	6.6_{a}	16.1	1.8_{a}	4.8	5.2_{a}	13.0
Fruity	5.1_{a}	8.7	6.5_{a}	8.1	7.6_{a}	10.7	5.8_{a}	9.1
Hully	4.1_{a}	7.7	7.0_{a}	13.9	2.6_{a}	6.3	7.5_{a}	15.6
Painty	2.3_{ab}	6.2	3.5_{a}	8.3	0.5_{b}	2.2	1.0_{ab}	3.2
Waxy	4.3_{b}	7.0	8.6_{a}	9.2	4.5_{b}	6.8	3.8_{b}	5.6
Musty	2.3_{a}	5.1	0.8_{a}	2.6	0.6_{a}	1.7	1.9_{a}	4.4
Bitter	4.0_{a}	7.6	9.8_{a}	22.0	3.9_{a}	8.2	6.3_{a}	14.5
Texture								
Viscosity	37.6_{a}	18.6	32.3_{a}	19.7	25.1_{a}	18.4	26.8_{a}	17.3
Mouthcoat	21.0_{ab}	11.0	26.7_{a}	15.2	20.3_{b}	10.9	21.1_{ab}	10.8

Means in each row with different letters are significantly different ($p < 0.05$).

Source: Leveaux and Resurreccion 1996. Reproduced with permission of Blackwell Publishing.

Consumer Acceptance of Canola Oils

Consumer acceptance determines the success of a food product in the marketplace and, in most cases, is the benchmark for quality standards. Establishing a tolerance level of consumers to degrees of oxidation in canola oils is useful for establishing shelf-life sensory limits. Vaisey-Genser and others (1994) observed that the consumer acceptance threshold for regular canola oil (RCAN) was 12 days lower than that for LLCAN when the oils were stored at room temperature, suggesting greater stability for the modified oil. In the same context, Malcolmson and others (1996) provided chemical and sensory characterization of stored RCAN and LLCAN at different levels of consumer acceptance (70%, 60%, 50%, and 40% acceptance for RCAN and 80%, 70%, and 50% acceptance for LLCAN). They found an increase in the painty odor intensity as consumer acceptance decreased. This same trend was found for chemical measurements of PV, total volatiles, total carbonyls, unsaturated carbonyls, and dienals. The presence of painty flavors in oxidized oils was also reported by Warner (1994), who related the painty odor formation to the oxidation of high-linolenic oils.

Imparting Flavors to Food Products

When canola oil is used as a salad oil, its flavor quality is determined by the oxidative stability of the bulk oil stored at room temperature. Information on this topic was described previously in this chapter. When canola oil is used as a frying oil, the quality of the fried material needs to be evaluated as a function of the oil used and storage conditions (time and temperature). The flavor quality of food products fried using different varieties of canola oil is described below.

The oxidative stability of tortilla chips fried in different oils (canola, corn, partially hydrogenated soy [(PHS]), partially hydrogenated canola (PHCAN), and LLCAN was determined by Hawrysh and others (1995) using sensory and chemical techniques. Chips were stored under Schaal conditions and also at room temperature. In the absence of light, off-flavors were more evident in PHCAN chips than in LLCAN chips. However, when samples were stored at room temperature, canola chips had the highest off-flavor while all other chips were similar. As expected, rancid, painty, buttery odor/flavor, and bitter flavor notes were detected in these samples. The PV and the *p*-anisidine value for oils extracted from Schaal-stored chips supported the panelists' data (Hawrysh et al. 1995). Similarly, Xu and others (1999) evaluated the frying performance of different oils during two 80-h deep-frying trials with potato chips. They found that the higher the linolenic acid content in high oleic oils, the lower the sensory ranking of fried foods and oxidative stability, suggesting a low flavor quality of the product. LLCAN and sunflower oil, however, obtained the highest sensory ratings, while partially hydrogenated canola oil received the lowest scores (Xu et al. 1999).

Warner and others (1994) studied the oxidative stability of potato chips during storage at room temperature for 4 months. They found that potato chips fried in RCAN had the lowest flavor quality scores compared with chips fried in hydrogenated, low-linolenic (LLCAN), and high-oleic canola oils (HOCAN). Similarly, higher amounts of total volatiles were obtained in RCAN chips, while the lowest amount was found in HOCAN and LLCAN chips. Petukhov and others (1999) also evaluated the storage stability of potato chips. After frying the chips for a total of

40 h, chips fried in RCAN had greater peroxide, free fatty acid, conjugated dienoic acid, and polar compound values and developed higher levels of total volatiles over the storage period than chips fried in LLCAN, HOCAN, and hydrogenated canola oil. All chips developed a painty odor during storage, with the exception of chips fried in hydrogenated canola oil, which developed an intense stale/musty odor during storage.

The sensory data presented above suggest that canola oil varieties, especially LLCAN and HOCAN, can be efficiently used in frying products, providing a desired flavor to the food and maintaining a good oxidative stability during storage.

SUMMARY

The flavor of fresh RBD canola oil is characterized by nutty and buttery notes. When the oil is stored in the dark, slight oxidation develops, resulting in a rancid, fishy, metallic, and a characteristic painty flavor, which is usually present in oxidized high-linolenic acid oils. The main factor that affects canola oil flavor is oxidation, either by simple exposure to light and oxygen or by increasing the temperature during frying processes. The flavor quality of canola oil can be measured using instrumental techniques or sensory evaluation tests. Both types of methods are useful; however, sensory techniques provide a direct measurement of consumers' perception. The flavor stability of canola oil can be optimized either by modifying its chemical composition (breeding), by mixing with other vegetable oils, or by adding antioxidants.

Overall, canola oil, with its bland nutty and buttery flavor, is ideal for cooking, providing good nutritional value and flavor profile to foods.

REFERENCES

AOCS Cg-2-83. 1997. Flavor panel evaluation of vegetable oils. In *Official Methods of the American Oil Chemists' Society*. Champaign, IL: American Oil Chemists' Society.

ASTM E 1346-90. 2000. *Bulk Sampling, Handling and Preparing Edible Vegetable Oils for Sensory Evaluation*. West Conshohocken, PA: American Society for Testing and Materials.

ASTM E 1627-94. 2004. *Standard Practice for Sensory Evaluation of Edible Oils and Fats*. West Conshohocken, PA: American Society for Testing and Materials.

Bengtsson L, Von Hoften A, Loof B. 1972. Botany of rapeseed. In Appelqvist L, Ohlson R (eds.), *Rapeseed Cultivation and Composition, Processing and Utilization*. London: Elsevier Publishing, pp. 36–48.

Bett KL, Dionigi CP. 1997. Detecting seafood off-flavors: Limitations of sensory evaluation. *Food Technol* 51(8):70–6.

Carlson BL, Tabacchi MH. 1986. Frying oil deterioration and vitamin loss during foodservice operation. *J Food Sci* 51:218–21.

Coppin EA, Pike OA. 2001. Oil stability index correlated with sensory determination of oxidative stability in light-exposed soybean oil. *J Am Oil Chem Soc* 78:13–18.

Daun JK. 1984. Composition and use of canola seed, oil and meal. *Cereal Foods World* 29:291–6.

Daun JK, Adolphe DF. 1997. A revision of the canola oil definition. *Bull GCIRC* 14:134–41.

Dimakou CP, Kiokias SN, Tsaprouni IV, Oreopoulou V. 2007. Effect of processing and storage parameters on the oxidative deterioration of oil-in-water emulsions. *Food Biophys* 2:38–45.

Dobbs JE, Vaisey-Genser M, Diamant R. 1978. Unpleasant odors of rapeseed oil heated to frying temperatures. *J Inst Can Sci Technol Aliment* 11:66–70.

Dodd GH, Bartlett PN, Gardner JW. 1992. Odours-the stimulus for an electronic nose. In Gardner JW, Bartlett PN (eds.), *Sensors and Sensory Systems for an Electronic Nose. NATO ASI Series*. Dordrecht: Kluwer Academic Publishers, pp. 1–11.

Durance-Tod S, Przybylski R, Eskin NAM, Vaisey-Genser M. 1988. The flavor and oxidative stability of canola oil blends. *J Am Oil Chem Soc* 65:528–9.

Eskin NAM, Vaisey-Genser M Durance-Tod S, Przybylsky R. 1989. Stability of low linolenic acid canola oil to frying temperature. *J Am Oil Chem Soc* 66:1081–4.

Faraji H, McClements JD, Decker E. 2004. Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *J Agric Food Chem* 52:4558–64.

Fomuso LB, Corredig M, Akoh CC. 2002. Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. *J Agric Food Chem* 50:2957–61.

Frankel EN. 1998. Stability methods. In *Lipid Oxidation*. Dundee Scotland: The Oily Press, pp. 99–114.

Frankel EN, Huang SW. 1994. Improving the oxidative stability of polyunsaturated vegetable oils by blending with high-oleic sunflower oil. *J Am Oil Chem Soc* 71:255–9.

Fritscher CW. 1994. Lipid oxidation—the other dimensions. *Inform* 5(4):423–36.

Gray JI. 1978. Lipid oxidation. *J Am Oil Chem Soc* 55:539–46.

Hawrysh ZJ, Erin MK, Kim SS, Hardin RT. 1995. Sensory and chemical stability of tortilla chips fried in canola oil, corn oil, and partially hydrogenated soybean oil. *J Am Oil Chem Soc* 72:1123–30.

Hawrysh ZJ, Erin MK, Lin YC, Hardin RT. 1992. Propyl gallate and ascorbyl palmitate affect stability of canola oils in accelerated storage. *J Food Sci* 57:1234–40.

Hawrysh ZJ, Shand PJ, Tokarska B, Lin C. 1989. Effects of tertiary butylhydroquinone on storage stability of canola oil. II. Practical storage. *Can Inst Food Sci Technol J* 22:40–5.

Hawrysh ZJ, Shand PJ, Tokarska B, Lin C. 1988. Effects of tertiary butylhydroquinone on storage stability of canola oil. I. Accellerated storage. *Can Inst Food Sci Technol J* 21:549–54.

Holmes MRJ, Bennet D. 1979. Effect of nitrogen fertilizer on the fatty acid composition of oil from low erucic rape variaties. *J Sci Food Agric* 30:264–6.

Hu M, McClements JD, Decker EA. 2003. Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate and soy protein isolate. *J Agric Food Chem* 51:1696–700.

Jonsson R. 1975. Breeding for improved fatty acid composition in oilseed crops. 2. Investigation on the effect of some environmental factors on the fatty acid pattern in winter rape (Brassica napus L. var. biennis L.). *Sver Utsdestfor Tidskr* 85:9–18.

Let MB, Jacobsen C, Pham KA, Meyer AS. 2005. Protection against oxidation of fish-oil-enriched milk emulsions through addition of rapeseed oil or antioxidants. *J Agric Food Chem* 53:5429–37.

Leveaux VD, Resurreccion AVA. 1996. Descriptive sensory profiling of freshly processed commercial peanut, cottonseed, canola and soybean oils. *J Food Qual* 19:265–77.

Litwinienko G. 2005. Analysis of lipid oxidation by differential scanning calorimetry. In Kamal-Eldin A, Pokorny J (eds.), *Analysis of Lipid Oxidation*. Chapmaign, IL: AOCS Press, pp.152–93.

Malcolmson LJ, Vaisey-Genser M, Przybylski R, Eskin NAM. 1994. Sensory stability of canola oil: Present status of shelf life studies. *J Am Oil Chem. Soc* 71:435–40.

Malcolmson LJ, Vaisey-Genser M, Przybylski R, Ryland D, Eskin NAM, Armstrong L. 1996. Characterization of stored regular and low-linolenic canola oils at different levels of consumer acceptance. *J Am Oil Chem Soc* 73:1153–60.

Malcolmson LJ, Vaisey-Genser M. 2008. Canola Oil: Performance properties of canola oil. Canola Council of Canada. http://www.canola-council.org.

Mancuso JR, McClements JD, Decker EA. 1999. The effects of surfactant type, pH and chelators on the oxidation of salmon oil-in-water emulsions. *J Agric Food Chem* 47:4112–16.

McDonald BE. 2004. Food uses and Nutritional Properties. In Gunstone FD (ed.), *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*. Oxford: Blackwell Publishing, pp. 131–53.

Mei L, McClements JD, Decker EA. 1999. Lipid oxidation in emulsions as affected by charge status of antioxidants and emulsions droplets. *J Agric Food Chem* 47:2267–73.

Meilgaard MC, Civille GV, Carr BT. 2007a. Descriptive analysis techniques. In *Sensory Evaluation Techniques*, 4th Ed. New York: CRC Press, pp. 173–88.

Meilgaard MC, Civille GV, Carr BT. 2007b. Affective tests: Consumer tests and in-house panel acceptance tests. In *Sensory Evaluation Techniques*, 4th Ed. New York: CRC Press, pp. 255–312.

Moreira RG, Castell-Perez ME, Barrufet MA. 1999. Fired product quality. In *Deep-Fat Frying*. Gaithersburg, MD: Aspen Publishers, pp. 75–110.

Nawar WW. 1996. Lipids. In Fenema OR (ed.), *Food Chemistry*, 3rd Ed. New York: Marcel Decker, pp. 226–314.

Ohloff SY, Miyashits K, Miyazawa T, Fujimoto K, Kaneda T. 1987. Autoxidation of ethyl eicosapentanoate and docosahexanoate. *J Am Oil Chem Soc* 64:876–9.

Okuda S, McClements JD, Decker EA. 2005. Impact of lipid physical state on the oxidation of methyl linolenate in oil-in-water emulsions. *J Agric Food Chem* 53:9624–8.

Önal B, Ergin G. 2002. Antioxidative (effects of α-tocopherol and ascorbyl palmitate on thermal oxidation of canola oil. *Nahrung/Food* 46: 420–6.

