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Dr Adel Kader

Few people have had as much of an impact on a field of scientific study as Dr Adel Kader has had on the discipline of postharvest physiology and technology. Through his numerous publications and students, Dr Kader has influenced an entire generation of researchers, teachers and extension personnel. Just as important as this tangible evidence of his long and illustrious career is the intangible influence he has had on shaping the focus and direction of postharvest research and extension activities worldwide. While most of his time at the University of California, Davis (UCD) was spent on improving the more obvious aspects of postharvest quality of fruits, he became increasingly interested in the less obvious characteristics of flavor and nutritional quality. He realized that an improvement in people's diets through the consumption of more fruits and vegetables would only occur when the appearance quality of fruits and vegetables was matched by an increase in their flavor and nutritive quality. He became a vocal proponent of coupling the traditional studies of appearance quality with studies of flavor and nutritional quality changes during harvest, storage, transport and marketing of horticultural commodities.

During his more than 35 years at UCD he continued his teaching, research and extension activities in the area of postharvest biology and technology of horticultural crops. His prodigious output includes over 230 technical publications and many book chapters. Almost more important than his written output are his mentoring of 36 graduate students and 60 scientists who participated in his ongoing research programs. His commitment to continuing education is exemplified by his participation in the annual two-week Postharvest Technology Short Course which he was instrumental in organizing in 1979 and which has

been attended by over 2 500 people. Never one to rest on his laurels, Adel launched in 1998 the postharvest website (http://postharvest.ucdavis.edu) which has become the premier source of postharvest information worldwide.

Dr Kader received his BSc in Horticulture from the Faculty of Agriculture at Ain Shams University in Egypt in 1959. He then moved to UCD where he received his MSc in Vegetable Crops in 1962 and his PhD in Plant Physiology in 1966. After receiving his doctorate, he returned to Egypt where he held the position of Assistant Professor in the Faculty of Agriculture at Ain Shams University, Cairo, from 1966 to 1971. While there he was engaged in teaching and research on postharvest horticulture. He then became a lecturer and consultant in the Kuwait Institute for Scientific Research from 1971 to 1972. Again his emphasis was on teaching and consulting on the postharvest handling of horticultural crops. He returned to UCD in 1972, first as an Assistant Researcher, and later as an Assistant, Associate, and full Professor, and he retained this position until his retirement in 2007. He became a US citizen in 1976. During his retirement he and his wife, Aileen, plan to travel and spend time with their children.

Dr Kader is currently an Emeritus Professor at UCD, where he was for 35 years. During that time he served as Chairman of the Department of Pomology and received a number of awards. A listing of just a few of them will illustrate the breadth of his accomplishments. He was elected a Fellow of the American Society for Horticultural Science (1986), and served as President-elect (1994–95), President (1995–96), and Chairman of the Board of Directors (1996–97) of the Society. He received from UCD the Outstanding Teaching Award in Extension (1989), the Award of Distinction from the College of Agricultural and Environmental Sciences (2000), the Alumni Citation of Excellence from the Cal Aggie Alumni Association (2000), and the Academic Senate's Distinguished Graduate Mentoring Award (2003). He was also selected as the Outstanding Horticulturist of 1997 by the Horticultural Research Center at Laval University, Quebec, Canada.

Although retired, Dr Kader still maintains an active interest in postharvest programs worldwide and frequently participates in seminars at UCD and international meetings. He continues to do some consulting to raise funds for the UC Davis postharvest endowment. The absence of his experience and sage advice creates a void that is difficult to fill.

Professor Mikal Saltveit
University of California, Davis, USA

Foreword

Mangoes, mangosteens, and papayas may have been around for as long as apples and oranges, but you would never have known that based on a visit to a typical North American or European supermarket 20 years ago. Back then, the average grocery store carried only a handful of tropical and subtropical fruits – primarily oranges, bananas, and pineapples. Today, mangoes, papayas, avocados, and kiwifruits are a staple on grocery shelves all over the world, and it's even possible, here and there, to find a Safeway, a Kroger or an Ahold supermarket with an entire section in the produce department devoted to once exotic tropical and subtropical fruits. It is very unlikely that another 20 years will elapse before such displays are expanded to include mangosteens, cherimoyas, sapodillas, longans, loquats, and rambutans.

Much of the steadily growing appeal of tropical and subtropical fruits can be attributed to the new and nutritious tastes and flavors they offer to consumers in the Northern Hemisphere. But any proper explanation would have to also recognize the essential role played by growers, governments, and academics, as well as the vendors, transporters, and other industries that support the sale and distribution of these delectables.

Since 1982 I have had the privilege to serve as president of both the International Association of Refrigerated Warehouses (IARW) and the World Food Logistics Organization (WFLO), both of which have since become partners in the Global Cold Chain Alliance (GCCA). The history of IARW dates back 120 years, to a time when large-scale food storage was, for the most part, limited to keeping products surrounded by blocks of ice. With the advent of mechanical refrigeration in the last quarter of the nineteenth century, beer brewers were, apparently, among the first significant users of industrial refrigeration. By the turn of the century, ammonia refrigeration was adopted by the meatpacking industry and before long it was widely embraced by fruit, vegetable, and dairy producers as well.

Today, there are more than 350 IARW members in 67 countries on six continents, offering a nearly unimaginable variety of freezing, cooling, tempering, storage, handling, and shipping services to the global food industry. To help carry out the important work of feeding much of the world, cold storage companies look to the non-profit WFLO for guidance. Founded in 1943, WFLO is dedicated to the proper handling and storage of perishable products, and to developing best practices for the safe, efficient, and reliable movement of food.

As it endeavored to achieve these goals, WFLO developed a Commodity Storage Manual (CSM) and created a Scientific Advisory Council (SAC) comprised of approximately 15 distinguished food scientists and specialists in engineering, agricultural economics, and logistics. The CSM contains detailed information on hundreds of different meats, fish, dairy products, vegetables, and fruits, including a number of tropical and subtropical fruits. SAC members and other food scientists provide CSM readers with details about storage conditions, shelf life, color, odor transfer, product diseases, and additional useful information, all of which is product-specific.

During the past 65+ years, SAC members have also conducted or supervised more than 150 research projects addressing such topics as the effect of storage temperatures on quality, mobility threshold temperatures (glass transition), use of an 'electronic nose' to measure quality attributes of seafood, the sensitivity of product quality to fluctuating temperatures, and temperature control in frozen food transportation. While much of this research attention has focused on low temperature issues, SAC members are, of course, also very mindful of such other preservation techniques as temperature elevation (blanching, pasteurization, etc.), water reduction, ionizing radiation, and so on.

I have had the pleasure of working with Elhadi Yahia in his capacity as a member of the WFLO Scientific Advisory Council, where he has generously shared his expertise in the postharvest technology of perishable foods. Through his work on the council, Dr Yahia has enabled hundreds of cold storage operators and refrigerated transportation providers to better understand and address a wide range of matters related to food preservation, food safety, and food quality.

And now, with the publication of *Postharvest Biology and Technology of Tropical and Subtropical Fruits*, Professor Yahia makes an enduring contribution available to many more people around the globe. This sourcebook contains a wealth of information about every conceivable aspect of tropical and subtropical fruit production, harvesting, and health. It comprehensively addresses the causes and cures of traditionally significant postharvest losses, whether due to biochemical, environmental, economic, or regulatory factors. The book also discusses a variety of control measures and preservation processes that will enable growers and researchers to achieve both higher quality and higher quantities of market caliber fruits. As if that were not enough, the information and guidance included in this publication take into account food safety concerns and are fully compatible with the principles of sustainability.

Accompanying this volume are three other tomes containing a total of more than 60 chapters, each one devoted to a particular tropical or subtropical fruit.

These comprehensive discussions typically cover development, physiology, quality indices, preharvest conditions, diseases, postharvest handling, processing, and other valuable guidance.

Tropical fruits occupy a special niche in the world of agriculture. For one thing, more than 90% of tropical fruit production takes place in developing countries. This often connotes the presence of fewer good roads, less reliable power sources, and greater dependence on low-tech storage solutions. Second, harvested crops are frequently subjected to a convoluted supply chain in which the fruit is handled time and again by farm collectors, middlemen, market agents, exporters, and sellers before ever reaching the consumer. These circumstances inflict a great deal of stress on products that are typically more perishable to begin with than fruits from more temperate climates.

The UN Food and Agriculture Organization (FAO) states that the reduction of postharvest food loss needs to be a major agricultural goal in a hungry and increasingly competitive world. FAO goes on to point out that investments made to save food postharvest are invariably less costly – for the grower and the consumer – than efforts to increase production by comparable magnitudes. The agency further contends that even a partial reduction in postharvest losses can lessen dependence on marginal land and other scarce resources.

Dr Yahia's encyclopedic work will help growers to better understand latent hazards and how to deal with them. It will help growers and distributors alike to utilize best practices to minimize product losses and maximize quality. It will both help feed more local communities and ensure that fragile tropical fruits are as well suited as possible to long distance shipment. And by better informing food handlers throughout the supply chain it will ultimately boost trade and bring the benefits of tropical and subtropical fruits to millions of people who have never enjoyed the rich flavors and health benefits of mangosteens or breadfruits.

Having worked closely with the food industry for a quarter of a century, I know that this project was anything but easy. Dr Yahia's mission involved mastering the details on literally thousands of tropical and subtropical varieties. It entailed dealing with the newest and most advanced food science as well as 'nuts and bolts' applications of that science. And it tackles these issues in the context of increased international trade and a global economic shift that rewards winners handsomely and penalizes others decisively. I think it is fair to say that this milestone publication could not have come at a better time.

J. William Hudson
President and CEO
World Food Logistics Organization

Preface

Introduction to tropical and subtropical fruits

Tropical and subtropical fruits include a large number of crops such as acerola, aonla, avocado, bael, banana, black sapote, breadfruit, breadnut, carambola, cherimoya, chili plum, citrus fruit, cocona, coconut, date, durian, feijoa, fig, golden apple, grape, guava, jaboticaba, jackfruit, kiwifruit, longan, loquat, litchi, macadamia, mamey sapote, mango, mangosteen, nance, noni, olive, papaya, passion fruit, pecan, persimmon, pineapple, pistachio, pitahaya, pitanga, pomegranate, rambutan, salak, sapodilla, soursop, star apple, tamarillo, tamarind, wax apple, among many others (see volumes 2, 3 and 4 of this collection). They are important commodities with multiple uses, and their contribution to human nutrition and health and lives is very significant. Tropical and subtropical fruit plants come from a wide range of botanical families, are of different types, including vines (e.g. passion fruit), herbaceous crops (e.g. bananas) and woody plants (e.g. oranges), and produce various kinds of fruit such as berries (e.g. avocados), drupes (e.g. mangoes), nutlets (e.g. litchis) and compound fruits (e.g. pineapples). It is not straightforward to make a distinction between tropical and subtropical fruits, and, indeed, several can be cultivated both in the tropics and subtropics. Citrus, for example, is of tropical origin, yet achieves better quality when grown in the subtropics, or at higher elevations in the tropics. The geographical boundaries of the tropics may not, in fact, be the best indication of whether a fruit is tropical or subtropical, as some areas within the tropics have a climate that is not typically considered tropical, due to factors such as altitude. In general, tropical areas are those which remain constantly warm. In other words, they are areas in which the warmest month is only a few degrees hotter than the coldest, temperature differences between day and night are greater than those

between summer and winter and there is little variation in day length. Subtropical regions generally have a greater difference in temperature between summer and winter and are less humid.

Though there are tropical and subtropical fruit native to almost all continents, the majority of the most widely traded species originate from the American continent (e.g. papayas, guavas) or Asia (e.g. most citrus fruits, mangoes and bananas), some exceptions being dates from Africa and coconuts from Oceania. Tropical and subtropical fruits have long been part of the diet in certain areas. While the general public may just now be discovering many tropical fruits, these delicacies have served as staples for some cultures for generations. Mango has been cultivated and consumed in India for more than 4000 years, the Chinese have harvested litchis for thousands of years, carambolas (starfruit) have been popular in Malaysia for centuries, papaya was enjoyed throughout Latin America long before Columbus's arrival, and dates have been the staple food of the Middle East and North Africa for thousands of years. Tropical and subtropical fruit are thought to have spread very early from their respective areas of origin. For example, the conquest of parts of the Iberian peninsula by Moorish Muslim armies from North Africa brought oranges to southern Europe, and the Europeans brought crops from the New World back to the Old World. In general, the establishment of trade routes facilitated the spread of crops, especially those whose fruit and planting material could survive long voyages.

Trade in tropical and subtropical fruits

The major traded tropical and subtropical fruits include citrus fruits, banana, mango, pineapple and papaya. Some other tropical and subtropical fruits, such as durian and mangosteen, are not so extensively cultivated or traded, but are still important economically in their own regional markets. There are also a significant number of tropical and subtropical fruits which are not cultivated commercially. According to 2010 FAO statistics, world production of certain fruit amounts to the following: banana – almost 60 million tonnes (mmt), mango – almost 25 mmt, papaya – more than 8 mmt, pineapple – more than 13 mmt and plantain – more than 30 mmt. About 98% of tropical fruits and significant quantities of subtropical fruits are produced in developing countries. More than 50% of tropical and subtropical fruits are consumed fresh, and the rest is consumed in many different processed forms. There has been a major drive to promote several minor fruits (including many tropical and subtropical fruits) in international trade, but in general, it can be postulated that a significant gap still exists between world per capita consumption and estimated consumption saturation. Immigrant communities in several countries (including North America and European countries) who have used minor fruits in their diet for generations, have played a role in the establishment of new markets in their adopted countries of residence. In addition, tourists and travelers from many Western countries have been exposed to many of these fruits in the tropics and subtropics and have either started to

demand that they are stocked in the shops and marketplaces they usually frequent, or have begun to acquire them from shops and marketplaces that cater primarily for immigrants. In addition, there are areas in North America (around the southern tip of Florida, Hawaii, Puerto Rico) and in Europe (such as in the Canary Islands) which have suitable climates for tropical fruits, and fruits grown in those regions have also found their way to markets and consumers elsewhere in the United States and Europe, therefore increasing the promotion of these minor fruits.