Paradis A. 1993. Nitrogen in total quality for snack food. *Inform* 4(12):1378–82.

Petukhov I, Malcolmson LJ, Przybylski R, Armstrong L. 1999. Storage stability of potato fried in genetically modified canola oils. *J Am Oil Chem Soc* 76:889–96.

Pokorny J. 1989. Flavor chemistry of deep fat frying in oil. In Min DB, Smouse TH (eds.). *Flavor Chemistry of Lipid Foods*. Champaign, IL.: AOCS Press.

Prevot A, Perrin JL, Laclaverie G, Auge PH, Coustille JL. 1990 A new variety of low-linolenic rapeseed oil: Characteristics and room-odor test. *J Am Oil Chem Soc* 67: 161–4.

Przybylski R, Mag T. 2002. Canola/rapeseed oil. In Gunstone FD (ed.) *Vegetable Oils in Food Technology: Composition, Properties and Uses*. Oxford: Blackwell Publishing, CRC Press, pp. 98–127.

Przybylski R, Malcolmson LJ, Eskin NAM, Durance-Tod S, Mickle T, Carr R. 1993. Stability of Low Linolenic Acid Canola Oil to Accelerated Storage at 60°C. *Lebenson Wiss Technol* 26, 205–9.

Raghavan SK, Connel DR, Khayat A. 1994. Canola oil flavor quality evaluation by dynamic headspace gas chromatography. In Ho C, Hartman TG (eds.), *Lipids in Food Flavors, ACS Symposium Series 558*. Washington, DC: American Chemical Society, pp. 92–300.

Ratnayake WMN, Daun JK. 2004. Chemical Composition of Canola and Rapeseed Oils. In Gunstone FD (ed.), *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*. Oxford: Blackwell Publishing, pp. 37–78.

Richards A, Wijesundera C, Salisbury P. 2005. Evaluation of oxidative stability of canola oils by headspace analysis. *J Am Oil Chem Soc* 82:869–74.

Rousseau D. 2004. Chemical and physical properties of canola and rapeseed oil. In Gunstone FD (ed.), *Rapeseed and Canola Oil: Production, Processing, Properties and Uses*. Oxford: Blackwell Publishing, pp. 87–110.

Sauer FD, Kramer JKG. 1983. The problems associated with the feeding of high erucic acid rapeseed oils and some fish oils to experimental animals. In Kramer JKG, Sauer FD, Pigden WJ (eds.), *High and Low Erucic Rapeseed Oils Production, Usage, Chemistry, and Toxicological Evaluation*. Toronto: Academic Press, pp. 254–92.

Scarth R, McVvetty PBE, Rimmer SR, Stefansson BR. 1988. Stellar low linolenic-high linoleic acid summer rape. *Can J Plant Sci* 68:509–12.

Silvestre MPC, Chaiyasit W, Brannan RG, McClements JD, Decker EA. 2000. Ability of surfactant headgroup size to alter lipid and antioxidant oxidation in oil-in-water emulsions. *J Agric Food Chem* 48:2057–61.

Thurgood JE, Ward R, Martini S. 2007. Oxidation kinetic of soybean oil/anhydrous milk fat blends: A differential scanning calorimetry study. *Food Res Int* 40:1030–7.

Tong LM, Sasaki S, McClements JD, Decker EA. 2000. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J Agric Food Chem* 48:1473–8.

Vaisey-Genser M, Malcolmson LJ, Ryland D, Przybylski R, Eskin NAM, Armstrong L. 1994. Consumer acceptance of canola oils during temperature-accelerated storage. *Food Qual Prefer* 5:237–43.

Warner K. 1994. Sensory evaluation of oils and fat-containing foods. In Warner K, Eskin MNA (eds.), *Methods to Assess Quality and stability of Oils and Fat-Containing Foods*. Champaign, IL: AOCS Press.

Warner K, Frankel EN, Mounts TL. 1989. Flavor and oxidative stability of soybean, sunflower and low erucic acid rapeseed oils. *J Am Oil Chem Soc* 66:558–64.

Warner K, Mounts TL. 1993. Frying stability of soybean and canola oils with modified fatty acid compositions. *J Am Oil Chem Soc* 70:983–8.

Warner K, Nelsen T. 1996. AOCS collaborative study on sensory and volatile compound analyses of vegetable oils. *J Am Oil Chem Soc* 73:157–66.

Warner K, Orr P, Parrott L, Glynn M. 1994. Effects of frying oil composition on potato chip stability. *J Am Oil Chem Soc* 71:1117–21.

White PJ. 2000. Flavor quality of fats and oils. In O'Brien RD, Farr WE, Wan PJ (eds.), *Introduction to Fats and Oils Technology*. Champaign, IL: AOCS Press, pp. 341–70.

Xu XQ, Tran VH, Palmer M, White K, Salisbury P. 1999. Chemical and physical analyses and sensory evaluation of six deep-frying oils. *J Am Oil Chem Soc* 76:1091–9.

CHAPTER 54

Flavors of Palm Oil

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Palm oil was first introduced into then Malaya in 1875 in order to examine its economic potential. Cultivation of palm oil as a crop started in 1917, and in 1926 cultivation began to increase (Arnott 1959). The need to diversify the agriculture of Malaysia from natural rubber after the Second World War prompted Malaysia to further increase its oil palm plantation acreage. Currently, the oil palm acreage is more than 4.5 million ha. In 1960, Malaysia exported 92,000 tons of palm oil. Since then the production of palm oil increased progressively through the years, and in 2008 the production was 17.7 million tons (Malaysian Palm Oil Board 2009). The spectacular increase in production of palm oil has made it one of the most abundant oils in the world.

SYNTHESIS OF OIL IN PALM FRUITS

The biochemistry of palm oil in relation to fruit development and oil deposition has been reported by Hartley (1977), Thomas and others (1971), Oo and others (1985, 1986), Sambathamurthi and others (2000), and Azis (1984). Palm fruits of *Elaeis guineensis* are clustered tightly in bunch (Fig. 54.1). Each fruit consists of the kernel or endocarp, which is encapsulated by the kernel shell. Enveloping this structure, commonly referred to as nut, is the fleshy mesocarp, which is reinforced by strands of lignified vascular and nonvascular fibers running longitudinally from the base toward the fruit tip. The mesocarp, which is thicker in the Tenera variety, is morphologically divided into the fibrous pericarp and exocarp or the skin. Tenera is the progeny of the cross between the Dura and Pisifera varieties. The Dura variety has a distinctive thick shell and thinner mesocarp, while the mesocarp of the Pisifera variety does not have any distinct kernel shell. The Tenera has a distinct fiber ring when a cross section of a fruit is examined.

The kernel in the fruit (of Tenera and Dura) is fully developed at 13–14 weeks after anthesis, while the mesocarp takes about 20–22 weeks to be fully oil bearing (Azis 1984). The apex of development is determined with reference to the

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

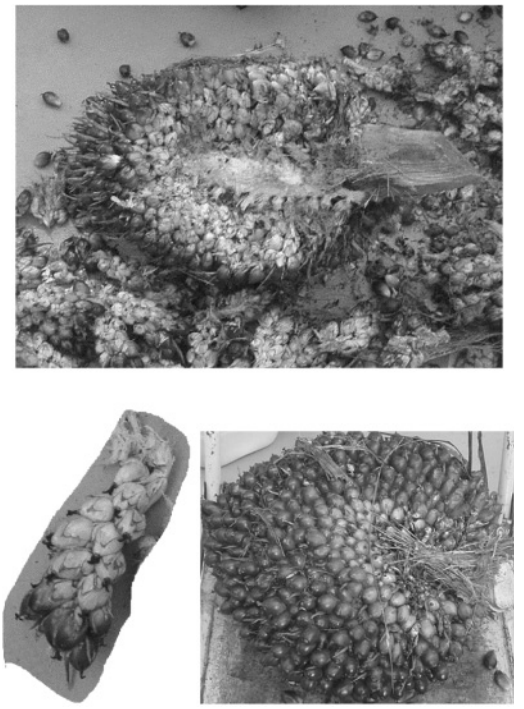


Figure 54.1. Palm fruit bunch.

TABLE 54.1. Fatty Acid Composition (%) of CPO and Fruits at Different Ages

Age of Fruit (weeks)	Myristic C14:0	Palmitic C16:	Palmito-Oleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3
4	0.99	43.0		5.79	16.92	25.7	6.0
6	0.8	38.9		5.9	18.9	23.1	11.9
7		35.6		4.8	18.9	25.8	14.1
9	0.8	34.6		4.5	18.0	25.2	17.5
11	1.2	32.2	2.1	4.6	16.9	23.8	17.5
12	0.6	38.9		5.1	18.4	25.5	10.8
13	0.8	37.2		5.2	22.0	24.4	9.8
14	0.5	39.6		4.9	32.6	19.3	2.7
16	1.0	40.8	0.3	5.5	37.6	14.1	***0.4***
18	1.4	42.6	0.3	5.8	36.5	12.9	***0.3***
20 (ripe)	1.7	44.9		4.5	39.0	***10.5***	***0.3***
21	1.3	44.7	0.1	4.7	39.3	***10.1***	
CPO	0.2	44.0	0.1	4.5	39.2	***10.1***	***0.4***

Source: Azis (1984).

Note: Shaded area indicates fatty acid composition of fruit as it ripens with peak at week 20.

consistently distinct fatty acid compositions of oil in the kernel and mesocarp (Table 54.1), respectively. The formation of storage of oil in the mesocarp occurs only after the 14th or 15th week of anthesis.

Morphologically, the fruit mesocarp is made up of many cells, vascular bundles, and fibers. Each cell consists of the cytoplasm, which contains numerous organelles.

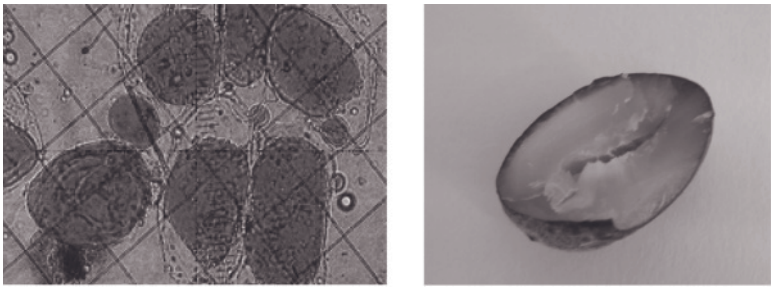


Figure 54.2. Palm mesocarp and palm kernel of the oil palm fruit.

TABLE 54.2. Ratio of Membrane to Lipid Storage of Developing Fruits

Age of Fruit (weeks)	Phospholipid (Membrane):Storage Oil
1	100:0
10	100:0
15	20:80
20	1:99

Source: Azis (1984).

TABLE 54.3. Intact and Undamaged Mesocarp Reaffirms that Storage Lipid is Pure Triglyceride

Triglyceride (Neutral Lipid) (%)	Diglyceride (%)	Monoglyceride (%)	Free Fatty Acid (%)	State of Fruit
99.8	—	—	0.025	Intact and undamaged
98	2	0.2	0.5	Very mildly damaged
91.43	4.3	1.7	4.7	Damaged
70.6	12.1	2.7	15.2	Extremely damaged

Source: Azis (1984).

Each organelle has its own specific function, and is prominent under the required circumstances. For example, chloroplasts, which help in photosynthesis and contribute toward the formation of fatty acids, are important during the developing stages. Storage oils (Fig. 54.2) of palm fruit mesocarp shows very distinct reductions in linolenic acid from the 14-week-old fruit of 2.7–0.3%, while for linoleic acid from 12.9% (18th week) to consistent 10.5% when the fruit is ripe (refer to Table 54.1). Both the kernel and mesocarp have cells that will accommodate the oil storage as oil globules.

The lipid of significance in palm, especially the fruits, are the storage lipids; triacylglycerols or triglycerides, and phospholipids for the membrane (Campbell et al. 1999). Most palm kernel and mesocarp lipids are storage lipids of triglycerides. Phospholipids are membrane lipids, and the ratio of membrane lipid to storage lipid of developing fruits is shown in Table 54.2. Storage oils in cells of both kernel and mesocarp are pure neutral lipids, that is, pure triglycerides (Table 54.3). Each

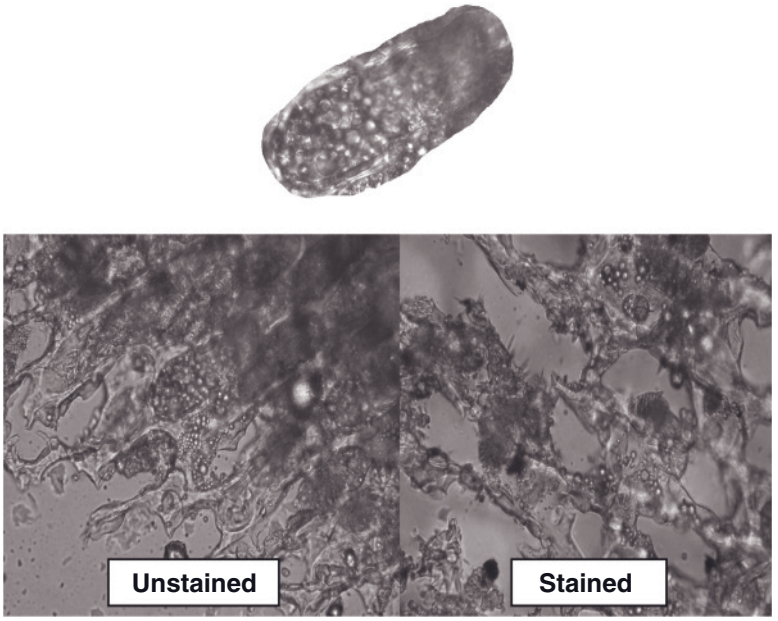


Figure 54.3. Oil globules in palm kernel cells (oil bodies unstained and stained with Sudan 3).

completely developed palm kernel cell contains many and separate oil bodies or globules (Figs. 54.3 and 54.4), and the mesocarp cells appear to contain a large single oil body (Figs. 54.5 and 54.6). Younger mesocarp cells, however, have many such oil bodies.

Extracted or isolated oils from either the mesocarp or kernel cells have been analyzed to contain triglycerides, diglycerides, monoglycerides, and free fatty acids. The composition is attributed to the catalytic reactions of endogenous and microbial enzymes on the triglycerides. Palm kernel oil is rich in saturated acid, with a saturated-to-unsaturated acid ratio of 80:20, while mesocarp oil or crude palm oil (CPO) has a balanced saturated-to-unsaturated ratio (50:50). Triglycerides constitute about 50% by weight of kernel, and the kernel in turn makes up 4–6.5% of fresh fruit bunch (FFB). In palm fruit mesocarp, the triglyceride content ranges from 25% to 30% on bunch weight. During the mill extraction process, some oil is lost. The inevitable physical damage to the fruits, and other reactions affecting the storage oil, leads to the reduction in the total triglyceride content, accompanied with parallel increases in mono- and diglycerides and free fatty acids.

PROCESSING OF OIL FROM PALM FRUITS

The palm oil is processed from bunches of palm fruits according to the following steps:

1. Extraction: pressing the oil from palm fruits, which is known as milling

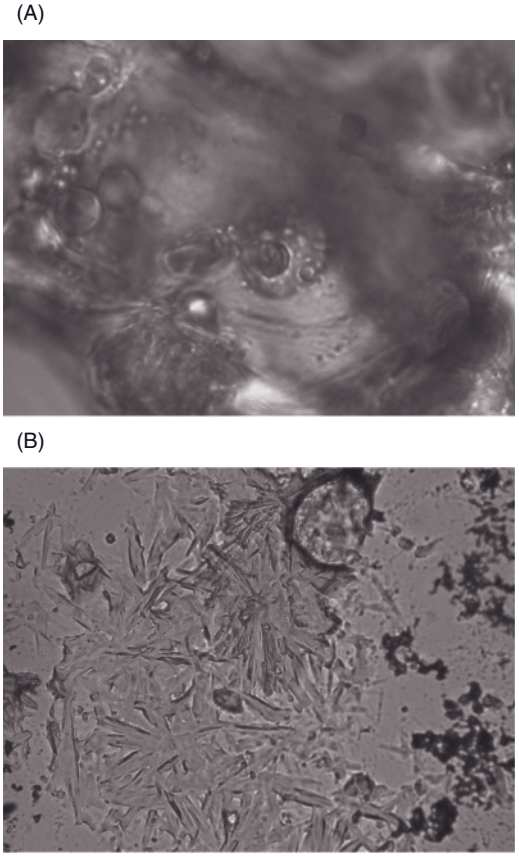
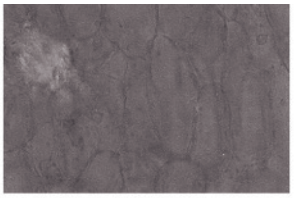


Figure 54.4. (A) Fluid globule storage oil of palm kernel cells. (B) Crystallized isolated storage oil. Both of the specimens observed at 24°C.

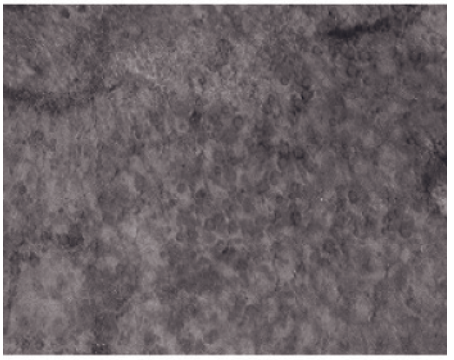
2. Refining: bleaching and deodorizing to remove colored materials and odoriferous substances
3. Fractionation: separation of the olein (liquid portion) from the stearin fraction (solid portion)

Milling

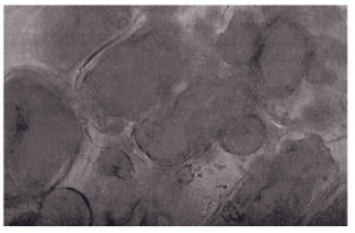
The ripened palm fruits are harvested as FFBs and transferred to the palm oil mill for immediate processing. Careful handling of the palm fruit bunch is important to prevent bruising. Bruising at any position on the fruits causes the free fatty acid to increase to 60% in a few minutes. Intact fruits have free fatty acid of less than 0.025%. The fruit bunches are first sterilized to inactivate the enzyme responsible for the formation of free fatty acid. Besides, it also assists in separating the fruits from the bunch. Sterilized FFBs are threshed to recover all the detached loose fruits. These fruits are pressed to recover the oil as pressed crude oil. Pressed crude oil contains oil, oil-soluble compounds, water, water-soluble compounds, and solid impurities. The oil is recovered via clarification and centrifugal separation. Also



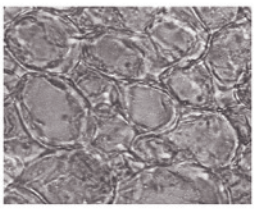
10-week-old mesocarp



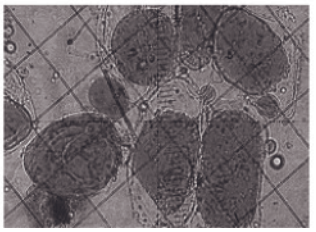
16-week-old mesocarp



Ripe mesocarp



Unstained



Stained

Figure 54.5. Oil globules in palm mesocarp cells (oil bodies unstained and stained with Sudan 3).

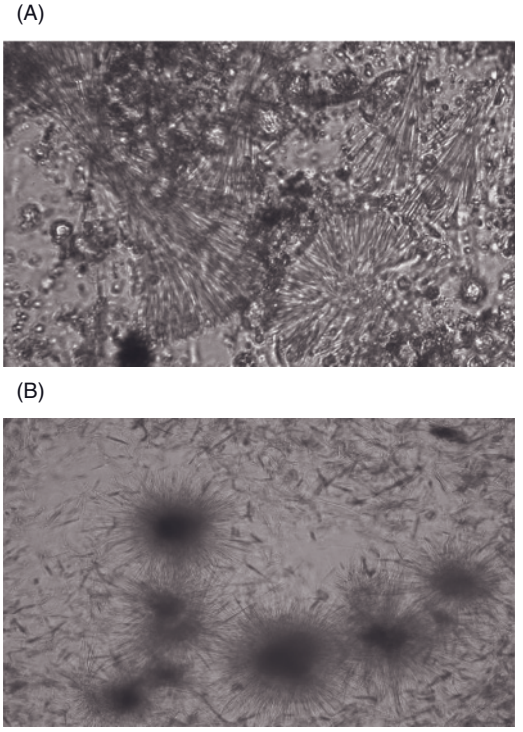


Figure 54.6. (A) Fluid globule storage oil of palm mesocarp cells. (B) Crystallized isolated storage oil. Both the specimens observed at 24°C. *Note*: Panel (B) shows the crystal structure of the saturated free fatty acids or highly saturated triglyceride in fluid oil or olein.

recovered are the important oil-soluble compounds, including carotene, tocopherols and squalene, phospholipids, and so on.

Refining

Refining of the CPO either involves degumming, neutralization (chemical refining), bleaching, deodorizing, and polishing, or simply degumming, bleaching, and deodorizing (physical refining). The physical refining technique is widely used in Malaysia.

The first step in this refining process is degumming, where the oil is treated with phosphoric acid to remove phosphatides, protein fragments, and gummy and mucilaginous substances. The oil is then bleached with 1–2% bleaching earth at 85°C. The bleaching earth is removed as spent earth residue. The eventual degummed and bleached oil is vacuum dried prior to being channeled into the deodorizer. Deodorization is where the oil is heated up to 180–260°C. At this stage, undesired odorous compounds and fatty acids are removed as palm fatty acid distillate.

Fractionation

Palm oil is a semisolid, made up of the saturated and unsaturated oil-rich glyceride fractions. Fractionation of palm oil leads to the separation of the liquid olein

TABLE 54.4. Fatty Acid Compositions of Palm Oil and Palm Kernel Oil (% of Total Fatty Acids)

Fatty Acid	RBD Palm Oil (RBDO)	Palm Kernel Oil (PKO)
6:0	—	0.3
8:0	—	4.2
10:0	—	3.7
12:0	0.2	48.3
14:0	1.1	15.6
16:0	44.0	7.5
18:0	4.5	1.8
18:1	39.2	14.8
18:2	10.1	2.6
18:3	0.4	—
20:0	0.4	—
Others	—	0.1

Source: Siew (2005).

from the solid stearin. Fractionation process allows the separation of olein from stearin.

There are three fractionation techniques:

1. Dry fractionation: palm oil is allowed to cool and is followed by filtration to separate the fractions
2. Detergent fractionation: partially crystallized palm oil is mixed with surface active agent (sodium lauryl sulfate), followed by centrifugation
3. Solvent fractionation: palm oil crystallizes in the presence of the solvent such as hexane, isopropyl alcohol, acetone, nitropropane, and so on, and is followed by filtration

The two fractions, olein and stearin, are used for different food applications because of the difference in their physical and chemical properties.

PALM OIL COMPOSITION

CPO appears as a deep reddish orange-colored viscous solution. The reddish color is attributed to carotene (500–700 ppm). At ambient temperature, it is semisolid, the solid crystalline form at the bottom and the liquid portion on top. Carotenes are discarded during the refining process. The fatty acid compositions of refined, bleached, and deodorized (RBD) palm oil and palm kernel oil are shown in Table 54.4 (Hall 1968; Sundram 2006). The triglyceride composition of palm oil is shown in Table 54.5 (Siew 2005). The high-molecular-weight tocopherols and sterols are generally not removed during the deodorization step.

Palm oil also contains natural antioxidants such as tocopherols (600–1000 ppm), of which the major types are α-, β-, and δ-tocopherols and tocotrienols (Siew 2005; Sundram 2006). Tocopherols make palm oil resistant to oxidative deterioration. The sterol content in palm oil is 300 ppm, of which 14% is cholesterol. The presence of

TABLE 54.5. Typical Triglycerides Composition of Palm Oil

Triglycerides	Composition (%)
LOL	0.3
PLL/MOL	2.2
MPL/MOM	0.7
OOL	1.4
POL	9.1
PLP/MOP	10.1
MPP	0.5
OOO	2.8
POO	21.5
POP	30.9
PPP	5.6
OOS/LSS	2.3
POS	5.1
PPS	1.0
SOS	0.5

Source: Siew (2005).

L, Linoleic; O, oleic; P, palmitic; M, myristic; S, stearic.

these natural antioxidants (α-, β-, and δ-tocopherols and tocotrienols) (Siew 2005; Sundram 2006) have increased the acceptability of palm oil in food. The total carotene content of CPO is 673 ppm, with the major carotenoid being α- and β-carotenes.

FLAVOR OF PALM OIL

Hall (1968) defines flavor as the sensation produced by a material taken in the mouth, perceived principally by the senses of taste and smell, and also by the general pain, tactile, and temperature receptors in the mouth. Vegetable oils and fats have distinct, characteristic flavors, which play an important role in the flavor of different foodstuffs. However, these accepted natural flavors may be masked by the development of off-flavors, resulting from processes such as hydrolysis and auto-oxidation. The true off-flavors of palm oils are, in most cases, caused by volatile components, and their main precursors are the fatty acid portions of the triglycerides, especially the unsaturated ones such as oleic acid, linoleic acid, linolenic acid, and arachidonic acid.

Crude vegetable oils have a natural protection against deterioration and frequently exhibit a characteristic flavor. After refining, much blander, odorless oil is obtained. However, even after deodorization, experts are able to discuss different types of oils such as soybean, sunflower, corn, canola, and so on, which means that small concentrations of volatiles are still present. The development of a characteristic odor in freshly refined oil is called "reversion." Flavor reversion takes place especially in unstable oil, containing linoleic or higher unsaturated fatty acids such as linseed oil (40–60% linolenic acid), soybean oil (5–9% linolenic acid), and rapeseed oil (about 15% linolenic acid) (Hoffmann 1960). It is clear that volatile

compounds play an important role in contributing to the flavors of vegetable oils either positively (natural flavor) or negatively (off-flavors, rancidity). These volatiles belong to the different classes of compounds: alkanes, alkenes, alkynes, aldehydes, ketones, alcohols, acids, esters, terpenes, lactones, and aromatic hydrocarbons. The flavors of these volatiles can be described as painty, trainy, fishy, tallowy, beany, nutty, musty, cardboardy, grassy, green, and so on.

The volatile composition of vegetable oils has been studied by several authors. One of the most widely studied vegetable oil volatiles is soybean oil (Chang et al. 1961, 1966; Feenstra and Meijboom 1971; Ho et al. 1978; Hoffmann 1960, 1961a,b, 1966; Keppler et al. 1965; Meijboom et al. 1970; Seals and Hammond 1966, 1970; Sessa and Plattner 1979; Smagula et al. 1979; Smouse 1966; Smouse and Chang 1967; Smouse et al. 1965). Studies had also been carried on other oils such as olive oil (Flath et al. 1973; Olias et al. 1977, 1978), coconut oil (Allen 1965; Pai et al. 1979), and corn oil (Kavada et al. 1967; Krishnamurthy and Chang 1967; Swoboda and Lea 1965). Study on the volatile composition of palm oil was carried out by several people. The unsaturated fatty acids of palm oil are oleic, linoleic, and linolenic acids. The possible volatiles that can arise from these fatty acids are shown in Table 54.6. *n*-Octanal and *n*-nonanal are the two major aldehydes from oleic acid, while the major aldehydes for linolenic acid are hexanal, followed by 2-octenal, 2-heptenal, 2-octenal, and 2,4–decadienal.