The increasing interest in and marketing prospects for tropical and subtropical fruits worldwide have made the science underpinning postharvest technologies for these fruits a priority research and development area in several countries, including many developing countries. There is increasing demand for tropical and subtropical fruit handling information from shippers, importers and trade organizations. However, making handling information available is often of limited importance to whole regions (this applies especially to information on some very minor and exotic fruits). This reflects the characteristics of several tropical and some subtropical fruit industries: they are comparatively small, dispersed and fragmented and production is highly seasonal. Production also mostly takes place in developing countries, where adequate distribution systems are yet to be established. It is in the economic and national interest of food/fruit exporting countries to possess and maintain an international commercial reputation as a reliable supplier of products of acceptable sensory and safety quality, and therefore the establishment of adequate postharvest handling systems for these fruits is essential.

Postharvest handling systems for tropical and subtropical fruits

Most tropical fruits and several subtropical fruits are highly perishable and can only be maintained for short periods of time (from a few days to a very few weeks), but some fruits can be maintained for extended period of time (such as oranges, some cultivars of table grapes, nuts and dried fruits). The three major postharvest problems that affect tropical and subtropical fruits (especially tropicals) are chilling injury, decay and insect damage. Chilling injury (CI) is a common problem in most tropical and several subtropical fruits. Most temperate fruits can be stored for the longest period of time at around 0°C (just above the freezing point), and are injured only if they freeze. However, many tropical and subtropical fruits are injured by low, nonfreezing temperatures in the range 0°–13°C. CI results in surface pitting, browning of the peel and flesh, and faster postharvest decay development. Thus, postharvest temperature control is even more critical for tropical and subtropical fruits than for temperate fruits. The high perishability of these fruits (especially tropical fruits) is due not only to the higher storage temperature requirements, but also to their general nature: most tropical fruits are climacteric and produce high quantities of ethylene, but some, such as citrus, are ‘non-climacteric’. Citrus fruit ripen slowly, without going through a burst of respiration, softening, and color change right at the end of development,

and can be stored on the tree for several weeks, widening the window in which the crop can be marketed and allowing for greater marketing flexibility.

Almost all tropical fruits and most subtropical fruits are produced in developing countries and postharvest handling systems in these areas are, in most cases, not adequately developed (see Plate Ia in the color section between p 238 and p 239). Therefore rates of deterioration and losses are commonly high. Common inadequate handling practices causing significant qualitative and quantitative losses include a lack of adequate ripening indices and harvesting methods, a non-existent or inadequate cold chain and inadequate storage and marketing practices (Plate Ia). However, there have recently been improvements in the postharvest handling in particular of fruit for export, and also of locally marketed crops. Plate Ib shows fruits of excellent quality in a local supermarket in a developing country. Fruit intended for export is commonly handled better in developing countries, but improvements have also been seen in postharvest handling of locally consumed commodities. Export of fresh tropical and subtropical fruits is mainly done by ship or surface transport. These transport techniques have improved very significantly, and refrigerated boats (some even providing modified and controlled atmospheres and quarantine facilities) move these commodities from producing countries to their ultimate markets with relative ease. A small proportion of some tropical fruits, such as mangosteen and rambutan, are sometimes still transported by air, especially when they are destined for gourmet or niche markets or for celebrations at certain times of the year, such as Christmas and New Year, when they command higher prices.

Several important postharvest techniques are available for the handling of tropical and subtropical fruits; some of them are very simple and can be adapted in any location. The most common pre-cooling techniques for these fruits are forced-air and hydrocooling. Modified and controlled atmospheres are used for storage of a very few fruit (such as kiwifruit), for transport of several fruits (such as mango, banana, avocado, papaya), and for packaging of some minimally processed products. Ethylene is used for the ripening of some fruits (such as banana), and 1-MCP (1-methylcyclopropene) is efficient in controlling ripening of some fruits. Several treatments are legally established for quarantine control, such as low temperature (for citrus and grapes), heat (for mango and grapefruit), and irradiation (for mango and guava). Plate II (also in the color section) shows hot water quarantine treatment of mango in Mexico. Heat treatments (especially hot water treatments) are effective in controlling postharvest decay and are commercially used on the mango, among other fruits. Tropical and subtropical fruits are suitable for the development of many minimally processed and processed products. Biotechnology is also another technique that has excellent potential for the improvement of various product characteristics and for solving several problems associated with tropical and subtropical fruit.

Conclusions

Major research efforts and activities are still needed to solve some problems (especially related to chilling injury, decay and insects), and to establish better

postharvest handling practices, especially for tropical fruits. Major extension and technology transfer efforts are also still needed, especially in developing countries, to establish better mechanisms by which to transfer the results of research and encourage the adoption of the new handling practices developed. Many more details can be found in the following chapters of the four volumes of this collection. The following 78 chapters demonstrate the diversity of tropical and subtropical fruit, their characteristics and importance, and the knowledge that has accumulated on their handling after harvest. Excellent postharvest knowledge has been generated in the past few decades through the efforts of many excellent scientists and extension specialists, such as Prof. Adel A. Kader to whom we dedicate the book for his significant contributions and efforts. I would like to thank all the authors for their excellent efforts and patience. Many thanks are also due to Mr Bill Hudson, Prof. Jules Janick, Prof. Ian Ferguson and Prof. George Wilson for agreeing to write the forewords to the four volumes, to Prof. Mikal Saltveit for writing the dedication, and to Prof. Jeffrey Brecht for his help during the initial preparation of the book proposal. The help and support of the Woodhead team, especially Ms Sarah Whitworth and Ms Vicki Hart, is very much appreciated. Finally, the importance of adequate postharvest handling and prevention of qualitative and quantitative losses of these important food commodities (and any other food commodity) is very important, and I think it could not be explained in a simpler manner than by the following Chinese proverb that states: 'if you make your plans for a year, plant rice, if you make them for one decade, plant trees, and if you make them for a lifetime, do not waste food.'

Elhadi M. Yahia

Plate I (Preface) (a) Handling of some tropical fruits in an open market in a developing country; (b) a supermarket in Bogotá, Colombia.

Plate II (Preface) Hot water treatment for quarantine insect control of mango in Mexico.

1

Economic importance of tropical and subtropical fruits

Z. Mohamed, I. AbdLatif and A. Mahir Abdullah, University of Putra Malaysia, Malaysia

Abstract: Asian countries are among the largest producers of tropical and subtropical fruits in the world. The top five tropical and subtropical fruits in terms of production volume are watermelon, orange, grape, banana and tangerine/mandarin. China is the largest producer of watermelon, Brazil of oranges, Italy of grapes and India of bananas. Most of the fruits are consumed as food in fresh and processed form. EU countries are the main destination for tropical and subtropical fruits, consuming nearly 50% of the total world export, while also supplying temperate and subtropical fruits to the global fruits market. The importance of fruit production for the economic development of a country can be seen in its contribution to the gross domestic product (GDP) and employment through agriculture. Most less-developed countries depend on agriculture as a major source of income and employment. Price demand elasticities for most fruits are elastic.

Key words: tropical and subtropical fruits, producers, GDP, employment, consumption, elasticities and prices.

1.1 Introduction

There are a very large number of different tropical and subtropical fruits, yet only 50 or so are well known in most parts of the world (Martin *et al.*, 1987). The estimated gap between world per capita consumption (54.9 kg per year) and estimated consumption saturation (100 to 120 kg per year) is considerable (TFNet, 2003). According to Galán Saúco (1996) and Katz *et al.* (2003), tropical and subtropical fruit can be divided into three groups based on production and trade figures, (with some overlap between categories). The first group, 'major fruits',

include crops such as banana and plantain, citrus, coconut, mango and pineapple. These are cultivated in most tropical and subtropical countries and are found in international markets as well as local ones. The next group, 'minor fruits' (e.g. avocado, breadfruit, carambola, cashew nut, durian, guava, jackfruit, litchi, macadamia, mangosteen, papaya, passion fruit, sapodilla and soursop) are not cultivated as extensively. Trade in these crops (and therefore also consumption) is likely to be less widespread, both geographically and quantitatively. Nevertheless, some of these minor fruits, such as carambola, durian and mangosteen from South-East Asia are of considerable economic significance in regional markets (Anang and Chan, 1999). The last group, 'wild fruits', are not cultivated commercially. They come from various botanical families and have generally not been well characterized (Katz *et al.*, 2003).

1.2 World fruit production and contribution to gross domestic product (GDP)

1.2.1 Global production of tropical and subtropical fruits

According to the world food balance sheets published by the Food and Agriculture Organization (FAO), the total world fruit supply in 2005 was 517 million tonnes. It comprised 514 million tonnes of fruit production, 107 million tonnes of imports and 106 million tonnes of exports (stock variations accounted for 2 million tonnes). Asia contributed 46% of the total world fruit production, while the Americas contributed 26%. Europe, Africa and Oceania held 14%, 13% and 1% of the share of world fruit production in 2005, respectively (Fig. 1.1) (FAOSTAT, 2008).

The total world production for the ten most popular fruit types in 2008 was over 431 million tonnes. As shown in Fig. 1.2, watermelons, bananas, grapes, oranges, mangoes and tangerines are among the most produced around the globe. Other important fruits include pineapples, papayas, other citrus fruits and dates (FAOSTAT 2008).

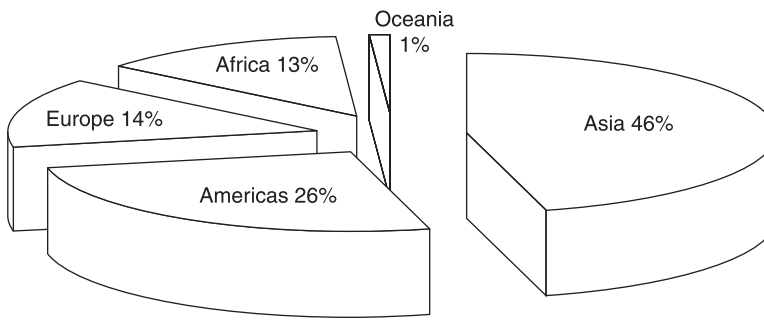

Fig. 1.1 World fruit production share by continent.

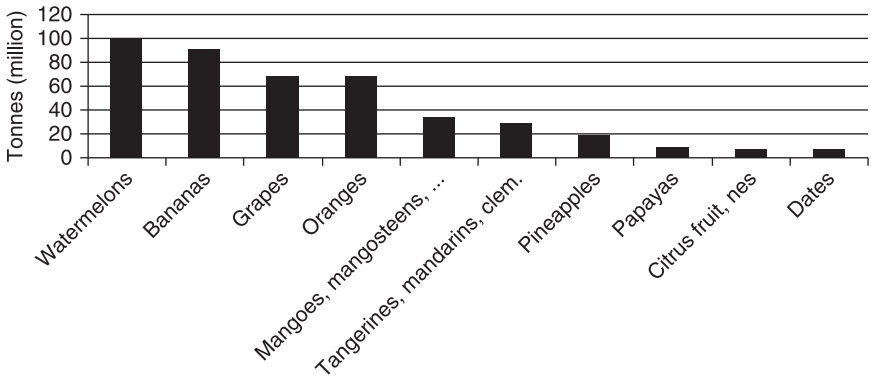

Fig. 1.2 World production of selected tropical fruits.

World fruit production has increased over the years. There has been an increase of 17% for the ten fruit types for the past five years, as shown in Table 1.1. The production increase was a response from producers to the general increase in demand for tropical and subtropical fruits worldwide. As illustrated in Fig. 1.3, higher production growth was observed for bananas and watermelons, in contrast to a lower growth rate for grapes and oranges from 2003 to 2008. The nature of the crops in question could account for this: short-term crops such as banana and watermelon can respond quickly to increased demand, while longer-term crops such as grapes and oranges cannot.

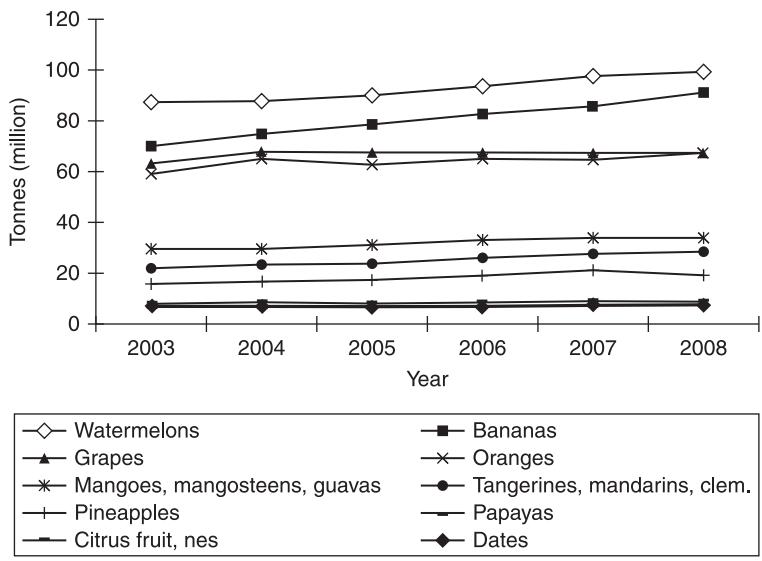

Fig. 1.3 Production trends for ten most popular fruits.