Gaddis and others (1961) performed paper chromatography of the 2,4-dinitrophenylhydrazine (2,4-DNPH)-derivatives and isolated $C_{2,3,6,8,9}$ alkanals, C_{5-11}

TABLE 54.6. The Possible Hydroperoxides and Secondary Autoxidation Products in Unsaturated Fatty Acid

Fatty Acids	Isomeric Hydroperoxides	Expected Aldehydes
Oleic acid	8-OOH	*n*-Decanal, 2*c*-undecenal
	9-OOH	*n*-Nonanal, 2*t*-decenal
	10-OOH	*n*-Nonanal
	11-OOH	*n*-Octanal
Linoleic acid	8-OOH	2,5-Undecadienal, 3-nonenal
	9-OOH	2,4-Decadienal
	10-OOH	3-Nonenal
	11-OOH	2-Octenal
	12-OOH	2-Heptenal
	13-OOH	*n*-Hexanal
	14-OOH	*n*-Pentanal
Linolenic acid	8-OOH	2,5,8-Undecatrienal
	9-OOH	2,4,7-Decatrienal
	10-OOH	3,6-Nonadietnal
	11-OOH	2,5-Octadienal
	12-OOH	2,4-Heptadienal, 3-hexenal
	13-OOH	3-Hexenal
	14-OOH	2-Pentanal
	15-OOH	2-Butenal
	16-OOH	*n*-Propanal
	17-OOH	Acetaldehyde

Source: Badings (1960), and Ho and Shahidi (2005).

alkenals, $C_{7,9,10}$ alka-2,4-dienals in unheated palm oil. In heated palm oil, he further isolated $C_{7,8,9,11}$ alkanals, $C_{7–12}$ alken-2-enals, and $C_{7,9,10,11}$ alka-2,4-dienals. Also, Badings (1960) identified 2-undecanone in palm oil by derivatization with 2,4-dinitrophenylhydrazine. Hoffmann and Keppler (1960) determined the stereo configuration of the 2,4-decadienals in palm oil and groundnut oil as that of *trans*-2,*cis*-4 and *trans*-2,*trans*-4-decadienal. In bleached palm oil, γ- and δ-lactones were present at concentrations of less than 1 ppm (Van der Ven and De Jong 1970). Dirinck and others (1977) identified $C_{5–9}$ alkanals, $C_{7,9,11}$ alk-2-enals, $C_{7,9,10}$ alka-2,4-dienals, $C_{7,9}$ alka-2-ones, α- and β-ionones, and δ-heptalactone in the steam distillate of palm oil. Aromagrams showed the following odors: green odors (*n*-hexanal), oily, fatty odors (*n*-heptanal), odors of oxidized burnt fat, frying odors (deca-2,4-dienal isomers), and musty odors (butyl acetate and β-ionone). Miura and others (1980) fractionated palm oil by vacuum distillation and saponification. They found that the carbonyl free fractions had a distinct palm oil-like flavor, and from heated β-carotene they found eight fractions that had a palm oil-like taste.

Volatile compounds found in palm oil contribute to the flavor of the oil. Kuntom (1982) carried out studies using headspace analysis of palm oil products. The chromatographic volatile profile of CPO is shown in Figure 54.7 (Dirinck et al. 1983, 1984; Kuntom 1982). The volatiles identified were *n*-acetaldehyde, 2-butenal, *n*-butanal, 3-methylbutanal, 2-methylbutanal, 2-pentanone, *n*-pentanal, 4-methyl-2-pentanone, *trans*-2-pentenal, *n*-hexanal, 2-heptanone, *n*-heptanal, benzaldehyde, *n*-octanal, and *n*-nonanal. The threshold values of some of the aldehydes and ketones due to degradation of the fatty acid components of the oil are shown in Table 54.7.

Headspace analysis of volatiles extracted from samples of CPO collected at the port in Malaysia and on arrival at the port in England showed the presence of such volatiles as aldehydes, butanal, methylbutanal, pentanal, 2-pentenal, hexanal, 2-hexenal, benzaldehyde, *n*-octanal, and *n*-nonanal (Figs. 54.8 and 54.9). The amount of the headspace volatiles in CPO shipped at the port in Malaysia with peroxide value of 1.50 and anisidine value of 1.19, had on arrival in England higher peroxide value (6.38) and anisidine value (4.23) as shown in Table 54.8. The amount of

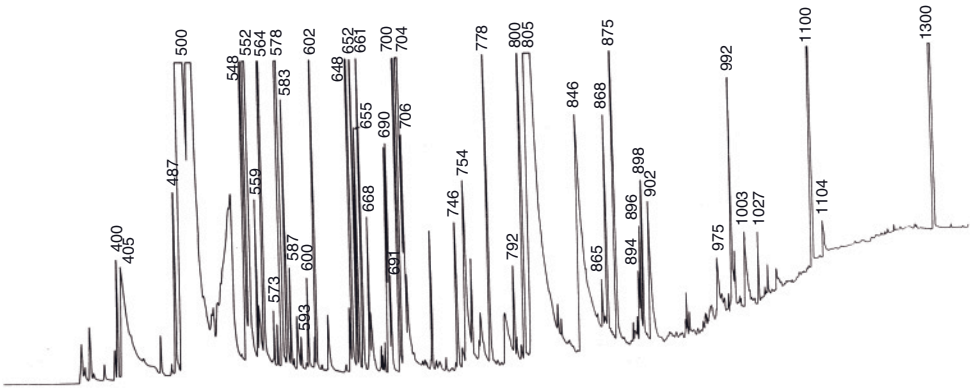


Figure 54.7. Chromatogram of volatile compounds that contribute to palm oil flavor. 405: acetaldehyde; 559: 2-butenal; 578: *n*-butanal; 648: 3-methylbutanal; 661: 2-methylbutanal; 691: 2-pentanone; 704: *n*-pentanal; 746: 4-methyl-2-pentanone; 754: *trans*-2-pentenal; 805: *n*-hexanal; 894: 2-heptanone; 902: *n*-heptanal; 975: benzaldehyde; 1003: *n*-octanal.

TABLE 54.7. Odor Threshold of Some Volatile Aldehydes and Ketones in Oil and Air

Compounds	Odor Threshold in Oil (μg/kg)[a]	Odor Threshold in Air (mg/kg)[b]
Acetaldehyde	0.22	0.041
n-Propanal	—	0.02
n-Butanal	—	0.042
n-Pentanal	240	0.072
n-Hexanal	320	0.039
n-Heptanal	3200	0.033
n-Octanal	55	0.015
n-Nonanal	13,500	0.03
2-Butenal	—	0.42
2-Pentenal	2300	—
2-Hexenal	—	0.034
2*t*,4*c*-Decadienal	10	—
2*t*,4*t*-Decadienal	180	—
2-Butanone	—	63–70[c]
2-Pentanone	—	350[d]
2-Heptanone	—	0.84
2-Nonanone	—	0.075

Source: [a]Belitz and others (2004b); [b]Teranishi and others (1974); [c]Backmann (1917); [d]Laffert (1960).

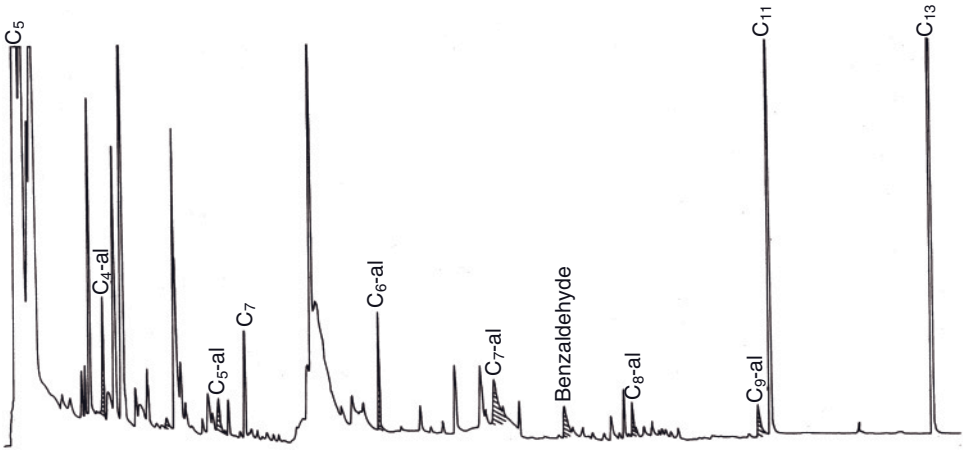


Figure 54.8. Chromatogram of volatile compounds of crude palm oil shipped at the port in Malaysia.

volatiles increased during shipment, and there was also the development of new volatile *n*-nonanal. Hexanal, for example, at 0.08 and 1.16 mg/kg in the CPO are higher than the threshold value of 320 μg/kg. Other aldehydes too were found to be higher in CPO on arrival at port in England than at the point of shipment in Malaysia. The headspace volatiles of chemically refined palm oil are less than the physically refined palm oil as shown in Figures 54.10–54.13, but the resultant refined products from both processes are bland to the taste buds. Hence, physical refining is just as efficient as chemical refining in removing the volatiles that contribute to the flavor.

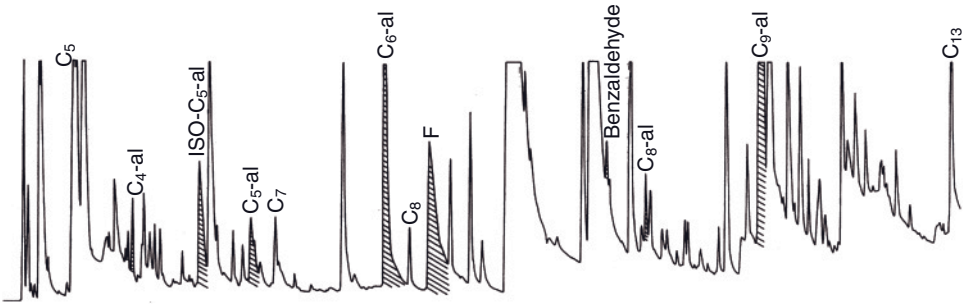


Figure 54.9. Chromatogram of volatile compounds of crude palm oil on arrival at the port in England.

TABLE 54.8. Headspace Volatiles of Crude Palm Oil Shipped at the Port in Malaysia and on Arrival at the Port in England

	Concentration of Headspace Volatiles (mg/kg)	
	CPO Shipped at Port in Malaysia	CPO on Arrival at Port in England
Volatiles	*PV—1.50*; *AV—1.19*	*PV—6.38*; *AV—4.23*
n-Butanal	0.16	0.20
Methylbutanal	—	0.61
n-Pentanal	0.04	0.24
2-Pentenal	—	—
n-Hexanal	0.08	1.16
2-Hexenal	—	0.28
Benzaldehyde	0.05	0.35
n-Octanal	0.02	0.29
n-Nonanal	—	4.83

Source: Kuntom (1982).

PV, peroxide value; AV, anisidine value.

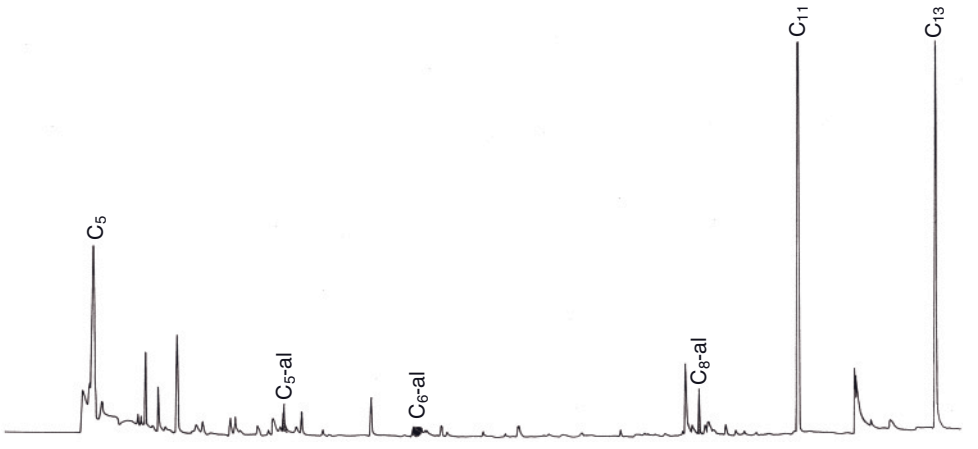


Figure 54.10. Chromatogram of volatile compounds of physically refined palm oil shipped at the port in Malaysia.

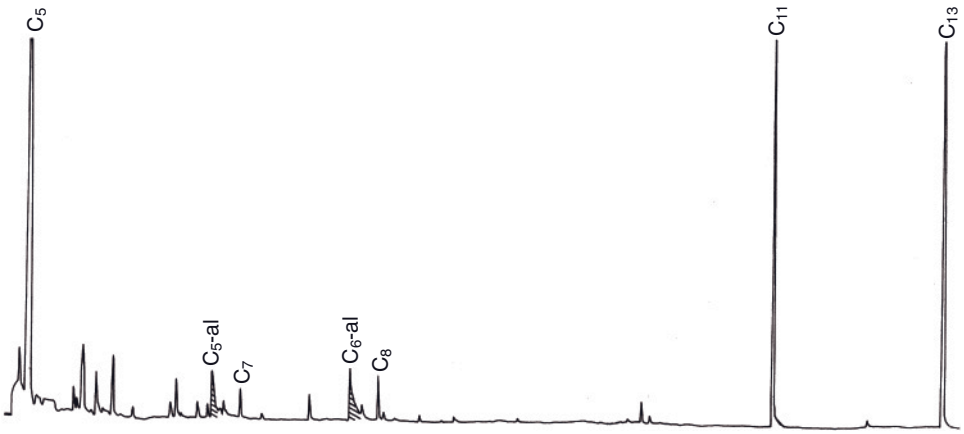


Figure 54.11. Chromatogram of volatile compounds of chemically refined palm oil shipped at the port in Malaysia.