Table 1.1 World tropical and subtropical fruit production by fruit types (tonnes), 2003–2008

Fruit type	2003	2004	2005	2006	2007	2008
Watermelons	87 289 487	87 833 969	90 176 238	93 709 170	97 434 562	99 194 223
Bananas	70 209 520	74 845 330	78 749 456	82 887 488	85 855 856	90 705 922
Grapes	63 685 749	67 565 568	67 190 031	67 333 393	67 221 000	67 708 587
Oranges	59 491 522	64 709 042	62 829 839	65 319 196	64 763 648	67 695 802
Mangoes, mangosteens, guavas	29 359 957	29 087 337	31 251 420	32 995 171	33 866 557	34 343 083
Tangerines, mandarins, clementines	22 030 899	23 412 226	23 735 119	26 234 839	27 864 626	28 556 834
Pineapples	16 096 896	16 667 953	17 856 630	19 210 902	20 911 077	19 166 560
Papayas	7 930 846	8 594 281	8 066 114	8 913 064	9 210 748	9 095 875
Citrus fruit	6 815 494	7 036 877	7 109 308	6 936 267	7 223 647	7 452 302
Dates	6 669 625	7 041 628	6 548 201	6 701 968	6 908 900	7 048 089
Total	369 579 995	386 794 211	393 512 356	410 241 458	421 260 621	430 967 277

Source: FAOSTAT (2008)

Tables 1.2 to 1.5 illustrate the main fruit producing countries by fruit types. The tables show that China is an important contributor to world fruit production. In 2008, the country produced 67% of the total world watermelon production, and almost 11% of the total world grape production, alongside 9% and 5% of world banana and orange production respectively. Other major producers of watermelons in the same year were Turkey, Iran, Brazil and the USA.

India was the leading producer of bananas in 2008, with approximately 26% of the global total. The second most important banana-producing country was the Philippines with a share of almost 10% of world production. Other significant banana producers were Brazil, Ecuador and Indonesia.

Table 1.2 Top ten watermelon producing countries 2008

Country	Tonnes
China	67 203 275
Turkey	4 002 280
Iran, Islamic Republic of	3 400 000
Brazil	1 950 000
United States of America	1 793 990
Egypt	1 485 939
Mexico	1 199 711
Russian Federation	1 100 000
Uzbekistan	981 200
Korea, Republic of	856 755

Source: FAOSTAT (2008)

Table 1.3 Top ten banana producing countries 2008

Country	Tonnes
India	23 204 800
Philippines	8 687 624
China	8 042 702
Brazil	7 116 808
Ecuador	6 701 146
Indonesia	5 741 352
Tanzania, United Republic of	3 500 000
Mexico	2 159 280
Thailand	2 000 000
Costa Rica	1 881 783

Source: FAOSTAT (2008)

Table 1.4 Top ten grape producing countries 2008

Country	Tonnes
Italy	7 793 301
China	7 284 656
United States of America	6 744 840
Spain	6 053 000
France	5 664 195
Turkey	3 918 440
Argentina	2 900 000
Iran, Islamic Republic of	2 900 000
Chile	2 350 000
Australia	1 956 790

Source: FAOSTAT (2008)

Table 1.5 Top ten orange producing countries 2008

Country	Tonnes
Brazil	18 389 752
United States of America	9 138 980
India	4 396 700
Mexico	4 306 633
China	3 454 125
Spain	3 367 000
Italy	2 527 453
Indonesia	2 322 581
Iran, Islamic Republic of	2 300 000
Egypt	2 138 425

Source: FAOSTAT (2008)

The leading grape producers of the world in 2008 were Italy (11.5% of total world grape production), China (11%), the USA (10%), Spain (9%), France (8%) and Turkey (6%). Other important grape producing countries were Argentina, Iran, Chile and Australia. The fifth major fruit produced in 2008 was oranges, with Brazil producing 27% of the global total, while 13.5% was contributed by the USA, and 6.5% by India. Mexico, China, Spain, Italy and Indonesia were among the other major orange producers.

1.2.2 Contribution of agriculture to GDP

The agriculture sector plays a very important role in the economic development of many countries, and especially in contributing to their GDP. In 2005 the agriculture sector was responsible for about 4% of world GDP (www.nationmaster.com) with the fruits subsector playing a significant role, particularly in less developed countries.

Table 1.6 shows that the major tropical fruit production areas stretch across many developed and less-developed countries. Fruits are thus extremely important as they can provide a major source of income to many nations as well as employment for the population of those countries. Less-developed countries including Ethiopia, the Republic of Congo and Sudan still depend on agricultural activities, including fruit production, as a major contributor to their GDP (see Table 1.7), with the agriculture sector generally responsible for about 30–55% of their GDP. Developing countries such as Vietnam, Pakistan, Syria, India, Bangladesh, Tanzania and Indonesia are increasingly coming to depend less on the agriculture sector, including fruit production, with around 18–30% of their GDP currently provided by agricultural activities; this figure is expected to continue to fall. Many developed countries are also major fruit producers, in particular, Japan, the EU countries and the USA, which is a leading producer and

Table 1.6 Production of major tropical and subtropical fruits in 2000

Fruit	World production ('000 t)	Important producing countries
Orange	66 055	Brazil, United States, India, Mexico, Spain, China, Italy, Egypt, Pakistan, Greece, South Africa
Banana	58 687	Burundi, Nigeria, Costa Rica, Mexico, Colombia, Ecuador, Brazil, India, Indonesia, Philippines, Papua New Guinea, Spain
Coconut	48 375	Indonesia, Philippines, India, Sri Lanka, Brazil, Thailand, Mexico, Vietnam, Malaysia, Papua New Guinea
Plantain	30 583	Colombia, Ecuador, Peru, Venezuela, Ivory Coast, Cameroon, Sri Lanka, Myanmar
Mango	24 975	India, Indonesia, Philippines, Thailand, Mexico, Haiti, Brazil, Nigeria
Pineapple	13 455	Philippines, India, Indonesia, China, Brazil, United States, Mexico, Nigeria, Vietnam
Papaya	8 426	Nigeria, Mexico, Brazil, China, India, Indonesia, Thailand, Sri Lanka
Avocado	2 331	Mexico, United States, Dominican Republic, Brazil, Colombia, Chile, South Africa, Indonesia, Israel, Spain

Source: http://www.fao.org

Table 1.7 Contribution of agriculture to Gross Domestic Product (GDP)

Country	GDP of agriculture (%)	GDP of industry (%)	GDP of services (%)
Congo, Dem Rep	55.0	11.0	34.0
Ethiopia	44.9	12.8	42.3
Sudan	31.0	34.7	34.3
Tanzania	27.1	22.5	50.4
Vietnam	22.0	39.9	38.1
Pakistan	20.4	26.6	53.0
Bangladesh	19.1	28.6	52.3
Syrian Arab Republic	18.5	26.9	54.6
India	17.6	29.0	53.4
Philippines	14.7	31.6	53.7
Guatemala	13.1	25.0	61.9
China	11.3	48.6	40.1
Malaysia	10.1	43.7	46.3
European Union	2.0	27.1	70.9
Japan	1.5	26.3	72.3
United States	1.2	19.2	79.6

Source: www.nationmaster.com

exporter of citrus fruits. Dependence on agriculture is lower in developed countries, with only around 1–2% of their GDP generally contributed by the agriculture sector. Although the percentage contribution of this sector to GDP is low, the production value is higher than in most developing and less-developed countries.

1.2.3 Contribution of agriculture to employment compared to other sectors

It is expected that employment activities will have a direct effect upon the composition of a country's GDP. In less-developed countries including Ethiopia, the Republic of Congo and Sudan it is likely that a major proportion of the labour force will be employed in agricultural activities. Indeed, as can be seen in Table 1.8, about 80% of the labour force of these countries is involved in the agriculture sector (which includes the fruit production subsector). Meanwhile, only 13% are employed in the service sector, and 7% in industry. On the other hand, developing nations like Guatemala have only about 50% of their labour force in the agricultural sector, with a similar trend observed in other countries such as Vietnam, India and Bangladesh.

Table 1.8 Contribution of agriculture sector to employment

Country	Employment in agriculture (%)	Employment in industry (%)	Employment in services (%)
Ethiopia	80.2	6.6	13.2
Tanzania	80.0	20.0	0.0
Sudan	80.0	7.0	13.0
Bangladesh	63.0	11.0	26.0
India	60.0	12.0	28.0
Vietnam	55.6	18.9	25.5
Guatemala	50.0	15.0	35.0
Pakistan	43.0	20.3	36.6
China	43.0	25.0	32.0
Philippines	35.0	15.0	50.0
Syrian Arab Republic	19.2	14.5	66.3
Malaysia	13.0	36.0	51.0
European Union	5.6	27.7	66.7
Japan	4.4	27.9	66.4
United States	0.6	22.6	76.8

Source: www.cia.gov

Developed countries including the USA, Japan and EU countries have consistently maintained a small labour force – only around 1–5% of the total – in agriculture. These figures indicate that developed countries are able to make use of advanced technology in fruit production methods (and in agriculture in general), while less-developed countries still rely on a large workforce.

1.3 Global consumption of tropical and subtropical fruits

Fruits are consumed in different forms around the world: some are consumed fresh, while others are used as animal feed or in making processed products such as dried and canned fruit and pickles. There are four categories of fruit usage: animal feed, processing, food and other usages, with food being the most popular form of consumption. The 2005 FAO world food balance sheets (source: FAOSTAT), show that out of a total fruit supply of 517 million tonnes, 5 million tonnes were used as animal feed, 53 million tonnes were processed, 415 million tonnes were used as food and the remaining 44 million tonnes were used in various other ways.

In the same year, Asia consumed 233 million tonnes of fruit; the Americas consumed 116 million tonnes, Europe 101 million tonnes, Africa 63 million

tonnes and Oceania 5 million tonnes. In all continents except Oceania the majority was used as food: 87% in Asia, 80% in the Americas, 77% in Africa and almost 67% in Europe. In Oceania 41% of fruits were processed, with a relatively large percentage also being processed in Europe. On the other hand, close to 4% of the fruits consumed in Africa was used as animal feed. Table 1.9 provides a detailed breakdown of fruit consumption. The world average per capita fruit consumption in 2003 was 78 kg (FAOSTAT), but the figures for individual countries vary considerably, from 1 kg to 317 kg per capita annually. These figures are shown in Table 1.10. The Americas have the highest per capita consumption of any continent, at 105.6 kg, followed by Europe at 92.5 kg, Africa at 53.4 kg, and Asia at 52.5 kg.

1.4 International trade in tropical and subtropical fruit

The market demand for fresh horticultural produce has been growing steadily since the 1980s. International trade in fruits and vegetables has expanded rapidly over the past two decades compared to trade in other agricultural commodities. FAOSTAT figures show that in 2005 around 44% of the world's acreage of fruit production was in Asia, but the quantity of export of fruits from Asia was only 19% of the world market, lagging behind the export quantities of other major fruit-producing regions such as the Americas, which took a 41% share of global exports and Europe, which provides a 33% share. (The remaining exports came from Africa and Oceania.)

The EU is the leading destination of temperate and subtropical fruit exports in the global fruit trade, and is also the principal source of supply. During 1999–2001, EU countries accounted for nearly 50% of the world's imports and over 40% of the exports of temperate and subtropical fruits. The EU imports almost one-third of its fresh subtropical fruit from banana-exporting countries and from South America, while only around 0.5% of the total tropical and subtropical fresh fruit imports (along with 3.3% of fruit and vegetable juices) come from Asian countries (source: FAOSTAT).

According to Filho *et al.* (2002), the temperate varieties of fruit mostly purchased by consumers in larger markets such as Europe and the United States dominate international trade. However, bananas were the fruit with the fastest growth in consumption during the 1990s (11% per year). The countries exporting the largest quantities of fresh fruit (counting temperate fruit together with tropical and subtropical fruit) are Spain, the United States, Italy, the Netherlands, France and Ecuador, which together account for 54% of sales by value. Main exporting countries are shown in Table 1.11. Spain is the world's largest exporter. In total, 47% of exports come from Europe while 36% come from the United States. Germany, the United States, Britain and France import 42% of the fresh fruit traded internationally. Germany is the largest importer (16%), followed by the United States (11%). Major fruit distribution centres in Europe are the Netherlands, Belgium, Luxembourg and France. France acts as a European distribution centre for pineapples and bananas.

Table 1.9 Fruit consumption by continent in 2005 (thousand tonnes)

Category	Asia	%	Americas	%	Europe	%	Africa	%	Oceania	%
Feed	873	0.37	1 163	1.00	350	0.35	2 387	3.76	3	0.06
Process	7 417	3.18	8 459	7.26	28 830	28.42	6 216	9.78	2 030	40.78
Food	203 432	87.27	93 446	80.18	67 613	66.65	48 698	76.62	2 845	57.15
Other	21 377	9.17	13 482	11.57	4 645	4.58	6 259	9.85	100	2.01
Total	233 099	100	116 550	100	101 438	100	63 560	100	4 978	100

Source: FAOSTAT (2008)

Table 1.10 Fruit consumption (excluding wine) by country (Kg/capita/year), 2003

Country	Kg	Country	Kg	Country	Kg	Country	Kg	Country	Kg
Dominica	317	Sweden	115	Argentina	83	Korea, Democratic People's Republic of	56	Sierra Leone	34
Belize	292	United Kingdom	115	Occupied Palestinian Territory	82	Libyan Arab Jamahiriya	55	Yemen	33
St Lucia	255	Germany	113	Peru	82	Vietnam	55	Nicaragua	32
Grenada	223	USA	113	Czech Republic	79	Azerbaijan	54	Myanmar	31
Bahamas	222	Dominican Republic	112	Former Yugoslav Republic of Macedonia	79	Japan	54	Pakistan	30
Uganda	193	Spain	112	Venezuela, Bolivarian Republic of	79	Malaysia	54	Sudan	30
Netherlands	182	Cyprus	109	Guyana	78	Kenya	53	Uzbekistan	30
Samoa	178	St Kitts and Nevis	108	Seychelles	77	Latvia	53	Angola	29
Antigua and Barbuda	168	Turkey	107	Paraguay	75	Swaziland	53	Congo, Democratic Republic of	29
Ecuador	167	Iceland	105	Honduras	74	Armenia	52	Tanzania, United Republic of	29
Iran, Islamic Republic of	158	Malta	105	Trinidad and Tobago	74	China	49	Netherlands Antilles	27
Costa Rica	157	Australia	103	Cameroon	73	Indonesia	49	Kyrgyzstan	25
Sao Tome and Principe	155	Colombia	103	Estonia	73	Russian Federation	48	Nepal	24
Jamaica	153	Philippines	103	Hungary	71	Turkmenistan	48	Cambodia	23
Rwanda	152	Switzerland	103	Uruguay	71	Chile	47	Namibia	21
Lebanon	150	Burundi	102	Moldova	70	Poland	47	Timor-Leste	19
Greece	147	Saudi Arabia	102	New Caledonia	70	Belarus	46	Mozambique	18

Denmark	146	Guinea	99	Côte d'Ivoire	69	Central African Republic	46	Mongolia	16
Gabon	146	Brunei Darussalam	98	Belgium	68	Liberia	46	Djibouti	15
Slovenia	142	St Vincent and Grenadines	97	Lithuania	66	Bulgaria	45	Kazakhstan	15
Cuba	141	Brazil	95	Nigeria	66	Cape Verde	45	Lesotho	14
Austria	137	France	95	Suriname	66	Guinea-Bissau	43	Senegal	14
Ireland	137	Bermuda	94	El Salvador	65	Madagascar	43	Mauritania	12
Italy	130	Vanuatu	94	Romania	64	Botswana	41	Zimbabwe	12
United Arab Emirates	128	Finland	91	Korea, Republic of	63	Mauritius	41	Chad	11
Norway	124	Albania	90	Guatemala	62	Kuwait	40	Bangladesh	10
New Zealand	121	Maldives	90	Morocco	62	South Africa	40	Ethiopia	10
Barbados	120	Egypt	89	Kiribati	61	Sri Lanka	40	Togo	9
Mexico	120	Croatia	88	Algeria	60	Fiji	39	Zambia	9
Canada	119	Haiti	86	Georgia	59	Malawi	38	Burkina Faso	5
Ghana	118	Panama	86	Slovakia	59	Solomon Islands	38	Niger	5
Serbia and Montenegro	118	Thailand	86	French Polynesia	58	India	37	Gambia	3
Portugal	116	Comoros	85	Jordan	58	Ukraine	36	Tajikistan	3
Bolivia	115	Tunisia	85	Bosnia and Herzegovina	57	Lao People's Democratic Republic	35	Mali	2
Israel	115	Syrian Arab Republic	84	Congo	56	Benin	34	Eritrea	1

Source: FAOSTAT (2008)

Table 1.11 Selected top five tropical fruit exporting countries (quantity and value) 2007

Area	Quantity (tonnes)	Value (US$'000)
Mango, Mangosteen and Guava		
India	240 858	163 622
Mexico	236 004	119 187
Netherlands	80 598	114 408
Brazil	116 271	90 102
Peru	82 512	63 674
Pineapple		
Costa Rica	1 353 027	486 860
Belgium	263 811	268 803
Netherlands	190 626	194 555
Philippines	270 054	147 807
USA	89 269	88 526
Tangerine		
Spain	1 652 428	1 682 204
China	399 986	174 750
Morocco	243 983	159 209
Turkey	257 935	111 625
Netherlands	95 689	107 118
Papaya		
Mexico	101 306	55 327
Brazil	32 267	34 404
USA	9 604	17 715
Netherlands	8 625	16 907
Belize	33 341	13 101
Banana		
Belgium	1 167 511	1 303 559
Ecuador	5 174 565	1 282 036
Philippines	1 793 930	856 447
Costa Rica	2 272 332	675 406
Colombia	1 639 833	531 765
Watermelon		
Mexico	484 676	191 050
Spain	288 673	179 295
USA	177 793	91 591
Panama	168 259	87 435
Netherlands	79 977	54 072

Source: FAOSTAT (2008)

Spain is the leading example of specialization. This country supplies the world table orange market, which is a niche market as most oranges are processed into juice. Spain accounts for 49% of world table orange supply. Despite the fact that its production has been declining at an annual rate of 2%, exports have risen by an average of 5% per year over the past decade. It exports close to 1.6 billion tonnes and earned US$1.6 billion from exports of tangerines in 2007.

Ecuador, the world's third largest producer of bananas, is another case of specialization. This country's contribution to world fruit trade is limited to this crop, yet its share of the market is 17%. Due to its involvement in the banana trade, Ecuador is the world's fourth largest international exporter of fresh fruits. The United States is the leading example of diversification, making a significant contribution to world production, import and export (with the exception of banana production). According to these authors, the US position in the orange market is particularly notable. Production has been boosted at a rate of 7% per year, while imports have fallen (by 13% per year) and exports risen (by 11% per year), in a market that has grown at 5% per year (Filho *et al.*, 2002)

According to Rabobank International, four trading companies controlled 80% of world trade in fruits in 1997. Larger players in the European and US fruit markets achieve annual revenues in excess of US$1 billion, notably the two US groups, Dole Foods (US$6.9 billion in 2007) and Chiquita (US$3.6 billion in 2008) (www.unctad.org/infocomm/francais/orange/Doc/brazil.pdf).

The World Trade Organization (WTO) agreement reached in Marrakesh on 15 April 1994, which followed the conclusion of the Uruguayan Round of the General Agreement on Tariffs and Trade (GATT) talks is particularly relevant for the development of trade in tropical and subtropical fruit. According to Katz and Weaver (2003), these agreements essentially established the principle of free trade not exposed to arbitrary market entrance taxes, and furthermore obligate signatory countries to use only sanitary and phytosanitary quarantine measures based on solid scientific information. Therefore the use of these measures as a loophole to arbitrarily restrict imports was effectively halted.

1.5 Price of tropical and subtropical fruit

1.5.1 Price trends of selected fruits

The increased awareness of the health benefits of fruit consumption in the 1980s has led to an expansion of fruit trade especially from developed countries. This resulted in a double-digit growth in the export of mangoes and avocados during 1989–2001, while the overall fruit exports grew by 4.2% during the same period. Most tropical fruit exporting countries have taken advantage of the growing fruit trade, with Thailand, China and the Philippines taking the lead for Asian production. The expansion in production and supply exerted a downward pressure on prices and tended to depress margins due to intense competition among exporting developing countries. Mangoes are a prime example of this, with prices in Europe falling by 30% since 1988 in conjunction with a 66% increase in import volumes.

Table 1.12 Price trends of selected tropical fruits

	2003	2004	2005	2006	2007	Average price
Papaya	351	443	607	695	1038	627
Pineapple	481	489	547	653	723	579
Tangerine	426	447	493	528	593	497
Lemon	461	434	447	507	588	487
Orange	390	403	460	509	508	454
Banana	360	379	430	472	560	440
Pomelo	336	341	367	415	446	381
Watermelon	223	245	266	279	309	264

Source: FAOSTAT (2008)

But since 2000, the prices of most tropical fruits have recovered, changing the overall market scenario for certain fruits (Sing Ching Tongdee, 2004).

As can be seen from Table 1.12, despite the differences in price received by fruit producers, the general trend of world fruit prices has shown a consistent increasing trend since 2003. The price of papaya has increased from US$351/tonne in 2003 to US$1038/tonne in 2007 while pineapple prices have increased from US$481 to US$723/tonne during the same period. Among the citrus fruits, tangerine prices have increased from US$426/tonne to US$593/tonne during 2003 to 2007. The average prices of selected popular fruits have ranged from US$264 to US$627/tonne as shown in Table 1.12: papaya and pineapple have always commanded higher prices as compared to pomelo and watermelon.

1.5.2 Demand and supply of tropical and subtropical fruit

There have been several studies on the price demand elasticities for tropical and subtropical fruits. Most of the studies have analysed the demand and expenditure/income elasticities for some of the more popular tropical fruits. It is expected that when prices increase, consumer demand will decrease and similarly, when prices decrease, consumer demand will increase, provided that other variables that can influence demand remain constant. If the price elasticity is elastic it means that consumer demand is very responsive to price changes. For example, if the price decreases by 1%, demand will increase by more than 1%, and if the price increases by 1%, demand will decrease by more than 1%. Similarly, expenditure/income elasticity measures changes in consumer demand caused by changes in income. It is expected that demand will increase when income increases. It is important to understand the concept of price and income elasticities as they have the potential to influence demand for fruits in the global market.

Fatimah *et al.* (2005) analysed the Malaysian household budget and estimated income elasticities for the individual fruits. Fruits are generally considered to be a complementary product with respect to all other food items. Starfruit has the highest income elasticity, at 1.104, suggesting that as Malaysian household

incomes rise by 1%, the budget share of starfruit will rise by 1.104%. In other words, Malaysian consumers will consume more fruits as per capita income increases. Melon and jackfruit have the lowest income elasticities, at 0.257 and 0.225 respectively. In general, the income elasticities of imported fruits and processed fruits are higher than those of local fruits. Among the imported fruits, grape has the highest income elasticity of 0.961, while processed fruits have an elasticity of 0.918.

Mubarak *et al.* (2006) studied the demand elasticities for fruits and vegetables in Vietnam. The own-price elasticities of cereals and vegetables are inelastic, implying that they are an integral part of the Vietnamese diet. Fruits have an own-price elasticity of −0.55, indicating that consumers in Vietnam are not very responsive to changes in fruit prices. The elasticity implies that as price of fruits decreases by 1%, the demand for fruits will increase by only 0.55%. The cross price elasticities were mostly negative, suggesting separability among the food groups.

Dong *et al.* (2009) used retail purchase data to investigate the US demand for organic and conventional fresh fruits. All expenditure elasticities are positive, ranging from 0.81 for organic bananas to 1.03 for organic oranges and from 0.98 for conventional bananas to 1.01 for other major conventional fruits. The results show that the expenditure elasticities for organic and conventional fruits tend to be close to unity, implying that given an increase in the spending on fresh fruits, consumers would allocate approximately the same proportional increase to their purchase of conventional and organic fresh fruits. Thus, households with higher incomes are more likely to purchase some organic fruits and spend more on fresh fruits as a whole.

An FAO study (Anon., 1998) estimated elasticity (own-price, cross-price and income) for three major fresh tropical fruits (mangoes, avocados and papayas). Results indicate that consumers in all three markets – the USA, the EU and Japan – react quite strongly to price changes affecting these fruits. By contrast, for fruits with smaller volumes (mangosteens, longans and litchis), consumption was price inelastic in most markets. Similarly, substitution and income effects of the fruits were significant factors affecting consumption.

As can be seen in Table 1.13, of the three fruits imported to Europe, papaya was the most responsive to a change in its own price (own price elasticity of −2.73), indicating that as price decreases by 1%, the demand for papaya will increase by 2.73%. Similarly the cross-price elasticity of papaya with other fruits is elastic, implying that as the prices of other fruits increase, the demand for papaya will also increase. Thus, there is a substitutability effect between papaya and other fruits (cross-price elasticity of 1.92) indicating that as the prices of other fruits increase, consumer demand for papaya will also increase. In the EU, the demand response to an increase in income results in a significant increase in demand for avocados and mangoes, but not for papayas.

Consumer response to price changes in the United States differed from that observed in other markets. Own-price elasticities were much lower for papayas at −0.07, while avocados proved more price elastic at −1.48. Cross-price elasticities

Table 1.13 Estimated own-price, cross-price and income elasticities

	EC's import demand		
	Own-price	Cross-price	Income
Avocados	−2.58	1.07	1.74
Mangoes	−2.49	1.78	1.73
Papayas	−2.73	1.92	0.66
	United States import demand		
	Own-price	Cross-price	Income
Avocados	−1.48	0.34	3.23
Mangoes	−0.74	5.59	1.39
Papayas	−0.07	2.20	0.91
	Japan's import demand		
	Own-price	Cross-price	Income
Avocados	−2.01	0.20	1.22
Mangoes	−1.41	0.34	3.23
Papayas	−2.95	0.95	0.4

Source: Dong and Lin (2009)

for mangoes were surprisingly high at 5.59. As expected, the income effect was low for papayas and highest for avocados, with mangoes in-between.