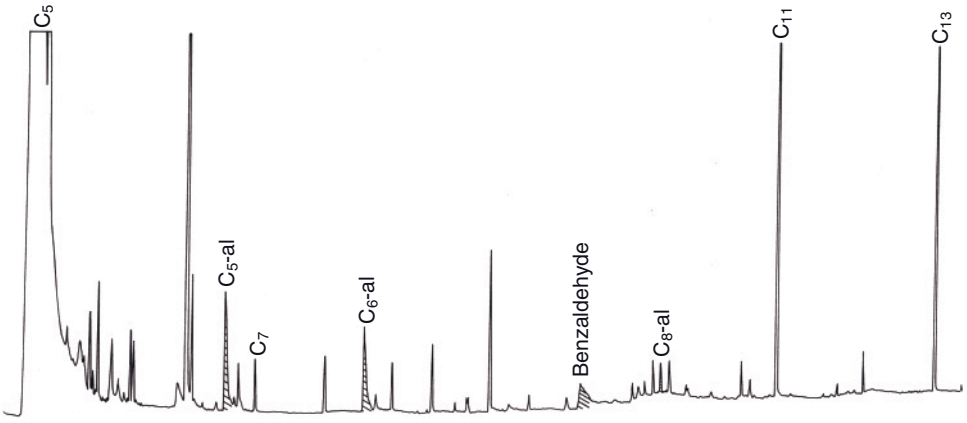


Figure 54.12. Chromatogram of volatile compounds of physically refined palm oil on arrival at the port in England.

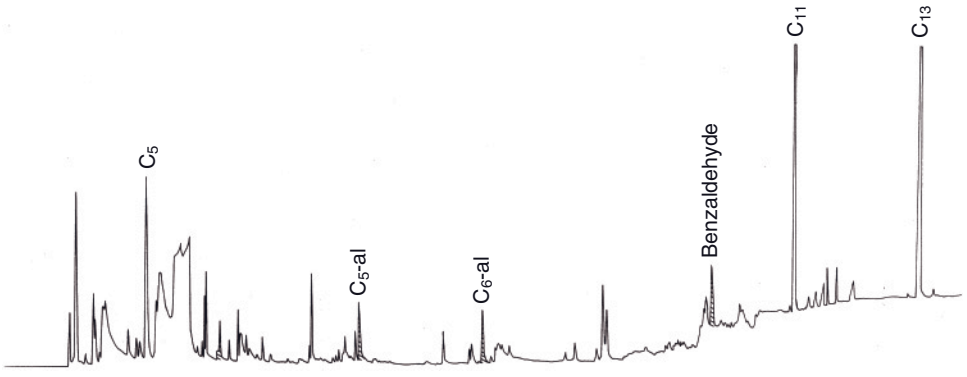


Figure 54.13. Chromatogram of volatile compounds of chemically refined palm oil on arrival at the port in England.

In a different study, Kuntom (1982), Dirinck and others (1983), and Belitz and others (2004b) established a correlation between volatiles developed in oxidized palm oil and sensory evaluation for five types of palm oil products for 15 days. During the oxidation process, the volatile aldehydes monitored were butanal, pentanal, 2-pentenal, hexanal, 2-hexenal, *n*-heptanal, *n*-octanal, and *n*-nonanal. The concentration of these aldehydes was combined as total aldehydes. Figure 54.14 shows the flavor intensity score of the five types of palm oil products ranked (on a scale of 1–7) as a function of oxidation time. The ranking was according to rancidity, fatty-oily, and natural palm oil flavor. There was an increase in the rancid flavor intensity with oxidation time for all types of palm oil products. The increase was linear for CPO and exponentially for other palm oil products. For the CPO, a noticeable flavor was observed and this was attributed as the character of palm oil and defined as palm oil-like flavor. CPO was observed to have the fastest development of rancid off-flavors.

In a separate study, Kuntom and others (1989) analyzed the volatiles from steam distillate of CPO using gas chromatography in tandem with mass spectrometry. Aroma of the compounds present in the steam distillate was characterized by sniffing the eluting products from the column at the sniffing port of the gas chromatograph. Figures 54.15 and 54.16 show the chromatogram and aromagram of the steam distillate, respectively. An empirical relationship was observed between the aromagram peaks and chromatographic peaks. The palm oil-like flavor was found to be the area corresponding to aldehydes, ketones, and terpenes such as *n*-nonanal, 2,2,6-trimethylcyclohexanone, 3,3,5-trimethylhex-2-enone, *n*-nonanone, linalool, *trans*-allo-ocimene, and cyclocitral. The compound 3,3,5-trimethylhex-2-enone had a distinct nutty, palm aroma. A few peaks suspected to be mono-oxygenated terpenes were not positively identified. Likewise, ionol (2,6-ditertiarybutyl-4-methylphenol) was also detected. Besides the compounds that contributed to the palm oil aroma, other compounds identified were *n*-pentanol, *n*-hexanal, and 2-methyl-2-hepten-6-one. The oxidized palm oil showed the aldehydes and ketones generally identified in oxidized oil such as $C_{4–10}$ aliphatic aldehydes, $C_{6–9}$ unsaturated trans-2-alkenals, *trans*-2-nonenone; *trans*,*cis*-2,4-decadienal; *trans*,*trans*-2,4-decadienal and *trans*,*cis*-2,4-hexadienal, $C_{5,9,10}$ 2-alkanones; 2,2,6-trimethylcyclohexanone; oct-3-en-2-one and γ-heptalactone.

Volatile aroma compounds identified when French fries were deep fried in palm were methanethiol, methional, methylpropanal, 2-methylbutanal, *trans*-4, 5-epoxy-(*E*)-2-decenal, 3-methylbutanal, (*E*,*Z*)-2,4-decadienal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2,3-diethyl-5-methylpyrazine, (*E*,*E*)-2,4-decadienal, 2,3-butanedione, 2-ethyl-3,5-dimethylpyrazine, 2-ethenyl-3-ethyl-5-methylpyrazine, 3-isobutyl-2-methoxypyrazine, and 2-ethyl-3,6-dimethylpyrazine (Belitz et al. 2004a). Omar and others (2006) carried out a study on the changes of aroma constituents of palm olein after frying French fries (potato chips). The volatile constituents, extracted by means of the solid phase microextraction (SPME) headspace technique, were analyzed using gas chromatography in tandem with mass spectrometry. Hexanal was identified as the compound responsible for the off-odor (rancid) and was found to increase with frying time, while 2*t*,4*t*-decadienal, the flavor contributing compound of the French fries, decreased when the frying time was prolonged up to 40 h. The fried product was evaluated by the sensory panel, and preference was for French fries with higher 2*t*,4*t*-decadienal content and a lower hexanal content.

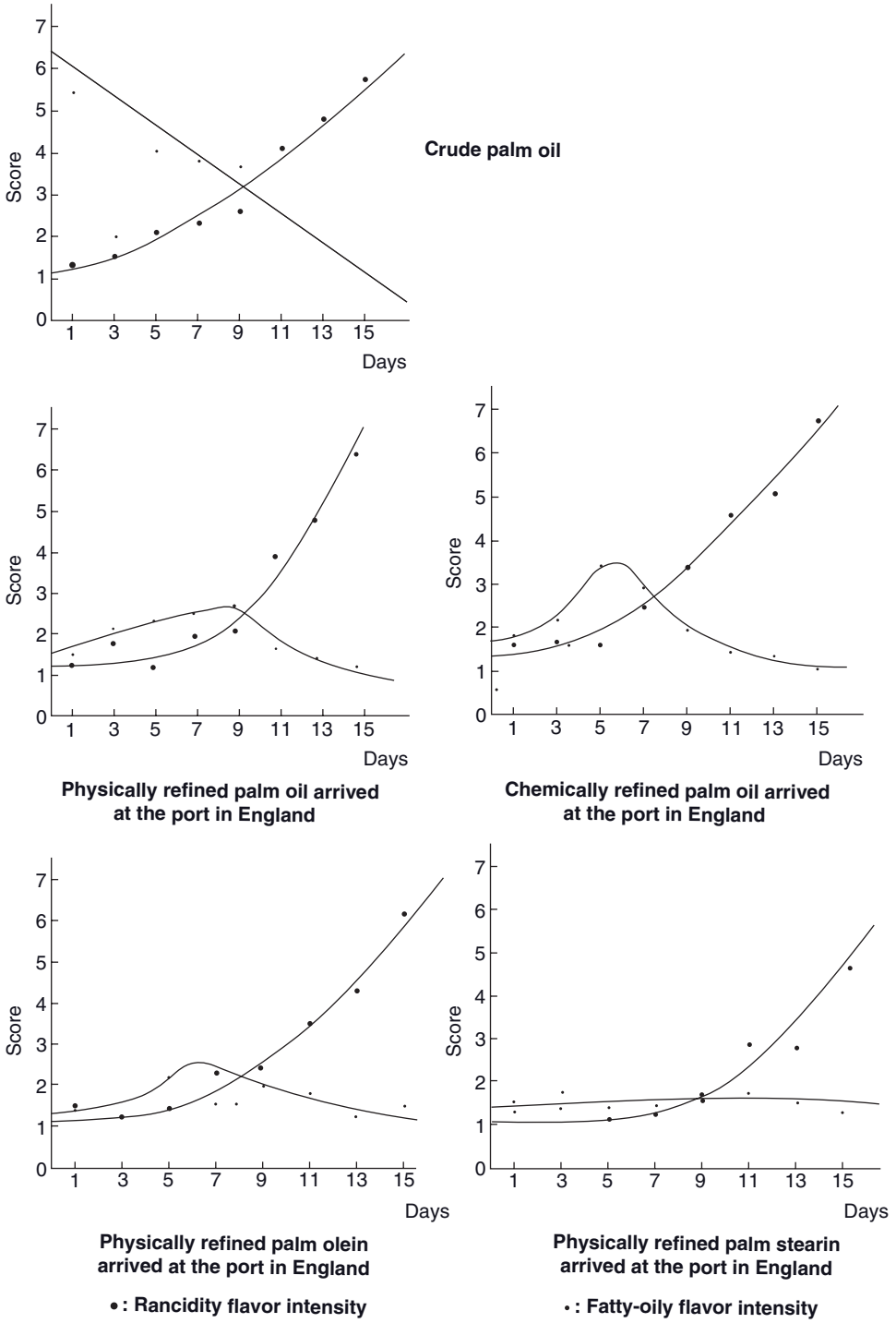


Figure 54.14. Flavor intensity score versus oxidation time for five different types of palm oil products.

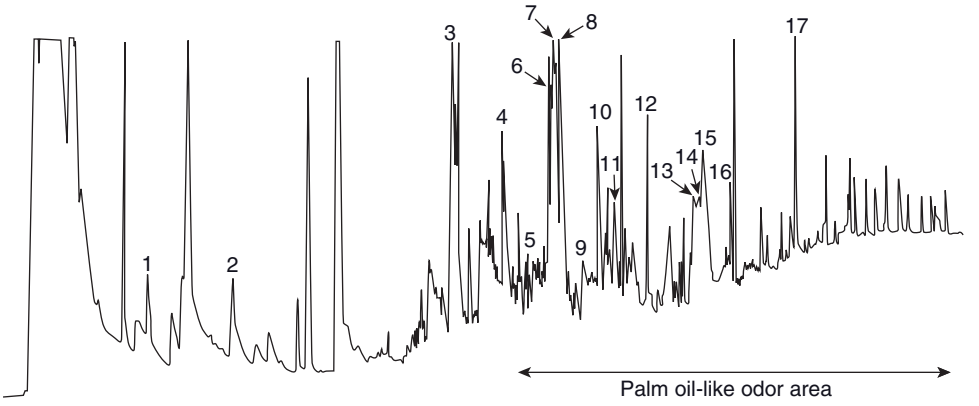


Figure 54.15. Chromatogram of flavor volatiles from steam distillation of crude palm oil. 1: *n*-pentanal; 2: *n*-hexanal; 3: 2-methyl-2-hepten-6-one and 2-pentylfuran; 4: 2,2,6-trimethylcyclohexanone; 5: 3,5,5-trimethylcyclohexen-2-one; 6: 2-nonanone; 7: *n*-nonanal; 8: linalool; 9: *trans*-allo-ocimene; 10: unidentified (mol wt 154); 11: ethyl benzoate; 12: β-cyclocitral; 13: unidentified (mol wt 156); 14: unidentified (mol wt 152); 15: unidentified (mol wt 152); 16: unidentified (mol wt 152); 17: ionol.