In Japan, the own price elasticities for avocados, mangoes and papayas are highly sensitive to price changes, particularly papayas (−2.95). Of the three major markets, the Japanese market shows the lowest cross-price effects, indicating that these fruits have few substitutes. The response to income changes is again highest for mangoes and lowest for papayas.

Similar results were observed for papayas in all three markets. However, for avocados and mangoes, the demand response to increases or decreases in income differed across the three markets. In Japan, the highest demand response was for mangoes, while in the United States it was for avocados; in the EU, mangoes and avocados showed similar results.

1.6 Conclusions

In general there has been an increasing trend towards greater production of major tropical and subtropical fruits over the years. This could be due to higher productivity or alternatively could be the result of fruits being cultivated in more areas. The per capita consumption of fruits varies considerably between countries, with the highest being 317 kg per capita and the lowest 1 kg per capita. It is interesting to note that there is no clear correlation between per capita consumption figures and the level of economic development of a country or region: in some less-developed regions the per capita consumption of fruits is in fact higher than

in some developed regions. Thus, there is potential for the tropical and subtropical fruits industry to expand or to increase its market share in developed and developing regions. For example, per capita consumption in Japan and Kuwait – categorised as developed countries – is only 54 and 40 kg per capita respectively. Given the right promotion and compliance with trade regulations and the non-tariff barrier (NTB), the consumption of fruits in such countries could be increased in the future. In less-developed nations such as Togo, Niger and Mali, which have lower levels of consumption, educational and promotional programmers need to be put in place in order to increase fruit consumption among the population. This is particularly important since fruits are considered to be one of the principal sources of the vitamins and minerals necessary for a healthy diet.

Asia contributes almost half of the world production of tropical and subtropical fruits, with China producing the largest share of any country. About 30% is contributed by the Americas, and the rest is shared between other parts of the world. The most popular fruits both in terms of production and consumption include watermelons, bananas, grapes, oranges, mangoes, tangerines, pineapple, papaya, citrus and dates, to name a few. Most of these fruits are found in subtropical regions.

The cultivation of fruits forms part of the agricultural activities of a country and makes an extremely important contribution to GDP and employment levels, especially among the less developed nations which still depend on agriculture as their main economic activity. For example, in Ethiopia, the Republic of Congo and Sudan about 80% of the population is engaged in agriculture, while nations such as the US, Japan and the EU countries are less dependent on agriculture, which contributes only around 1–2% of their GDP.

Although Asia is the largest producer of tropical and subtropical fruits, exports from Asia account for only about 19% of the world market, possibly due to the high consumption levels among the Asian population. About 41% of global exports come from the Americas, with Dole Foods, Chiquita and Del Monte among the major players in the fruits market.

In general, the price demand elasticities of most fruits are price elastic, indicating that consumer demand for fruits is responsive to price fluctuations. An increase in prices will usually cause the demand to decrease, and a decrease in prices will cause demand to increase. Thus setting prices for fruits is crucial in ensuring an adequate level of consumer demand: there are always substitute products for fruits, and unlike tubers and grains, fruit is not a staple food in most parts of the world.

1.7 References

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2

Nutritional and health-promoting properties of tropical and subtropical fruits

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Abstract: A worldwide increase in the consumption of tropical and subtropical fruits has been observed recently. These fruits are very diverse and contain many nutritional components that have been associated with the prevention of diseases such as cancer and cardiovascular disease, among others. This chapter lists and describes some of the important nutritional components of tropical and subtropical fruits and their effects on health. The effects of pre- and postharvest treatments on the nutritional components of tropical and subtropical fruits are also discussed, including the effects of postharvest preservation technologies and processing. The effects of these compounds on human nutrition and health are emphasized, analyzing the mechanisms involved in their protective actions. Finally, the synergistic effect of the multiple bioactive fruit components is presented, as pure bioactive compounds do not exert the same health effects as the actual consumption of tropical and subtropical fruits.

Key words: postharvest, vitamins, minerals, fiber, pigments, antioxidants, phenolics, degenerative diseases, cancer, cardiovascular diseases, nutraceuticals.

2.1 Introduction

Increasing incidences of some chronic diseases such as cancer and cardiovascular problems have raised awareness of the importance of diet (Erbersdobler, 2003). Numerous epidemiological studies suggest that abstaining from smoking, increasing intake of fruits and vegetables, and preventing infectious diseases can have a major effect on reducing rates of several chronic diseases including

cardiovascular disease and different types of cancer (Percival *et al.*, 2006; Yahia, 2010). Therefore, interest in the health benefits of fruit and vegetable consumption, and in understanding the mechanism and mode of action of the different components that confer health benefits, is increasing.

Fruits are considered rich sources of some essential dietary micronutrients, fibers, and more recently they have been recognized as important sources of phytochemicals that individually, or in combination, may benefit health (Table 2.1) (Rechkemmer, 2001; Yahia, 2010). The nutritional compounds and phytochemicals present in tropical and subtropical fruits are very diverse and often include ascorbic acid, carotenoids and phenolic compounds (Table 2.1) (Percival *et al.*, 2006; Yahia, 2010). They have considerable antioxidative activity *in vitro* which can have important implications for health. A diet rich in fruits and vegetables increases antioxidants concentration in blood and body tissues, and potentially protects against oxidative damage to cells and tissues (Yahia, 2010). However, other more complex mechanisms also seem to be involved in the protective effects of these compounds (Yahia and Ornelas-Paz, 2010). Clarification of the potential protective roles of specific antioxidants and other constituents of tropical and subtropical fruits deserves major attention.

Table 2.1 Some nutrients and phytochemicals in some tropical and subtropical fruits that were established or proposed to have a positive effect on human health (modified from Kader, 2001)

Nutrient/phytochemical	Source	Established or proposed effects
Vitamin C (ascorbic acid)	Cantaloupe, citrus fruits, guava, pineapple, mango, papaya	Prevents scurvy, aids wound healing, healthy immune system, cardiovascular disease
Vitamin A (carotenoids)	Orange-fleshed fruits such as papaya, mango, pineapple, citrus	Night blindness prevention, chronic fatigue, psoriasis, heart disease, stroke, cataracts
Vitamin K	Nuts	Synthesis of procoagulant factors, oesteoporosis
Vitamin E (tocopherols)	Nuts, avocados, olives	Heart disease, LDL-oxidation, immune system, diabetes, cancer
Fiber	Most fruits and nuts	Diabetes, heart disease
Folate (folicin or folic acid)		Birth defects, cancer, heart disease, nervous system
Calcium	Almonds, orange, raisin, papaya	Osteoporosis, muscular/ skeletal, teeth, blood pressure
Magnesium	Banana, nuts	Osteoporosis, nervous system, teeth, immune system
Potassium	Banana, plantain, cantaloupe	Hypertension, nervous system, teeth, immune system
Phenolic compounds		
Proanthocyanins: tannins	Pomegranates	Cancer

Anthocyanidins: cyanidin, malvidin, delphinidin, pelargonidin, penidin, petunidin	Grape, pomegranate	Heart disease, cancer initiation, diabetes, cataracts, blood pressure, allergies
Flavan-3-ol: epicatechin, epigallocatechin, catechin, gallocatechin	Mango	Platelet aggregation, cancer
Flavonones: hesperetin, naringenin, eriodictyol	Citrus fruits	Cancer
Flavones: luteolin, apigenin	Guava	Cancer, allergies, heart disease
Flavonols: quercetin, kaempferol, myricetin, rutin	Papaya	Heart disease, cancer initiation, capillary protectant
Phenolic acids: caffeic acid, chlorogenic acid, coumaric acid, ellagic acid		Cancer, cholesterol
Carotenoids		
Lycopene	Papaya, Brazilian guava, red grapefruit	Cancer, heart disease, male infertility
β-Carotene	Mango, cantaloupe, papaya	Cancer
Xanthophylls: lutein, zeaxanthin, β-cryptoxanthin		Macular degeneration
α-Carotene	Cantaloupe, kiwifruit, mango, papaya,	Tumor growth
Monoterpenes: limonene	Citrus (grapefruit, tangerine)	Cancer
Sulfur compounds: glucosinolates, isothiocyanates, indoles, allicin, diallyl disulphide	Brassicas	Cancer, cholesterol, blood pressure, diabetes

It is argued that increasing intake of fruits and vegetables to 400–800 g per day is an adequate public health strategy. For this reason, the World Health Organization (WHO) recommends a daily intake of at least 400 g of fruits and vegetables per person, and health authorities worldwide follow this recommendation. This recommendation has been very difficult to reach in both developed and developing countries due to several reasons. The implementation of policies either at the economic, sanitary, trade or health level may help to reach this goal.

With regard to plant physiology, several factors affect the synthesis and metabolism of bioactive compounds in tropical and subtropical fruits. Climatic conditions, especially temperature and light, have major effects both before and after harvest. Factors such as soil type, irrigation, fertilization, mulching, rootstocks, and several other cultural practices are of great importance (Goldmann *et al.*, 1999). Maturity at harvest, harvesting methods, precooling, delays between harvest and consumption or processing, and several postharvest techniques (such as refrigeration, heat treatments, modified and controlled atmospheres, processing

techniques, and cooking) are other factors that can have major effects on nutritional and health components (Kader, 2001; Yahia, 2010).

This chapter will highlight the potential nutritional and health importance of tropical and subtropical fruit.

2.2 Consumption

Despite the relatively low caloric values of tropical and subtropical fruits (banana, plantain and avocado are the notable exceptions), they play an important role in human diet mainly because of their high and diverse content of vitamins, minerals and bioactive compounds. They have been of key importance in the tropics, being a large component of the diet there since ancient times, either by collecting fruit from the wild or by cultivating plants. More recently, they have also become an important part of the diet of people in developed countries, especially due to health and fitness consciousness. Whether these fruits are chosen as a healthy choice or a staple food they have excellent nutritional and health components. Nutritionists have long recommended a minimum of 400 g of fruit per day, and that it should be as varied as possible. Toward the end of the twentieth century marketing campaigns commonly recommended consumption of five fruit portions per day, which, while it may have more to do with commerce than with science, does reinforce the value of fruit as a part of the human diet.

Collection and transport from the field to the storage facility or marketplace do not significantly deplete the nutritional and organoleptic properties or the antioxidant capacity of fruits. This is not the case, however, for fruits that undergo lengthy storage duration, since several components such as vitamins and polyphenols are quite sensitive to modifications of enzymatic oxidases and chemical oxidation occurring during storage. The control of environmental conditions and the extent of the storage time of fresh fruits represent important steps for maintaining their nutritional quality (Ninfali and Bacchiocca, 2004).

Tropical and subtropical fruits are usually consumed fresh, although there are a few exceptions such as the case of breadfruit which is only eaten cooked. Nuts can be eaten directly or processed (roasted, candied). Salads, both savory and sweet types, are prepared with many tropical and subtropical fruits. Several processed products are made from fresh tropical and subtropical fruits, and include jams, jellies, juices, sauces, ice cream and sherbets, and other desserts and diverse confectionary items, infusions such as social beverages, medicinal remedies, etc. Baby foods are made with ‘healthy’ fruits like banana and mango, based on different kinds of puree. Flour is also made from some tropical and subtropical fruits such as durian, banana and plantain. Consumption of flour from mango kernels has been practiced for a long time in India. Pickles and chutneys are made from many fruits, the most famous being mango chutney, a staple in South Asian cuisine. Dips are also popular in many countries, of which the best known is avocado-based guacamole. Guava paste or spread is consumed, usually with bread and cheese, in many countries, particularly in Cuba, Brazil and the Canary Islands.

2.3 Health components of tropical and subtropical fruits

Tropical and subtropical fruits are rich sources of different nutrients and also have some medicinal properties. Many tropical fruits, notably mango and papaya, are good sources of β-carotene (provitamin A). An indication of the high content of this vitamin is the orange-yellow color of the flesh. Others, including all citrus fruits and the guava, are well known as good sources of ascorbic acid (vitamin C). In general they are not a good source of the B-group vitamins (thiamine, riboflavin and niacin) except for nuts, which are also a good source of vitamin E, proteins and fats (Yahia, 2011a). Tropical and subtropical fruits are also rich in pectin, fiber and cellulase, which promote intestinal motility. In common with other fruits, they are good sources of antioxidants, and some are also good sources of organic acids, which stimulate appetite and aid digestion.

Nuts and banana have high calorie content. Avocado has a high oil content (of the different avocado races, the West Indian types have the lowest) composed of highly digestible unsaturated fatty acids, rich in folic acid, and some cultivars contain good quantities of proteins, vitamin A, riboflavin, and phosphorus (Whiley and Schaffer, 2006). Fruits with greater caloric content such as the macadamia nut are also rich in protein, oil, iron, calcium, thiamine, riboflavin and niacin.

Guava, which is high in vitamin C, also has good amounts of niacin and iron. Papaya has high quantities of vitamins C and A as well as potassium and calcium, and it is low in carbohydrates. However, its outstanding feature, which distinguishes the papaya from all other fruits, is the fact that it contains papain, a proteolytic enzyme that promotes digestion (although papain content decreases as the fruit ripens). It is highly recommended for people with certain digestive disorders. Passion fruit is rich in provitamin A and carbohydrates and is an acceptable source of vitamin C and niacin. The pineapple is also rich in vitamin C and carbohydrates and is a good source of calcium, phosphorus, iron, potassium and thiamine (Yahia, 2010).