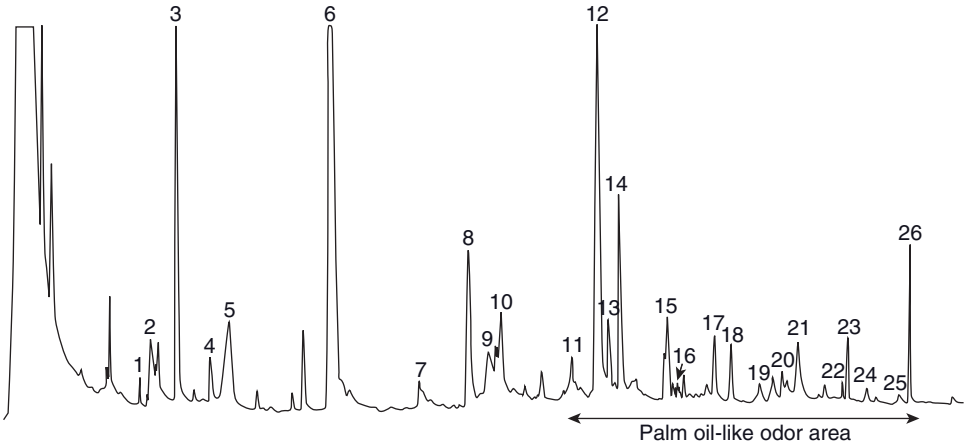


Figure 54.16. Aromagram of volatiles from steam distillation of crude palm oil. 1: sweet, floral; 2: perfumery; 3: aldehyde; 4: green, grassy; 5: caramel; 6: aldehyde; 7: green, aldehyde; 8: sweet, fruity, mushroom; 9: green, aldehyde; 10: metallic; 11: palm oil, nutty; 12: benzaldehyde (musty); 13: aldehyde, green; 14: leather, heptanal, fishy; 15: palm oil, nutty; 16: painty; 17: nutty, green, lemon-like; 18: painty; 19: terpene; 20: pears, tomatoes; 21: palm oil; 22: painty; 23: palm oil; 24: oily; 25: floral; 26: green.

The main fatty acids of palm oil are palmitic acid and oleic acid, with a small amount of linoleic acid; hence, the volatile of palm oil will be the degraded or auto-oxidized products from these fatty acids. The presence of tocopherols and tocotrienols protect or reduce the unsaturated fatty acids from auto-oxidation. The flavor of CPO is a combination of volatiles from the unsaturated fatty acids and the terpenes. During transportation of CPO to the importing countries, there is an increase

in the volatile content due to the auto-oxidation of the fatty acids. The process of refining removes the volatile aroma flavor compounds, and the resultant oil is bland and oily to the taste. However, when RBD palm oil is transported to the importing countries, there is a new buildup of a small amount of the volatile compounds. Heated palm oil behaves in the same manner as other fats and oils, with the production of *n*-hexanal, *n*-nonanal, and 2,4-decadienal.

ACKNOWLEDGMENTS

I would like to thank Y.Bhg. Dato' Dr. Mohd Basri Wahid, director general of the Malaysian Palm Oil Board, and Y.Bhg. Dato' Dr. Choo Yuen May, deputy director general of the Malaysian Palm Oil Board, for allowing me to contribute this chapter.

REFERENCES

Allen RR. 1965. *Chem Ind* 36:1560.

Arnott GW. 1959. Malaysian Palm Oil and the Analysis of Product. Department of Agriculture, Federation of Malaya, (Division of Agriculture No. 13).

Azis AA. 1984. The biochemical aspects of ripeness standards. Proceedings of the (1984) Symposium on Impact of the Pollinating Weevil on the Malaysian Oil Palm Industry, pp. 165–76, February 22, 2009, Kuala Lumpur, Malaysia.

Backmann EL. 1917. *Uppsala Laekareforen Forh* 22:319–470.

Badings HT. 1960. *Neth Milk Dairy J* 14:215.

Belitz HD, Grosch W, Schiebe P. 2004a. Aroma compounds. In *Food Chemistry*, 3rd Ed. Berlin: Springer, p. 361.

Belitz HD, Grosch W, Schiebe P. 2004b. Lipids. In *Food Chemistry*, 3rd Ed. Berlin: Springer, p. 202.

Campbell NA, Lawrence GM, Jane BR. 1999. *Biology: Concepts and Connections*, 3rd Ed. Menlo Park, CA: Benjamin/Cummings Publishing.

Chang SS, Brobst KM, Tai A, Ireland CE. 1961. *J Am Oil Chem Soc* 38:671–4.

Chang SS, Krishnamurthy RG, Mookherjee BD, Reddy BK. 1966. *Chem Ind* 46:1926–7.

Dirinck P, Kuntom A, Schamp N. 1983. Investigation of the oxidation deterioration of palm oil by aroma analysis. Proceedings of the Second European Conference on Food Chemistry, March 15–18, 1983, Rome, Italy.

Dirinck P, Kuntom A, Schamp N. 1984. Oxidative dynamic headspace analysis for objective measurement of rancidity in oils. In Adda J (ed.), *Progress in Flavour Research*. Amsterdam: Elsevier Science Publishing, pp. 505–13.

Dirinck P, Schreyen L, De Schoenmaeker L, Wychuyse F, Schamp N. 1977. *J Food Sci* 42(3):645–8.

Feenstra WH, Meijboom PW. 1971. *J Am Oil Chem Soc* 48:387–90.

Flath RA, Forrey RR, Guadagni DG. 1973. *J Agric Food Chem* 21(6):948–52.

Gaddis AM, Ellis R, Currie GT. 1961. *J Am Oil Chem Soc* 38:371–5.

Hall RL. 1968. *Food Technol* 22:1388.

Hartley CWS. 1977. *The Oil Palm*, 2nd Ed. New York: Longman, pp.67–9, 690–703.

Ho CT, Shahidi F. 2005. Flavors component of fats and oils. In Shahidi F (ed.), *Bailey's Industrial Oil and Fats Products*, 6th Ed. New York: John Wiley & Sons, pp. 391.

Ho CT, Smagula MS, Chang SS.1978. *J Am Oil Chem Soc* 55:233–7.

Hoffmann G. 1960. Symposium on foods: Lipid and their oxidation. In Schultz HW, Day EA, Sinnhuber RO (eds.), *Vegetable Oils*. Westport, CT: AVI Publishing, p. 215G.

Hoffmann G. 1961a. *J Am Oil Chem Soc* 38:1–3.

Hoffmann G. 1961b. *J Am Oil Chem Soc* 38:31–2.

Hoffmann G. 1966. *J Am Oil Chem Soc* 39:439–44.

Hoffmann G, Keppler JG. 1960. *Nature* 125:310–11.

Kavada T, Krishnamurthy RG, Moorkherjee BD, Chang SS. 1967. *J Am Oil Chem Soc* 44:131–5.

Keppler JG, Schols JA, Feenstra WH, Meijboom PW. 1965. *J Am Oil Chem Soc* 42:246–9.

Krishnamurthy RG, Chang SS. 1967. *J Am Oil Chem Soc* 44:136–40.

Kuntom A. 1982. Investigation of the oxidative deterioration of palm oil by aroma analysis. PhD thesis, Laboratory of Organic Science, Faculty of Agriculture, State University of Ghent, Belgium.

Kuntom A, Dirinck P, Schamp N. 1989. *Elaeis* 1(1):53–61.

Laffert P. 1960. *Some New Data on Physico-Chemical Determinations of Theories of Odour and Odour Measurement*. Bebek, Istanbul: Robert College, pp. 247–70.

Malaysian Palm Oil Board (MPOB). 2009. Malaysian Oil Palm Statistics, 2008, 28th Edition, MPOB Publication, February 2009, pp. 4, 50.

Meijboom PW, Jongenotter GA, Maarleveld A. 1970. *J Am Oil Chem Soc* 47:414.

Miura K, Usuki R, Kaneda T. 1980. *Yukagaku* 29(2):97–101.

Olias JM, del Barrio A, Gutierrez R. 1977. *Grasas Aceites* 28(2):102–12.

Olias JM, Dobargares Garcia MC, Gutierrez F, Rosales R, Gutierrez R. 1978. *Grasas Aceites* 29(3):211–18.

Omar MN, Idris NA, Kuntom A. 2006. Changes of aroma volatile constituents of palm olein after frying of French fries. 97th AOCS Annual Meeting and Expo, April 30–May 3, America's Center, St. Louis, MO.

Oo KC, Lee KB, Ong ASH. 1986. *Phytochemistry* 25:405–7.

Oo KC, Teh SK, Khor HT, Ong ASH. 1985. *Lipids* 20:205–10.

Pai JS, Lomanno SS, Nawar WW. 1979. *J Am Oil Chem Soc* 56:494–7.

Sambathamurthi R, Sundram K, Tan YA. 2000. *Prog Lipid Res* 39:507–58.

Seals RG, Hammond EG. 1966. *J Am Oil Chem Soc* 43:401.

Seals RG, Hammond EG. 1970. *J Am Oil Chem Soc* 47:278.

Sessa DJ, Plattner RD. 1979. *J Agric Food Chem* 27(1):209.

Siew WL. 2005. Chemical and physical characteristic of palm and palm kernel oils and their crystallization behaviour. In *Selected Readings of 26th Palm Oil Familiarization Programme, Malaysian Oil Palm Products, Palm Oil—The Golden Gift—Your Natural Choice*. Bangi, Malaysia: Malaysian Palm Oil Board Publication, pp. 294–311.

Smagula MS, Ho CT, Chang SS. 1979. *J Am Oil Chem Soc* 56:516–19.

Smouse TH. 1966. Isolation and identification of chemical components in reversion flavor of soybean oil. Dissertation Abstr., 26:7016 order no. 16–1205.

Smouse TH, Chang SS. 1967. *J Am Oil Chem Soc* 44:509–14.

Smouse TH, Mookherjee BD, Chang SS. 1965. *Chem Ind* 29:1301–3.

Sundram K. 2006. Nutritional properties of palm oil and its fractions: A review. In *Selected Readings of 26th Palm Oil Familiarization Programme, Malaysian Oil Palm Products, Your Choice of Sustainable Resource*. Bangi, Malaysia: Malaysian Palm Oil Board Publication, pp. 160.

Swoboda PAT, Lea CH. 1965. *J Sci Food Agric* 16:680–9.

Teranishi R, Buttery RG, Guadagni DG. 1974. *Ann N Y Acad Sci* 237:209–16.

Thomas RL, Sew P, Mok CK, Chan KW, Essau PT, Ng SB. 1971. *Ann Bot* 35:1219–25.

Van der Ven B, De Jong K. 1970. *J Am Oil Chem Soc* 47:534–8.

CHAPTER 55

Sesame Oil

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INTRODUCTION

Sesame is an ancient oilseed, first recorded as a crop in Babylon and Assyria over 4000 years ago. Sesame is widely cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein. Sesame has different names according to the region of production (Kanu et al. 2007; Salunkhe et al. 1991); in some areas it is known as sesamum (China, Mexico, South and Central America), gingelly (South India, Burma), benniseed (Sierra Leone, Guinea in West Africa), sim-sim (Middle East), and till (East and North Africa). China, India, Sudan, Mexico, and Burma are the major producers of sesame seeds in the world, contributing to approximately 60% of its total world production (USDA 2004).

The chemical composition shows that the sesame seed is an important source of oil (54–60%) and protein (25%). Sesame oil is considered to be a health-promoting food, owing to the fact that it contains a higher proportion of monounsaturated fatty acids than saturated ones, dominated by oleic (C18:1) and linoleic (C18:2). The fatty acid composition of sesame oil has been reported by Elleuch and others (2007) as follows: oleic acid, 44.06%; linoleic acid, 35.56%; palmitic acid, 11.18%; and stearic acid, 6.40%. Sesame oil contains very useful phytochemicals such as resveratrol, flavonoids, tocopherol, ethyl protocatechuate, phytosterols, lectins, sesamin, sesamolin, and others that could be utilized as functional ingredients (Kanu et al. 2007).

Dark sesame oil, which is made by roasting sesame seeds under selected conditions, before cold pressing, is one of the highly regarded specialty oils, particularly in the Orient. The resultant oils present delicate, but definite, flavors (Jung et al. 1997) and are known as being resistant to oxidative deterioration. Furthermore, sesame oil prepared from roasted sesame seeds has an extended shelf life (Fukuda et al. 1986).

Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui

ANALYTICAL IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS (VOCs)

Analytical instrumentation for detection and identification of VOCs has become smaller, more reliable, and increasingly sensitive. Gas chromatography (GC) tools have undergone important improvements such as development of open tubular columns with bonded stationary-phase material, providing improved chromatography and decreased fragility compared with packed-column GC. Mass spectrometry (MS) hardware for electron impact (EI) MS has grown smaller and increasingly sensitive. According to Hook and others (2002), while these technological improvements in hardware have made GC-MS analysis possible, sampling and sample preparation methods continue to rely on proven reliable techniques. These traditional methods do not easily support combined rapid sampling and analysis carried out completely. The major methods for preparing a sample for analysis are liquid-liquid, gas-liquid, solvent, gas-phase, and supercritical fluid extractions. These preparation methods are time consuming and require the use of additional analytical equipment and hazardous materials. Furthermore, changes in environmental regulations place increasingly severe restrictions on solvent use in laboratories worldwide.

According to Havenga and Rohwer (1999), the technique of solid phase microextraction (SPME) was introduced by Pawliszyn in 1989 and has shown advantages such as solvent-free extraction, relatively short analysis turnover time, and possibilities for automation. SPME is a technique that combines sampling, extraction, concentration, and instrument introduction into a single step, eliminating complicated sample preparation methods described previously. SPME passively extracts organic compounds and concentrates them onto a thin, fused-silica fiber coated with a stationary-phase material. Headspace sampling with SPME is limited to substances with sufficient vapor pressure, especially when sampling is performed at room temperature.

There are three different extraction modes for SPME—direct, headspace, and membrane. In the direct mode, the fiber is placed in the water or air sample and the analytes are adsorbed onto or absorbed into the fiber coating directly from the sample matrix. In the headspace mode, a sample of soil or water is placed in a vial. The SPME fiber is placed in the air directly above the water or soil, and analytes partition from the sample matrix through the air to the fiber coating. The air in the vial serves as a barrier between the SPME fiber and the sample matrix to protect the SPME fiber and eliminate fouling by high-molecular-weight compounds and other nonvolatile interferences in the sample media. The third mode uses a membrane to protect the SPME fiber from heavily contaminated samples that may damage the fiber (Havenga and Rohwer 1999).