The litchi, the longan, most of the Annonaceae, and the durian are all good sources of carbohydrates and vitamin C. The durian also has fair amounts of iron and niacin. Mangosteen is considered by many to be one of the finest tasting fruits of all, and therefore it is known as 'queen of fruits' (Yaacob and Tindall, 1995). Although it has a comparatively low nutrititional value, it has reasonable amounts of calcium, phosphorus, ascorbic acid and carbohydrates. The carambola is low in calories and rich in vitamin C, and it is an adequate source of vitamin A. It is not recommended for people with kidney problems (specifically stone formation) due to its high oxalic acid content, but new cultivars have been selected for lower oxalic content while maintaining sugar and vitamin levels (Galán-Saúco, 2002). Dates are rich in carbohydrates, a good source of vitamin A, potassium and iron, but low in fat and sodium.

The coconut is high in phosphorus, iron, proteins and fats – in this case all saturated fatty acids, the consumption of which should be limited according to health recommendations. Coconut milk aids in balancing pH in the body due to its alkaline reaction. The next section describes in detail the effect of vitamins, fiber and minerals of importance in tropical fruits on consumers' health.

2.3.1 Vitamins

Vitamin A

Vitamin A includes a group of natural and synthetic compounds, generically named retinoids. Major retinoids and their metabolites are all-*trans*-retinol, 3,4-didehydroretinol, all-*trans*-retinal, 11-*cis*-retinal, all-*trans*-retinoic acid, 9-*cis*-retinoic acid and retinoyl-*β*-glucuronide (Yahia and Ornelas-Paz, 2010). Fruits do not contain vitamin A; however, they are rich in carotenoids, which are precursors of this vitamin. Only carotenoids containing a *β*-ionone ring without oxygenated groups can be considered precursors of vitamin A. The main dietary provitamin A carotenoids are *β*-carotene (the major precursor), *α*-carotene and *β*-cryptoxanthin. Generally, fruits and vegetables provide 25–35% of total retinol intake. The content of provitamin A carotenoids and the vitamin A value of tropical and subtropical fruits are shown in Tables 2.2 and 2.3. It must be noted that carotenoids from tropical fruits are up to four times more bioavailable than those from vegetables (De Pee *et al.*, 1998). Recently, Ornelas-Paz *et al.* (2010) demonstrated in experimental rats that vitamin A content derived from *β*-carotene from 'Ataulfo' mango is 1.5 times higher than that from carrots. Differences in the method of carotenoids storage in the cells of fruits and vegetables seem to be

Table 2.2 Content (μg 100 g^{-1}) of provitamin A carotenoids in some tropical and subtropical fruits

Fruit	*β*-carotene	*α*-carotene	Lutein+zeaxanthin	*β*-cryptoxanthin
Avocado	62	24	271	28
Banana	26	25	22	
Cantaloupe	2020	16	26	1
Carambola	25	24	66	0
Fig	85	0	9	0.11
Grape	39	1	72	0
Grapefruit	14–686	3–8	5–10	3–6
Guava	374		0	0.73
Honeydew	30	0	27	0
Key lime	30	0	0	
Kiwifruit	43–52	0	114–122	1.4
Mandarin	155	101	138	407
Mango	445	17	0	11
Muskmelon	0–2020	0–16	26	0–1
Orange	71	11	129	11.6
Papaya	55	0	75	761
Passion fruit	743	0	0	41
Peach	30–1480	0–9	9–112	12–510
Pineapple	31–35	0	0	
Prickly pear	25	0		3
Soursop	1			
Tamarind	18	0	0	
Watermelon	303	0	8	78

Sources: USDA, 2005; van den Berg *et al.*, 2000

Table 2.3 Vitamin content in some tropical and subtropical fruits per 100 g of fresh tissue

Fruit	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)	Pantothenic acid (mg)	Vitamin B-6 (mg)	Total folates (µg)	Choline (mg)	Vitamin A, IU1	Vitamin E (α-tocopherol) (mg)	Vitamin E (γ-tocopherol, mg)	Vitamin K (phylloquinone) (µg)
Avocado	0.05–0.1	0.2–0.14	1–2.9	8–15	1.389	0.257	81	14.2	146	2.07	0.33	21
Banana	0.02–0.19	0.03–0.026	0.4–1	1.1–15	0.33	0.36	20	9.8	64	0.1	0.02	0.5
Bitter orange	0.11	0.03	0.4	51								
Black sapote	0.03	0.04	0.04	21								
Blackberry	0.02	0.03–0.04	0.02–0.4	15–83								
Chico sapote	0–0.02	0.02–0.09	0.2–0.6	8.9–14.7	0.25	0.037	14		60			
Coconut	0.01–0.5	0.01–0.1	0.1–2.3	0–4.5	0.3	0.05	26	12.1		0.24	0.53	0.2
Fig	0.01–0.06	0–0.05	0.3–0.7	0.6–15	0.3	0.11	6	4.7	142			4.7
Grape	0.01–0.069	0.01–0.13	0.1–0.5	1.4–11	0.05	0.086	2	5.6	66	0.19	0.07	14.6
Grapefruit	0.034–0.043	0.01–0.031	0.19–0.26	31.2–50.6	0.26–0.283	0.042–0.053	9–13	7.7	10–1150	0.13	0	0
Guava	0.03–0.07	0.03–0.06	0.4–1.4	7.2–250	0.45	0.11	49	7.6	624			2.6
Key lime	0.02–0.04	0.01–0.06	0.2–1.3	21–45	0.12	0.04	8	5.1	50	0.22		0.6
Kiwifruit	0.02–0.024	0.02–0.05	0.28–0.5	92.7–98	0.18–0.5	0.05–0.06	25–34	5–7.8	72–87		0.01–0.03	5.5–40.3
Lemon	0.02–0.09	0.01–0.06	0.2–0.6	30–77								
Mamey	0.02–0.04	0.03–0.06	0.4–1.5	14–59	0.1	0.1	14		230			
Mandarin	0.04–0.11	0.02–0.08	0.1–2	23–72	0.21	0.07	16	10.2	681	0.2	0	
Mango	0.03–0.06	0.02–0.11	0.2–0.6	19–80	0.16	0.13	14	7.6	765	1.1		4.2
Muskmelon	0.04–0.05	0.019–0.04	0.23–0.9	14.8–74	0.084–0.1	0.07–0.16	8–21	7.6	0–3382	0.05	0.11	2.5
Orange	0.04–0.1	0.01–0.12	0.1–0.5	42.2–92.3	0.25	0.05–0.063	17–39	8.4	225–230	0.18		0
Papaya	0.027–0.03	0.02–0.07	0.3–0.4	31.2–75	0.21	0.019	38	6.1	1094	0.73		2.6
Passionfruit	0–0.01	0.13–0.17	0.8–1.5	20–237		0.1	14	7.6	1272	0.02		0.7
Pineapple	0.078–0.09	0.029–0.05	0.2–0.57	10–56.4	0.19–0.217	0.106–0.114	11–19	5.4–5.6	52–58	0.02	0	0.7
Pomegranate	0–0.09	0.1–0.13	0.2–1.6	5–30	0.37	0.07	38	7.6		0.6		16.4
Pomelo	0.01–0.06	0.02–0.07	0.2–0.6	25–53								
Prickly pear	0.01–0.014	0.02–0.06	0.3–0.5	9–25.5		0.06	6		43			
Soursop	0.04–0.07	0.05–0.07	0.6–1.7	19–21	0.25	0.05	14	7.6	2	0.8		0.4
Tamarind	0.2–0.43	0.15–0.19	0.7–2.5	1–20	0.14	0.06	14	8.6	30	0.1		2.8
Watermelon	0.02–0.08	0.01–0.04	0–0.2	3–10	0.22	0.04	3	4.1	569	0.05	0	0.1

Note: [1]International units
Source: USDA, 2005

responsible for these results. Vitamin A and its bioactive metabolites exert pleiotropic effects in vertebrate organisms, modulating cell development, proliferation, metabolism and apoptosis (Wolf, 1984). Vitamin A is needed for growth (Hadi *et al.*, 2000) and is essential for reproduction (Clagett-Dame and DeLuca, 2002). This vitamin is important in the visual process and prevents several diseases of the eye, including night blindness, xeroftalmy, conjunctive xerosis and corneal diseases (Krinsky *et al.*, 2003). Vitamin A is also involved in immune function (Humphrey *et al.*, 2006). Other biological actions of vitamin A have been described elsewhere, including its anticancer effects (Wolf, 1984).

Vitamin C

Vitamin C or ascorbic acid has long been known for its ability to cure and prevent scurvy. Dehydroascorbic acid, the oxidized form of ascorbic acid, retains vitamin C activity (Johnston *et al.*, 2007). This vitamin is biosynthesized by plants and the majority of vertebrates. A few mammalian species (primates, humans, guinea pigs, etc.) are unable to biosynthesize ascorbic acid and therefore they have to take it from dietary sources. Ascorbic acid is biosynthesized from carbohydrates (Davey *et al.*, 2000). Tropical and subtropical fruits are rich in ascorbic acid. Acerola fruit contains the highest known ascorbic acid content among all fruits (1000–3300 mg 100 gm^{-1} fresh weight), and some other good sources of vitamin C include guava, litchi, papaya and passion fruit (Yahia, 2006). Mango fruit is also a rich source of vitamin C (Carrillo-Lopez *et al.*, 2010; Siddappa and Bhatia, 1954; Thomas, 1975; Yahia, 2006). Citrus fruit and their juices are rich sources of vitamin C: an 8 fl-oz serving of citrus juice was reported to supply the entire Recommended Daily Intake (RDI) amount of vitamin C (Rouseff and Nagy, 1994). However, there is considerable variation in the vitamin C content of juices of different citrus fruits. Grapefruit, tangerine and lemon generally contain between 20 and 50 mg 100 ml^{-1} juice (Yahia, 2006). The content of ascorbic acid in commonly consumed fruits is shown in Table 2.3.

Many biological actions have been attributed to ascorbic acid. It is one of the most effective and least toxic antioxidants identified in mammalian systems. Ascorbic acid protects lipids in plasma and low-density lipoproteins (LDL) against peroxidative damage (Davey *et al.*, 2000). Several studies have demonstrated that ascorbic acid may be important in protecting against oxidative stress-related diseases and degeneration associated with aging, including coronary heart disease, cataract formation, and cancer (Table 2.1). Ascorbic acid is also essential for collagen and carnitine synthesis. Vitamin C helps to maintain various enzymes in their required reduced forms. Ascorbic acid is a cofactor for the dopamine-β-hydroxylase and peptidylglycine α-amidating monooxygenase systems, which participate in the biosynthesis of norepinephrine and various α-amidated peptides (Sauberlich, 1994). Ascorbic acid is also involved in neural maturation, neuronal transmission, learning/memory and locomotor activity (Harrison and May, 2009). It participates in the normal functioning of fibroblasts and osteoblasts and immune function (Davey *et al.*, 2000).

Vitamin E

The term 'vitamin E' is used to describe a family of structurally related compounds, eight of which have been isolated from plants, fruits and vegetables (α, β, γ, and δ-tocopherol and α, β, γ, and δ-tocotrienol). α-Tocopherol is the most important form of vitamin E and possesses the highest antioxidant activity. This form is also the most effective in preventing vitamin E deficiency symptoms (Traber, 2007). Most fruits contain only low concentrations of vitamin E. The major sources of this vitamin are edible vegetable oils, including those from nuts and avocados (Yahia, 2010). The content of tocopherols in some commonly consumed tropical and subtropical fruits is shown in Table 2.3.

Vitamin E was discovered to be a nutritional factor that prevented the death and resorption of fetuses in pregnant rats. Other manifestations of vitamin E deficiency have been observed in different animal species, including testicular dystrophy in male rats, and injuries in the nervous system in chicks (Lang *et al.*, 1985). In humans, vitamin E deficiency has been related to an increased risk of suffering atherosclerosis and other degenerative diseases (Lass and Sohal, 2000). Extensive literature supports the fact that vitamin E has protective effects against cardiovascular disease, including the prevention of oxidative modification of low-density lipoproteins (LDL), modulation of the arachidonate cascade so as to reduce vasoconstriction and thrombosis, and inhibition of smooth muscle cell proliferation (Table 2.1) (Bramley *et al.*, 2000). Vitamin E also exerts protective effects against several forms of cancer (lung, esophagus, stomach, skin and large bowel) (McVean and Liebler, 1997). It protects the immune system, relieves the symptoms in patients suffering inflammatory conditions (arthritis, rheumatoid arthritis and osteoarthritis), and prevents cataract and age-related macular degeneration (Table 2.1). A possible protective effect of vitamin E on neurological disorders (Alzheimer's and Parkinson's diseases) has been proposed. Vitamin E is also known as the anti-ageing vitamin (Bramley *et al.*, 2000), and has good antioxidant properties.

Folates

The term 'folates' is used to describe a family of compounds consisting chemically of a pteridine ring attached to a *para*-aminobenzoate, which in turn is attached to the amino acid glutamate. These compounds differ in the oxidation state of the molecule, the length of the glutamate side chain, and the specific one-carbon units attached to the molecule. The number of glutamate units in the side chain of natural folates varies from five to eight. The fully oxidized monoglutamate form of the vitamin is referred to as folic acid, which rarely occurs in nature. Polyglutamyl folate is common in foods (Bailey, 2007). Fruits provide 35% of the total folate intake in adults in some European countries. Folate-rich fruits include avocados (6–8 μg 100 g^{-1}), bananas (14 μg 100 g^{-1}) and oranges (31 μg 100 g^{-1}) (Hall *et al.*, 1955; Scott *et al.*, 2000). The folates content in other fruits is shown in Table 2.3.