However, SPME, as any method, has limitations, including the selectivity of each fiber, the competition for linkage sites, and it has its mechanism based in physicochemical equilibrium of vapor and liquid phases, which generates particular partition constants depending on the structures of the compounds. Ikeda and others (2006) have combined in their work simultaneous distillation-extraction (SDE) or SPME with GC-olfactometry and to extract odorants from sesame and signalize first the differences in the results, and second the importance of the application of emerging techniques such as the retronasal aroma simulation (RAS) device, which is known to produce a measurable flavor release similar to that of the mouth and

to the solvent-assisted flavor evaporation (SAFE) method which allows careful isolation of volatiles and full characterization of all aroma-active components.

Formation and Composition of Volatile Fraction

Most of the flavor compounds in fats and oils are produced by the reaction of oxygen with unsaturated fatty acids in tricylglycerols or polar lipids. Hexanal, octanal, and nonanal are the primary oxidation products. On the other hand, some flavor compounds present in roasted sesame oil (2-acetyl-1-pyrroline and 2-furfurylthiol) are generated by the interaction of reducing sugars with amino compounds during thermal processing. 2-Acetyl-1-pyrroline has been also established as a key flavor compound in basmati rice, wheat bread crust, popcorn, and cooked sweets. Investigations on the precursors of those aroma compounds in wheat bread crust and bakers yeast by Schieberle (1991) have revealed the amino acid ornithine as a very specific precursor of 2-acetyl-1-pyrroline. 2-Furfurylthiol has also been detected as a key flavor compound in roasted coffee and popcorn. Results reported by Schieberle (1993) suggested that the 2-furfurylthiol, which is formed from a water-insoluble precursor, at higher roasting temperatures and longer roasting periods, favors the generation of the VOCs in sesame.

According to Krishnamurthy and Chang (1967), furan derivatives greatly affect the sesame oil aroma, giving rise to a pleasantly sweet effect. It has been suggested that most of them were developed by Maillard reaction, but they also can result from thermal oxidation of lipids and degradation of terpenic precursors already present in the unheated seeds. Furan, 2-pentyl may originate from linoleic acid and is perceived as earthy-moundy. It was observed a decrease in oil headspace amount of furan derivatives when the seeds roasting degree had been increased. Volatile phenols are reported as strong odorants in several foods, but very scarce information is available on their relation with the sensory quality of vegetable oil. In general, phenols are perceived as a smoky and woody/spicey odor and a bitter taste, and could be important to the body note of the roasted sesame seed aroma, depending on the amount present in the global mixture.

Pyrroles are primarily formed by thermal treatment, with few findings of their presence in raw foods. Several pathways have been proposed: reactions involving reducing amino acids or ammonia (Shibamoto and Bernhard 1977; Tressl et al. 1980); degradation of Amadori intermediates (Shigematsu et al. 1975); and reactions of furan compounds with α-amino acids, among others.

VOCs in Sesame Oil

By application of aroma extract dilution analysis (AEDA) on an extract of roasted white sesame seeds (180°C; 30 min), Schieberle (1993) was able to identify 2-furfurylthiol, 2-phenyl-ethylthiol, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone as the most odor-active compounds in roasted sesame seeds. Working with an extract prepared from moderately roasted sesame (180°C; 10 min) and using the same technique as in his previous work, Schieberle (1996) identified 41 odor-active volatiles, 31 of which could be recognized by comparison of their mass spectra, retention indices, odor quality, and threshold with reference compounds. Of the 18 aroma compounds showing very high flavor dilution factors, 10 compounds [2-furfurylthiol,

TABLE 55.1. Concentrations and Odor Activity Values (OAVs) of 10 Selected Flavor Compounds in Roasted Sesame

Flavor Compound	Odor Threshold (g/L oil)	Concentration (g/kg)	OAVs[a]
2-Acetyl-1-pyrroline	0.1	30	300.00
2-Furturylthiol	0.4	54	135.00
2-Phenylethylthiol	0.05	6	120.00
4-Hydroxy-2,5dimethyl-3(2*H*)-furanone	50	2511	50.22
2-Ethyl-3,5-dimethylpyrazine	3	53	17.67
2-Methoxyphenol	19	269	14.16
2-Pentylpyridine	5	19	3.80
Acetylpyrazine	10	26	2.60
4-Vinyl-2-methoxyphenol	50	72	1.44
(*E*,*E*)-2,4-Decadienal	180	89	0.49

Source: Schieberle (1993).

[a]OAVs were calculated by dividing the concentration by the odor threshold in sunflower oil.

2-phenylethylthiol, 2-methoxyphenol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 2-pentylpyridine,2-ethyl-3,5-dimethylpyrazine,acetylpyrazine,(*E,E*)-2,4-decadienal, 2-acetyl-1-pyrroline, and 4-vinyl-2-methoxyphenol] were quantified by means of stable isotope dilution assays and their odor activity values (ratio of concentration to odor threshold) were calculated (Table 55.1). Especially, 2-acetyl-1-pyrroline, 2-furfurylthiol, and 2-phenylethylthiol were identified as the most important contributors to the overall roasty, caramel-like flavor note of the moderately roasted sesame (180°C; 10 min). On the other hand, 2-acetyl-1-pyrroline (roasty), 2-furfurylthiol (coffee-like), 2-phenylethylthiol (rubbery), and 4-hydroxy- 2,5-dimethyl-3(2*H*)-furanone (caramel-like) were identified as important contributors to the overall roasty, sulfuric odor of the crushed sesame material. Those results imply that the global flavor differences observed in sesame samples heated, under different conditions, are undoubtedly due to significant variations in the concentrations of the key flavor compounds. Those differences are probably caused by the different thermal stabilities of the respective flavor precursors on one hand, and by the different thermal stabilities of the aroma compounds on the other (Schieberle 1996).

Nakamura and others (1989) have obtained an extract of the volatiles associated with the oil of roasted sesame seeds by steam distillation and applied a technique to separate the compounds into neutral, weakly acidic, acidic, and basic fractions. All fractions were analyzed by GC-flame ionization detector (FID)/flame photometric detector (FPD), GC-MS, and GC-FT infrared (IR). Two hundred twenty-one constituents were identified. One hundred forty-six of those compounds are being reported for the first time in the aroma of roasted sesame seeds, seven of which were newly related with naturally occurring matrix.

Volatile flavor compounds in commercially processed roasted sesame seed oil were investigated by Shimoda and others (1996). Sesame seed oil was steam distilled under reduced pressure, and volatiles from the distillate were separated by an adsorption column method. Among the 171 individual peaks detected, 134 peaks were definitely or tentatively identified by analysis of mass spectra and modified

Kovats indices. Aiming to elucidate which compounds directly contribute to the characteristic flavor, the odor concentrate was fractionated by silica gel thin-layer chromatography and preparative GC. The identified compounds included 25 pyrazines, 1 pyridinamine, 8 pyridines, 14 pyrroles, 3 thiophenes, 11 thiazoles, 2 thiols, 1 disulfide, 11 furans and furanone, 11 hydrocarbons, 2 esters, 14 aldehydes, 9 ketones, 6 alcohols, 3 phenols, 5 fatty acids, and 8 miscellaneous compounds. Among which 1-(5-methyl-2-furanyl)-1-propanone, 3-formylthiophene, 2-propyl-4-methylthiazole, 2-ethyl-4-methyl-1*H*-pyrrole, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 4,5-dimethylisothiazole, 4,5-dimeth-ylthiazole, 2,6-diethylpyrazine, 2-ethyl-2,5-dimethylpyrazine, 1-(2-pyridinyl)ethanone, and 1-(1-methyl-1*H*-pyrrol-2-yl) were considered to be the main contributors to sesame seed oil flavor.

Shimoda and others (1997) evaluated the effect of temperature during roasted seeds on volatiles profile of sesame oil. The mentioned authors described the seeds as been light (150°C/10 min) and deep (180°C/10 min) roasted, and volatiles were separated from the commercially extracted oil by a combination of steam distillation under reduced pressure (90 mmHg) and column concentration. The GC-MS identified compounds including 20 pyrazines, 9 pyrroles, 4 pyridines, 9 thiazoles, 7 furans, 10 aliphatic aldehydes, 3 alcohols, 2 ketones, 3 fatty acids, 8 aromatic compounds, and 6 miscellaneous compounds (Table 55.2). The ratio of the amount of volatile component in deep- and light-roasted oil have shown that almost all the volatiles have increased by two to seven times in deep-roasted oil in relation to light-roasted oil. Additionally, an important flavor compound, 2-furanmethanethiol, with an intense coffee-like odor, increased from 6 to 40 ppb in deep-roasted oil. Such thiol compound had contributed to the unfavorable odor of deep-roasted oil. The authors have concluded that lipid oxidation is one of the most important alterations affecting both oils and fats. It is responsible for the development of unpleasant taste and smell in foods, making them unsuitable for human consumption.

The VOCs from cold-pressed unroasted sesame oil, extracted by SPME, were reported by Haiyan and others (2007). The identified compounds (9) included acetic acid, pentanal, toluene, methylpentenal, hexanal, 2-pentylfuran, α-terpene, and nonanal. The authors assign that hexanal arises from linoleic acid, whereas octanal and nonanal are oleic acid derivatives. There were significantly fewer volatiles than reported by Schieberle (1996) and Shimoda and others (1997) for roasted sesame seeds. This is not surprising, given the importance of roasting temperature to the production of volatiles, particularly pyrazines.

The presence of volatile degradants (hexanal, octanal, 2-octenal, 2-decenal, and 2-undecenal) in capsule formulations containing sesame oil has been reported by Chen and others (2007). In this case, three peaks were identified as 2,4-decadienal by SPME-GC-MS. The authors conclude that the multiple peaks of 2,4-decadienal are due to four possible isomers, namely (*E*,*E*), (*Z*,*Z*), (*E*,*Z*), and (*Z*,*E*)-2,4-decadienals that result from free radical auto-oxidation of oils containing linoleic acid under specific conditions. Additionally, the authors pointed out the SPME-GC-MS technique as an effective way of identifying trace amounts of VOCs in complex sample matrices, such as pharmaceutical drug products.

Kouzui and others (2002) created a particular criterion and classified the volatile components of roasted sesame seed oils into three groups: nitrogenous compounds (NC), sulfur compounds (SC), and tricylglycerol decomposition products (TDC), released upon heating by headspace sampler-GC-MS. The results of the sensory

TABLE 55.2. Effect of Roasting Degree on the Formation of Volatile Compounds in Sesame Seeds

Kovats Index[a]	Compound	Content (ppb) Deep Roasted[b]	Composition (%) Light Roasted[c]	Deep/Light Ratio[d]
Pyrazines				
1271	2-Methylpyrazine	779	195	4.0
1329	2,5-Dimethylpyrazine	735	158	4.7
1334	2,6-Dimethylpyrazine	383	82	4.7
1338	2-Ethylpyrazine	153	35	4.4
1352	2,3-Dimethylpyrazine	101	23	4.4
1390	2-Ethyl-6-methylpyrazine	237	47	5.0
1397	2-Ethyl-5-methylpyrazine	212	40	5.3
1410	2,3,4-Trimethylpyrazine	346	65	5.3
1438	2-Ethenylpyrazine	46	12	3.8
1449	3-Ethyl-2,5-dimethylpyrazine	542	104	5.2
1462	2-Ethyl-3,5-dimethylpyrazine	154	45	3.4
1479	2-(1-Methylpropyl)pyrazine	23	5	4.6
1490	2-Ethenyl-6-methylpyrazine	55	9	6.2
1494	2,3-Diethyl-5-methylpyrazine	22	4	5.5
1496	3,5-Diethyl-2-methylpyrazine	92	21	4.4
1535	2-Methyl-6-(1-propenyl)pyrazine	47	8	5.7
1543	2-Isopropenylpyrazine	48	8	6.3
1627	2-Acetylpyrazine	178	35	5.1
1671	(*E*)-2-Methyl-6-(1-propenyl)pyrazine	51	11	4.6
1680	2,3-Dimethyl-5-isopentylpyrazine	28	tr[e]	
	Total amount of pyrazines	4232 (43.5)	907 (45.0)	4.7
Pyrroles				
1183	2-Ethyl-1*H*-pyrrole	19	3	5.6
1505	1*H*-Pyrrole	70	16	4.4
1569	3-Methyl-1*H*-pyrrole	26	tr	
1598	1-Methyl-1*H*-pyrrole-2-acetonitrile	40	7	5.6