Folates exert several functions in the human body. They are required in biochemical reactions involving one-carbon reactions such as amino acid metabolism, pyrimidine and purine synthesis, and methylation reactions following

the formation of the body's primary methylating agent, S-adenosylmethionine (SAM). Consumption of folic acid significantly reduces the risk of neural tube defects. Poor folate status has been associated with an increase in cancer risk, with the strongest support for colorectal cancer and its precancerous lesion, adenoma. Elevated plasma homocysteine concentration is a significant risk factor for vascular disease, and folic acid supplementation lowers plasma concentration of this metabolite (Bailey, 2007).

Vitamin K

Vitamin K was discovered by Henrik Dam as an antihemorragic agent. The term vitamin K is used to describe 2-methyl-1,4-naphthoquinone and all derivatives of this compound that exhibit antihemorrhagic activity in animals fed a vitamin K-deficient diet. Vitamin K can be found in some tropical and subtropical fruits, including avocados (40 μg 100 g^{-1}), grapes (3 μg 100 g^{-1}), cantaloupe (1 μg 100 g^{-1}), bananas (0.5 μg 100 g^{-1}), and oranges (0.1 μg 100 g^{-1}). Vegetables and oils are better sources of vitamin K than fruits (Traber, 2007). The content of vitamin K in other fruits is shown in Table 2.3. The most important biological action of vitamin K in humans is related to its antihemorrhagic activity, although a possible protective effect of this vitamin on bone health and against the mineralization of cardiovascular tissues has also been postulated.

Other vitamins

Tropical and subtropical fruits provide low amounts of thiamin. Thiamin-rich fruits include oranges (0.11 mg 100 g^{-1}), avocados, breadfruit and cherimoya (Yahia, 2006; Bates, 2007). Fruits, except almonds (0.93 mg/100 g), are poor sources of riboflavin (vitamin B2); however, avocados contain low amounts of this vitamin (Hall *et al.*, 1955; Rivlin, 2007). Whole fruits and vegetables contain about 5–40 mg total choline per 100 g (Garrow, 2007). Fruits and vegetables do not contain vitamin B_{12} (Green and Miller, 2007). Breadfruit and cherimoya contain good amounts of niacin (Yahia 2006). Avocados are rich in vitamin B6 (3.9–6.1 μg g^{-1} pyridoxine) and to a lesser extent in biotin and vitamin D (Hall *et al.*, 1955; Yahia, 2010). The content of thiamin, riboflavin, niacin, pantothenic acid, vitamin B_6 and choline in some tropical and subtropical fruits is shown in Table 2.3.

2.3.2 Pigments

Chlorophylls

The term 'chlorophyll' comes from the Greek roots *chlorós* (green) and *phyllon* (leaf). This term includes several forms of 'chlorophyll' and their derivatives. Chlorophylls *a* and *b* predominate in plants, occurring in the approximate ratio of 3:1. Chlorophyll-rich fruits are kiwi (6.8 mg Kg^{-1}), olives (740–857 nmol $fruit^{-1}$), avocados (38–101 mg Kg^{-1}), and pistachio (25–160 mg Kg^{-1}, on a dry basis) (Robertson, 1985; Ju *et al.*, 1999; Bellomo and Fallico, 2007; Gallardo-

Guerrero *et al.*, 2007). Ashton *et al.* (2006) identified chlorophylls *a* and *b*, and pheophytins *a* in the skin, flesh and oil of avocado fruit. Chlorophyllides *a* and *b* were identified in the skin and flesh of avocado (Ashton *et al.*, 2006). Chlorophyll *a* was the most abundant pigment in several cultivars/lines of *Cactus pear* with the highest quantity in the line '21 441' (Castellanos-Santiago and Yahia, 2008).

Natural chlorophylls and their derivatives (pheophytins and pheophorbides) have shown antimutagenic and antigenotoxic properties in many *in vitro* tests (bacterial, *Drosophila* wing spot, and cultures of mammalian cells). *In vitro* studies also suggest that sodium copper chlorophyllin (SCC) protects against several environmental and dietary mutagens (3-amino-1-methyl-5H-pyrido-[4,3-b]indole, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), chromium (VI) oxide, aflatoxin B1, benzo[a] pyrene, benzo[a]pyrene-7,8-dihydrodiol-9-10-epoxide, 3-methylchloroanthracene, and 7,12-dimethylbenzo[a]anthracene). *In vivo* studies in rainbow trout and mice have demonstrated that SCC and natural chlorophylls have protective activity against hepatocarcinogenesis, skin carcinogenesis and papillomagenesis. The consumption of chlorophyll-rich foods has been positively correlated with a reduction in the risk of colon cancer in humans. Antioxidant activity, mutagen trapping, modulation of detoxification pathways and induction of apoptosis have been proposed as the cause of the cancer protective effects of chlorophylls and their derivatives. The literature suggests that the protective activity of natural chlorophylls is higher than those of commercial available chlorophyll derivatives (Ferruzzi and Blakeslee, 2007).

Flavonoids

Flavonoids are the pigments responsible for the color and flavor of many fruits, vegetables, flowers, nuts and seeds and therefore they are an important part of the human diet. Flavonoids belong to the family of phenolic compounds. The term 'flavonoids' is employed to describe a large group of structurally related compounds, including chalcones, flavones, flavonols, flavanones, flavanols, anthocyanins and isoflavones (Harborne and Williams, 2000). The content of flavonoids and other pigments in fruits depends on many factors including exposure to light, genotype, environmental conditions, ripening stage, processing, storage, postharvest handling and agricultural practices. Grapes are one of the most important dietary sources of phenolic compounds, including flavonoids (Frankel and Mayers, 1998). The phenolic compounds found in *Vitis vinifera* include phenolic acids, stilbenes, and flavonoids, which include flavonols, flavanols and anthocyanins, and play an important role in the quality of grapes and wines (Downey *et al.*, 2006). Anthocyanins are directly responsible for the color of grape fruit and young wines, whereas astringency and structure of wines are related to catechins, and proanthocyanidins, and flavonols contribute to bitterness (Hufnagel and Hofman, 2008).

Grapes and their products, such as grape juice and wine, have attracted a great deal of attention in recent years, and therefore, the composition and properties of grapes have been extensively investigated and their consumption is increasing.

Studies in other fruits such as ripe and green papaya using HPLC-MS have identified other phenols, and other catechin conjugates (Mahattanatawe *et al.*, 2006), which is consistent with the small number of compounds reported previously in this fruit (Agrawal and Agrawal, 1982). Quercetin and kaempferol, previously reported in leaves and shoots of *C. papaya*, were found only in trace amounts in fruit peel extracts (Canini *et al.*, 2007; Miean and Mohamed, 2001). Recently Rivera-Pastrana *et al.* (2010) found that ferulic acid, caffeic acid and rutin were the most abundant phenols identified in 'Maradol' papaya fruit exocarp. Ma *et al.* (2004) isolated and identified seven phenolic compounds in *Pouteria campechiana, P. sapota* and *P virids*, namely gallic acid, (+)-gallocatechin, (+)-catechin, (−)-epicatechin, dihydromyricetin, (+)-catechin-3-*O*-gallate, and myricitrin. The highest level of the seven phenolic compounds was found in *P. sapota*, the second highest in *P. viridis*, and the lowest in *P. campechiana*. Mangosteen pericarp is rich in 1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone, a phenolic compound with high bioactivity (Yu *et al.*, 2009). Alothman *et al.* (2008) reported that polyphenol content of Thai seedless guava was 123 to 191 gallic acid equivalents 100 g^{-1} (GAE 100 g^{-1}), that of *pisang mas* was 24.4 to 72.2 GAE 100 g^{-1}, and that of honey pineapple was 34.7 to 54.7 GAE 100 g^{-1}. They observed that the high phenol content was significantly correlated with high antioxidant capacity. The content of total flavonoids in some tropical and subtropical fruits is shown in Table 2.4.

A number of phenolic compounds, particularly flavonoids, are efficient antiproliferative agents, being able to inhibit proliferation of tumor cells by interfering with cell-cycle proteins or inducing apoptosis (Yang *et al.*, 2001). Flavonoids can markedly improve the condition of patients suffering from various stages of cardiovascular diseases; they can reduce hypertension and ameliorate some of the consequences of diabetes mellitus. Other favorable properties include protective effects against gastrointestinal ulcers, and they also inhibit neuronal transmitter receptors from activating pain neurons, stimulate the immune function in humans, exert antiallergenic effects and prevent the development of Alzheimer's disease (Wilson *et al.*, 1991; Efrat *et al.*, 1994; Havsteen, 2002).

Flavonoids have shown anticancer activity against carcinomas largely consisting of cells of ectodermal (skin, breasts, oral epithelium, urogenital tract, and lungs), entodermal (gastrointestinal tract, mammary glands, gonads, uterus, lungs, prostate, colon, rectum, and mesenchyme) and mesodermal (blood cells, bone, and muscle) origin (Table 2.1). Flavonoids are also effective against some tumors formed by oncogenic viruses (Harborne and Williams, 2000; Moon *et al.*, 2006). They have also shown high activity against many bacterial, protozoan, and fungal infections (Havsteen, 2002). The mechanisms by which polyphenols act as antitumoral agents are manifold and have been shown to be connected with the functions of radical scavengers, detoxification agents, cell signaling modulators, inhibitors of cell-cycle phases, and activators of apoptosis (Fresco *et al.*, 2006). Some flavonoids are able to achieve this effect by inhibiting the enzyme DNA topoisomerase II, which is necessary for the survival and spread of cancerous

Table 2.4 Content of phenolic compounds (mg $100g^{-1}$) in some tropical and subtropical fruits

Fruit	Quercetin	Myricetin	Kaempferol	Luteolin	Apigenin	Naringenin	Hesperetin	(+)-Gallocatechin	(+)-Catechin	(−)-Epigallocatechin 3-gallate	(−)-Epigallocatechin	(−)-Epicatechin 3-gallate	(−)-Epicatechin	Petunidin	Peonidin	Pelargonidin	Malvidin	Delphinin	Cyanidin
Avocado	0	0	0	0	0	0	0	0	0	0.15	0	0	0.37	0	0	0	0	0	0.33
Banana	0	0	0	0	0	0	0	0	6.10	0	0	0	0.02	0	0	0	0	7.39	0
Blood orange	0	0	0	0	0	1.68	13.12	0	0	0	0	0	0	0	0	0	0	0	0
Fig	0.93	0	0	0	0	0	0	0	0.15	0	0	0	0.02	0	0	0	0	0	0
Grape juice	0.64	0.21	0.01	0.01	0.01	0	0	0	0.19	0	0	0	0	0	0	0.02	0	0.47	0.56
Grapefruit (pink)	0	0	0	0	0	17.19	0.78	0	0	0	0	0	0	0	0	0	0	0	0
Grapefruit (white)	0.05	0.05	0	0	0	20.06	3.42	0	0	0	0	0	0	0	0	0	0	0	0
Grape (black)	2.54	0.45	0	0	0	0	0	0	10.14	0	0	2.81	8.68	0	0	0	0	0	0
Grape (red)	1.38	0.01		1.30	0	0	0	0	0.82	0	0.08	0.17	1.2	2.11	2.89	0.02	34.71	3.67	1.46
Grape (green)	1.62	0.3	0	0	0	0	0	0.01	3.73	0	0.02	0.25	1.7	0	0	0	0	0	0
Kiwi	0	0	0	1.12	0	0	0	0	0	0.09	0	0.01	0.27	0	0	0	0	0	0
Kumquat	0	0	0	0	21.87	57.39	0	0	0	0	0	0	0	0	0	0	0	0	0
Lemon	0	0	0	1.90	0	0.55	27.90	0	0	0	0	0	0	0	0	0	0	0	0
Mango	0	0.03	0	0	0.01	0	0	0	1.72	0	0	0	0	0	0	0.02		0.02	0.1
Medlar	0	0	0	0	0	0	0	0	0.02	0	0.01	0.23	0.53	0	0	0	0	0	0
Cantaloupe	0	0	0	0.64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Honeydew	0	0	0	0	0	0	0	0	0	0	0.04	0	0.01	0	0	0	0	0	0
Orange	0.58	0.02	0.01	1.13	0.01	15.32	27.25	0	0	0	0	0	0	0	0	0	0	0	0
Papaya	0	0.03	0.01	0.02	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pineapple	0	0.01	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pomelo	0	0	0	0	0	24.72	8.40	0	0	0	0	0	0	0	0	0	0	0	0

Source: USDA, 2005

cells (Fresco *et al.*, 2006). Indeed, phenolic compounds show anti-inflammatory activity, which is mediated by inhibiting the formation of transcription factors closely linked to inflammation, such as NF-κB and enzymes such as xanthine oxidase, cytochrome oxidase, and lipoxygenase, which mediate the inflammatory process (Chu *et al.*, 2002). Flavonoids have also been shown to reverse vascular endothelial dysfunction by increasing endothelium-derived nitric oxide bioactivity (Duffy and Vita, 2003).