1610	1-Ethyl-1*H*-pyrrole-2-carboxyaldehyde	29	5	6.0
1657	1-(1-Methyl-1*H*-pyrrol-2-yl)ethanone	22	tr	
1970	1-(1*H*-Pyrrol-2-yl)ethanone	71	17	4.2
2030	1*H*-Pyrrole-2-carboxyaldehyde	127	143	0.9
2058	Methyl pyrrole-2-carboxylate	33	tr	
	Total amount of pyrroles	437 (4.5)	191 (9.5)	2.3
Pyridines				
1603	1-(2-Pyridinyl)ethanone	16	tr	
1643	4-Pyridinyl acetate	67	13	5.2
1782	Methyl 4-pyridinecarboxylate	55	10	5.5
2110	2-Pyridinemethanol	58	9	6.4
	Total amount of pyridines	196 (2.0)	32 (1.6)	6.1
Thiazoles				
1281	4-Ethylthiazole	35	7	5.1
1285	2,4-Dimethylthiazole	58	12	4.8
1326	2,5-Dimethylthiazole	115	20	5.8
1400	4,5-Dimethylisothiazole	39	8	5.1
1409	4,5-Dimethylthiazole	110	22	5.0
1467	4-Methyl-5-ethylthiazole	23	5	5.1
1486	2-Ethyl-5-methylthiazole	28	8	3.5
1695	2-Propyl-4-methylthiazole	123	21	5.9
1738	2-Butyl-5-methylthiazole	45	5	9.0
	Total amount of thiazoles	575 (5.9)	107 (5.3)	5.4
Furans				
1228	2-Pentylfuran	50	8	6.1
1456	2-Furfural	51	10	5.1
1521	2-Furanmethyl acetate	75	13	5.8
1574	5-Methyl-2-furfural	220	47	4.7
1664	Furfuryl alcohol	316	49	6.4
1686	1-(5-Methyl-2-furanyl)-1-propanone	70	10	7.0
1837	*R*-Methyl-*R*-vinyl-2-furanacetaldehyde	25	8	3.3
	Total amount of furans	807 (8.3)	145 (7.2)	5.6

TABLE 55.2. ***Continued***

Kovats Index[a]	Compound	Content (ppb) Deep Roasted[b]	Composition (%) Light Roasted[c]	Deep/Light Ratio[d]
Aliphatic Aldehydes				
987	Pentanal	36	15	2.4
1086	Hexanal	263	59	4.5
1095	2-Methyl-2-butenal	31	15	2.1
1186	Heptanal	30	7	4.4
1291	Octanal	26	6	4.2
1430	(*E*)-2-Heptenal	82	13	6.3
1528	(*E*)-2-Octenal	48	16	3.0
1706	(*E*,*E*)-2,4-Nonadienal	32	12	2.7
1766	(*E*,*Z*)-2,4-Decadienal	35	tr	
1811	(*E*,*E*)-2,4-Decadienal	154	36	4.3
	Total amount of aliphatic aldehydes	737 (7.6)	179 (8.9)	4.1
Aliphatic Alcohols, Ketones, and Acids				
1185	2-Heptanone	31	6	5.6
1354	Hexanol	79	12	6.6
1393	2-Nonanone	62	11	5.6
1562	Octanol	26	9	2.8
1834	Hexanoic acid	32	8	3.9
1953	Heptanoic acid	32	5	6.7
1977	Dodecanol	69	12	5.8
2062	Octanoic acid	86	tr	
	Total amount of aliphatic alcohols, ketones, and acids	417 (4.3)	63 (3.1)	6.6
Aromatic Compounds				
1512	Benzaldehyde	240	53	4.5
1651	1-Phenylethanone	142	24	5.9

1846	Guaiacol	321	32	10
1868	Benzenemethanol	69	16	4.3
1893	Benzeneethanol	47	10	4.9
1922	*R*-Ethylidenbenzeneacetaldehyde	47	tr	
2169	2-Methoxy-5-(1-propenyl)phenol	33	7	4.9
2200	1-(3-Methoxyphenyl)ethanone	177	37	4.8
	Total amount of phenyl compounds	1076 (11.1)	179 (8.9)	6.0
Miscellaneous Compounds				
891	Ethyl acetate	45	12	3.8
1194	*d*-Limonene	27	7	4.0
1617	3,5,5-Trimethyl-2-cyclopenten-1-one	37	tr	
1647	2,3-Dihydro-1*H*-indole	37	7	5.4
1693	3-Formylthiophene	38	6	6.1
1802	2-Furanmethanethiol	40	6	6.3
	Total amount of miscellaneous compounds	224 (2.3)	38 (1.9)	5.9
Unknown Compounds				
	Total amount of 28 compounds	1024 (10.5)	173 (8.6)	5.9
	Total	9726 (100)	2014 (100)	4.8

Source: Shimoda and others (1997).

[a]Modified Kovats indices calculated for DB-WAX capillary column on the GC system.
[b]Deep-roasted oil.
[c]Light-roasted oil.
[d]Ratio of the amount of volatile compounds in deep- and light-roasted sesame seed oil.
[e]Trace (tr) concentrations < 3 ppb.

evaluation were compared with the composition of the volatile groups in various roasted sesame seed oils. The technique presented 22 compounds for NC, 12 compounds for SC, and 29 compounds for TDC, and the aromatic sensory quality correlates positively with the amount of SC and negatively with the amount of TDC (the amount of NC was not correlated with the aromatic quality of oils).

Experimental study carried out by Arruda and others, using the HS-SPME-GC-MS analysis, indicated a very complex aroma profile concerning the roasted sesame oil. A total of 134 and 132 volatile constituents were identified in roasted sesame seeds oils obtained at 160 and 180°C, respectively: 23 pyrazines, 15 furans, 13 phenols, 12 pyridines and 2 pyridinamines, 10 pyrroles, 9 thiazoles, 9 hydrocarbon, 8 acids, 7 ketones, 6 alcohols, 3 terpenes, 3 disulfides, 2 thiophenes, 1 aldehyde, 1 thiol, and 1 ester. The pyrazines (pyrazine, 2,5-dimethyl-; pyrazine, 2,6-dimethyl-; pyrazine, ethyl-; pyrazine, 2-ethyl-5-methyl-; pyrazine, 3-ethyl-2,5-dimethyl-) and furans (furan, 2-pentyl, furfural; 2-furanmethanol, acetate; 2-furancarboxaldehyde, 5-methyl-; 2-furanmethanol) were considered to be the main contributors to the flavor of sesame seed oil.

The large concentration of alkylpyrazines in sesame seeds subjected to heating can override nutty-like, green, sweet, or roasted notes to the final product and were expected since those compounds are commonly arising from interactions between α-amino acids and carbohydrates. The increase in seed roast temperature seemed to raise the concentration of both pyridines and pyrazines, as also observed by Shimoda and others (1997). In this case, acetylpyrazines were present only in light sesame seed oil. Those compounds are normally related to an intense note reminiscent of popcorn.

The study carried out by Arruda and others (2009) has identified nine thiazoles, most of them alkylthiazoles, which often gives a pleasant meaty/nutty/green/musty aroma and which has been previously recognized of organoleptic importance in foods by Ho and Jin (1985). Among the 10 pyrrole compounds identified in the roasted sesame oil, alkylpyrroles have presented a slight increase at the higher sesame seed roasting temperature. Three extensively reported sulfur constituents of thermally processed food were also identified: dimethyl disulfide; 2-furanmethanethiol; and dimethyl trisulfide, presenting odor threshold around 0.01 and 0.06 ng/L air, indicative of concentration increase as the temperature of seed roast was raised. In spite of having substances with either strong pleasant as well as unpleasant impact in global flavor, their usual low concentration makes it difficult to achieve success in terms of extraction and detection levels. Nakamura and others (1989) has described 20 sulfur constituents, among them thiophenes and thiols/sulfides. Additionally, several indicators of lipid oxidation were identified by Arruda and others (2009): acids, ketones, alcohols, and aldehydes. A special attention should be given to 2,4-decadienal, which appeared in greater concentration. The food and agriculture literature (Chung et al. 2004; Kataoka et al. 1995; Kouzui et al. 2002) has reported that substance as one of the numerous aldehydic degradants from oils which contain linoleic acid. According to Schieberle (1996), 2,4-decadienal has an odor threshold of 0.05 ng/L air and confers a fatty, deep, fried, citrus attribute. An interesting fact is found noteworthy by Arruda and others (2009): the short chain aldehydes, as pentanal and hexanal (oleic and linoleic acid derivatives), were not detected in sesame oils extracted from seeds roasted at 160 and 180°C in laboratory scale by cold pressing. The presence of these and other aldehydes in commercial oil

is attributed to the oxidative degradation of the vegetable oils due to inadequate conditions during oil extraction and/or storage.

FINAL CONSIDERATIONS

The composition of volatile constituents in sesame seeds oils has been used to identify compounds that contribute to their flavor, to evaluate the effects of processing methods, and to improve the storage time of vegetable oils and by-products. Roasting of sesame seeds generates a distinctive and intense flavor characterized by roasty, burnt, meat-like, or sulfuric odor notes. The roasting of seeds at 160–180°C has enhanced flavor compound generation in sesame oil. Until 1988, only a few volatiles had been identified in extracts of roasted sesame. Later studies led to the identification of more than 170 volatiles (Arruda et al. 2009; Nakamura et al. 1989; Schieberle, 1993). The progress reached in the chemistry of volatile products of lipid oxidation has resulted from advances in separation techniques, especially SPME, and analytical methodologies, particularly GC-MS.

REFERENCES

Arruda NP, Resende CM, Freitas SP. 2009. Volatile profile from cold-pressed roasted sesame seed oil by HS-SPME-GC-MS. In *Proceedings of the 8th SLACA—Simpósio Latino Americano de Ciência de Alimentos*. UNICAMP: Sao Paulo, Brazil: UNICAMP, pp. 2–2.

Chen W, Zhou P, Wong-Moon KC, Cauchon NS. 2007. Identification of volatile degradants in formulations containing sesame oil using SPME/GC/MS. *J Pharm Biomed Anal* 44(22):450–5.

Chung J, Lee J, Choe E. 2004. Oxidative stability of soybean and sesame oil mixture during frying of flour dough. *J Food Sci* 69(7):C574–8.

Elleuch M, Besbes S, Roiseux O, Blecker C, Attia H. 2007. Quality characteristics of sesame seeds and by-products. *Food Chem* 103(2):641–50.

Fukuda Y, Nagata M, Osawa T. 1986. Chemical aspects of the antioxidative activity of roasted sesame seed oil, and the effect of using the oil for frying. *Agric Biol Chem* 50:857–62.

Haiyan Z, Bedgood DR Jr., Bishop AG, Prenzler PD, Robards K. 2007. Endogenous biophenol fatty acid and volatile profiles of selected oils. *Food Chem* 100(4):1544–51.

Havenga WJ, Rohwer ER 1999. Chemical characterization and screening of hydrocarbon pollution in industrial soils by headspace solid-phase microextraction. *J Chromatogr A* 848(1–2):279–95.

Ho CT, Jin QZ. 1985. Aroma properties of some alkylthiazoles. *Perfum Flavor* 9(1):15–8.

Hook GL, Kimm GL, Hall T, Smith PA. 2002. Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS). *Trends Anal Chem* 21(8):534–43.

Ikeda G, Tomizawa A, Imayoshi Y, Iwabuchi H, Hinata T, Sagara Y. 2006. Flavor design of sesame-flavored dressing using gas chromatography/olfactometry and food kansei model. *Food Sci Technol Res* 12(2):261–4.

Jung MY, Bock JY, Back SO, Lee TK, Kim JH. 1997. Pyrazine contents and oxidative stabilities of roasted soybean oils. *Food Chem* 60(1):95–102.

Kanu PJ, Zhua K, Kanub JB, Zhoua H, Qiana H, Zhu K. 2007. Biologically active components and nutraceuticals in sesame and related products: A review and prospect. *Trends Food Sci Technol* 18(12): 599–608.

Kataoka H, Sumida A, Nishihata N, Makita M. 1995. Determination of aliphatic aldehydes as their thiazolidine derivatives in foods by gas chromatography with flame photometric detection. *J Chromatogr A* 709(2):303–11.

Kouzui H, Kubota K, Nakajima S. 2002. Relationship between the volatile components and the aromatic quality of roasted sesame seed oils. *J Food Sci* 51(11):691–8.

Krishnamurthy R, Chang SS. 1967. Chemical reactions involved in deep fat frying of foods. Identification of nonacidic volatile decomposition products of corn oil. *J Am Oil Chem Soc* 44(2): 136–40.

Nakamura S, Nishimura O, Masuda H. 1989. Identification of volatile flavor components of the oil from roasted sesame seeds. *Agric Biol Chem* 53(7):1891–9.

Salunkhe DK, Chavan JK, Adsule RN, Kadam SS. 1991. Sesame. In *World Oilseeds: History, Technology and Utilization*. New York: Van Nostrand Reinhold, pp. 371–402.

Schieberle P. 1991. Primary odorants in popcorn. *J Agric Food Chem* 39(6):1141–4.

Schieberle P. 1993. Studies on the flavor of roasted white sesame seeds. In Schreier P, Winterhalter P (eds.), *Progress in Flavor Precursor Studies*. Carol Stream, IL: Allured Publishing, pp. 341–60.

Schieberle P. 1996. Odor-active compounds in roasted sesame. *Food Chem* 55(3):145–52.

Shibamoto T, Bernhard RA. 1977. Investigation of pyrazine formation pathways in sugar-ammonia model systems. *J Agric Food Chem* 25(3):609–14.

Shigematsu H, Shibata S, Kurata T, Kato H, Fujimaki M. 1975. Odor constituents formed on heating L-proline with D-glucose. *J Agric Food Chem* 23(2):233–7.

Shimoda M, Nakada Y, Nakashima M, Osajima, Y. 1997. Quantitative comparison of volatile flavor compounds in deep-roasted and light-roasted sesame seed oil. *J Agric Food Chem* 45(8):3193–6.

Shimoda M, Shiratsuchi H, Nakada Y, Wu W, Osajima Y. 1996. Identification and sensory characterization of volatile flavor compounds in sesame seed oil. *J Agric Food Chem* 44(12): 3909–12.

Tressl R, Grunewald KG, Kamperschroer H, Silwar R. 1980. Formation of pyrroles and aroma contributing sulphur compounds in malt and roasted coffee. *Prog Food Nutr Sci* 4(1):111.1–2.9.

USDA. 2004. National nutrient database for standard reference. Report No 17. New York: U.S. Department of Agriculture.

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Handbook of Fruit and Vegetable Flavors, Edited by Y. H. Hui