Carotenoids

The term 'carotenoids' is used to describe a group of lipophyllic pigments. They confer a yellow, orange and red color to some fruits, vegetables, flowers, grains and animals. Carotenoids can be classified as carotenes and xanthophylls. Carotenes' chemical structure includes carbon and hydrogen atoms, exclusively; while xanthophylls may have different oxygenated groups such as hydroxyl, ketonic, epoxy, etc. (Yahia and Ornelas-Paz, 2010). Fruits and vegetables provide 70–90% of the intake of carotenoids. Tropical and subtropical fruits are rich sources of carotenes and xanthophylls esters, and they have more carotenoids than temperate fruits which contain more anthocyanins. Mango and papaya are among the tropical fruits rich in carotenoids (Ornelas-Paz *et al.*, 2007; 2008a; 2008b; Rivera-Pastrana *et al.*, 2010). Carotenoids in these fruits are highly bioavailable since they occur dissolved in oil droplets (West and Castenmiller, 1998). β-Carotene (all-trans), β-cryptoxthanin (all-*trans* and *cis*), zeaxanthin (all-trans), luteoxanthin isomers, violaxanthin (all-*trans* and *cis*) and neoxanthin (all-*trans* and *cis*) have been identified in several mango cultivars (Mercadante *et al.*, 1997; Ornelas *et al.*, 2007; 2008a; 2008b). Ashton *et al*. (2006) identified lutein, α-carotene, β-carotene, neoxanthin, violaxanthin, zeaxanthin and antheraxanthin in the skin, flesh and oil of avocado fruit. It has also been reported that avocado is a good source of lutein (containing up to 248 mg $100g^{-1}$) (Mazza, 1998). Lycopene, β-cryptoxanthin and β-carotene were identified as major carotenoids in mesocarp of 'Maradol' papaya fruit (Rivera-Pastrana *et al.*, 2010). Several cultivars/lines of cactus pear fruit had a similar carotenoid profile, in which lutein was the most abundant compound in 'Camuesa', while neoxanthin was the most abundant compound in the line '21 441' (Castellanos-Santiago and Yahia, 2008). The carotenoid content in some common tropical and subtropical fruits is shown in Table 2.2.

Epidemiological evidence has demonstrated that the increased consumption of fruits and vegetables rich in carotenoids is associated with a reduced risk of cancer. Carotenoids are involved in the elimination of singlet oxygen and peroxyl radicals, prevent cardiovascular diseases and modulate immune function (Table 2.1). They exhibit biological actions that impact cellular signaling pathways, gene expression or inhibiting certain enzymes. They are found in the macula of the eye, where they decrease the risk of developing age-related macular degeneration and cataracts. These and other biological actions of carotenoids have been described elsewhere (Yahia and Ornelas-Paz, 2010). There has been major interest in the effects of lycopene in the reduction of

different diseases especially those related to heart disorders. However, in humans, unlike *β*-carotene, lycopene is not transformed into vitamin A, as it lacks the *β*-ionone ring required for conversion into retinoids. Additionally, lycopene has been associated with decreased incidence of prostate cancer (Itsiopoulos *et al.*, 2008)

Bioavaibility of carotenoids differs depending on fruit cultivar and matrix in which they are contained. Recently, Ornelas *et al.* (2010) reported that bioavaibility of *β*-carotene of 'Ataulfo' mango was higher than that of carrots. These authors suggested that differences could be attributed to variation in firmness, fiber content between mango and carrots, how carotenoids are stored in chromoplasts and absorptive interactions among carotenoids. Ripe mango fruit is softer than carrots and therefore mechanical and enzymatic disruption of mango tissue during digestion could be more efficient than that of carrots, leading to an increase in carotenoids released from food. Disruption of cell walls from foods is closely related to the carotenoids bioavailability (West and Castenmiller, 1998).

Betalains

Betalains are water-soluble nitrogen-containing pigments. They can be red-violet (betacyanins) or yellow (betaxanthins). They are immonium conjugates of betalamic acid with *cyclo*-dopa and amino acids or amines, respectively. The chromophore structure in all betalains is the betalamic acid. Betalains are found in some flowers, fruits and fungi and have been considered a new class of dietary cationized antioxidants (Kanner *et al.*, 2001).

Betalains are accumulated in the vacuoles of vegetative and reproductive tissues, and they are found mainly in epidermal and subepidermal tissues. Their presence in reproductive tissue such as petals, seeds and fruits depends generally on the developmental stages, but their presence in vegetative tissue appears to be regulated to a greater extent by environmental conditions. Major dietary sources of betalains include cactus pear (6.6–114 mg 100 g^{-1}) and red-purple pitaya (Stintzing and Carle, 2007; Castellanos-Santiago and Yahia, 2008). Cactus pears contain mainly indicaxanthin, betanin and isobetanin, and can have up to 24 different betalains (Castellanos-Santiago and Yahia, 2008). Red-purple pitaya contains betanin, isobetanin, phyllocactin, hylocerenin and isohylocerenin (Strack *et al.*, 2003; Stintzing and Carle, 2007). The ratio and concentration of betalain pigments in cactus pear fruit are responsible for the color of specific cultivars, showing the highest betalains content in fruit of purple color, comparable to that found in red beet (*Beta vulgaris* L. cv. Pablo) (Castellanos-Santiago and Yahia, 2008).

Several studies have demonstrated that betalains present in these fruits exert antioxidant activity, which is twice that reported in apple, pear, tomato, banana, white grape and orange (Wang *et al.*, 1996; Butera *et al.*, 2002). It has been considered that betalains exert anti-carcinogenic effects (Sreekanth *et al.*, 2007). Gentile *et al*. (2004) demonstrated that betalains also modulate the expression of adhesive molecules in endothelial cells. This property along with their antioxidant

activity is the probable cause of the protective effects of betalains in some diseases such as atherosclerosis, atherotrombosis and ischemia.

2.3.3 Fibers

The term 'fiber' can be defined in several ways, depending on the extraction and quantification method. This term can also be defined on the basis of physiological issues. In general, it includes a group of compounds of plant origin that cannot be hydrolyzed by human digestive enzymes. These compounds include non-starch polysaccharides (cellulose, hemicelluloses, pectins and mucilages) and lignin, a molecule of polyphenylpropane units. Compounds such as sugar alcohol, polydextrose, and other oligosaccharides are not digested by human digestive enzymes and are thought to function like dietary fiber, although they are not considered in the traditional measurements of fiber. Resistant starch, the sum of starch and starch-degradation products, is neither digested in the small intestine nor considered in the fiber quantification (Slavin, 2003). Tropical and subtropical fruits are rich in fibers (Table 2.5). Fiber is commonly classified as soluble and insoluble. Soluble fiber has been shown to have beneficial effects on carbohydrate and lipid metabolism. As indigestible polysaccharides, the fiber health benefits have been linked to the formation of a gelatinous matrix that increases fecal mass. This contributes to a reduction in the concentration of harmful biliary acids and other potential cancerous compounds in excrement. Health-related effects for fibers include lowering serum lipid and blood cholesterol, which exert protective effects against cardiovascular diseases (hyperlipidemia, hypertension and coronary heart disease). Fibers modify gut function, helping against intestinal disorders. They help in controlling blood glucose and therefore protect against diabetes. Insoluble fibers are most effective for relaxation assistance (Slavin, 2003). However, several studies have demonstrated that the ingestion of fibers decreases carotenoids bioavailability. Horvitz *et al.* (2004) demonstrated that carrot fibers reduce carotenoids bioavailability in humans. Pasquier *et al.* (1996) showed that several dietary fibers increased the viscosity of reconstituted duodenal medium and affected fat emulsification and lipolysis, two indispensable steps for carotenoid micellarization and absorption (Faulks and Southon, 2005).

Several possible mechanisms have been proposed for the effect of fibers on colon cancer, including the dilution of potential carcinogens and speeding their transit through the colon, binding carcinogenic substances, altering the colonic flora, reducing the pH, or serving as the substrate for the generation of short-chain fatty acids that are the preferred substrate for colonic epithelial cells (Slavin, 2003). Currently, the health-protective mechanisms of fiber intake are under close examination in the search for a more complete picture that includes other molecules bound to polysaccharides. Higher intake of fibers has also been hypothesized to reduce risk of breast cancer by interrupting the enterohepatic circulation of estrogens (Willett, 2000). However, the role of dietary fiber in cancer prevention remains controversial. Therefore more evidence is necessary to understand the possible role of fiber in deteriorative biological processes.

Table 2.5 Content of fiber (g $100g^{-1}$) and other compounds in some tropical and subtropical fruits

Fruit	Water	Protein	Fat	Ash	Dietary fiber	Sugars, total	Sugars available	Energy (kcal)
Avocado	73.2–79.7	1.4–2	12.7–18.4	0.8–1.6	6.7	0.6–9.7		145–198
Banana	54–78.3	0.5–4.4	0.1–1.8	0.4–3.6	0.5–22.8	12.2–43.3		81–185
Bitter orange	82.4–87.7	0.6–1.5	0.2–0.6	0.4–0.8		11.1–14.7		49–70
Black sapote	82	0.8	0.1	1.1	16	82		68
Chico sapote	71–79.7	0.4–1.7	0.3–1.1	0.3–1.2	2.6–5.3	18.8–26.2		23.6–83
Citron	89.5	0.8	0.1	0.3		9.3		41
Coconut	46.9–94.2	1.8–3.8	0.2–34.7	0.7–1.4	9	4.6–30.2		21–382
Fig	76.1–79.1	0.7–1.4	0.1–0.4	0.2–1.1	2.9	9–22.1		38–95
Grape	77–90.5	0.2–0.9	0–0.6	0.3–0.7	0.4–2.3	8.6–21.3	8.2–20.1	36–92
Grapefruit	88.1–91.6	0.5–0.9	0.1–0.4	0.3–0.4	1.1–1.6	6.9–8.8		30–42
Guava	76–87	0.5–2.5	0.1–0.9	0.5–1.3	4.9–5.4	8.9–22.4	6.3	32–96
Key lime	87.6–92.8	0.4–0.9	0.1–0.5	0.3–0.4	2.8–4.5	1.6–10.8	5	26–51
Kiwifruit	79.7–83.2	1.0–1.2	0.4–1.4	0.5–0.8	2–3	8.9–18	11.6	60–81
Lemon	83.8–90.3	0.5–1.2	0.3–0.6	0.3–0.5		8.1–14.7		39–50
Mamey	77.3–88.9	0.4–1.2	0.1–0.5	0.3–0.8	3	9.7–20.6		42–92
Mandarin	77.1–90.1	0.5–0.9	0.1–0.4	0.4–3.8	1.8	8.6–21.2		40–92
Mango	79.6–91.6	0.3–0.8	0.0–0.3	0.1–0.6	1.2–1.8	10.8–19.5	15.4	33–81
Muskmelon	90.2–92.9	0.5–1.1	0.1–0.2	0.4–0.7	0.9	5.7–26		26–34
Orange	85–88.6	0.5–1.2	0.1–0.3	0.4–0.6	2.4–2.7	9.14–13.5	9.3	42–59
Papaya	85.9–91.5	0.4–1.4	0.1–0.2	0.4–0.7	1.4–1.8	5.9–12.8	6.1	28–54
Passionfruit	72.9–85.9	1.5–2.2	0.5–0.7	0.5–1.4	10.4	11.2–22.8		56–106
Pineapple	85.1–89.3	0.4–0.6	0.1–0.4	0.2–0.4	1.2–1.4	9.8–14		43–59
Pomegranate	77.9–84.4	0.5–1.6	0.1–1.2	0.4–1.4	4	12.9–22.8		62–106
Pomelo	86.4–90.6	0.5–0.8	0.1–0.4	0.3–0.5	2.3	8.4–12.5	6.1	28–54
Prickly pear	81.4–91	0.3–1.1	0.1–0.5	0.3–1.6	3.6–8.2	8.5–16.6		41–74
Soursop	81–84	0.9–1.1	0.2–0.3	0.6–0.7	3.3	13.5–14.6		63–66
Tamarind	18.4–31.4	2.8–5.4	0.5–1.7	2.5–2.9	5.1	57.4–73.2		239–323
Watermelon	90.5–95.7	0.4–0.7	0.2–0.4	0.2–0.4	0.3–0.4	3.7–8.5		16–37

Source: USDA, 2005

2.3.4 Minerals

Tropical and subtropical fruits are mineral-rich foods (Table 2.6). Humans can be deficient in some minerals, either due to insufficient intake or to poor absorption from food, which can cause suffering and even death. Oligoelements, a group of minerals, are transitional metals that have a role in the synthesis and structural stabilization of proteins and nucleic acids (Halliwell and Gutteridge, 1989). Numerous studies have been developed to investigate the role of minerals in preventing or slowing 'oxidative damage'. Minerals have a function in the mechanism of preventive defense against free radicals performed by antioxidant enzymes. These enzymes work in a coordinated and integrated manner, centered on the availability of oligoelements and NADPH, which is the source of reducing equivalents against oxygen radicals. The most important of these minerals are magnesium, copper, zinc, manganese, iron and selenium (Jackson and Combs, 2008). The content of these compounds varies among species.

Table 2.6 Content of some minerals (mg $100g^{-1}$) in some tropical and subtropical fruits

Fruit	Na	K	Ca	P	Fe	Zn
Avocado	4–7	485–604	10–39	52–604	0.5–0.6	0.4–0.64
Banana	1–3	348–370	4–42	20–54	0.3–1.4	0.15–0.2
Bitter orange	1	200	36–65	17–26	0.7–0.8	
Black sapote			47	26	1.6	
Chico sapote	12	193	21–46	12–28	0.8–2.4	0.1
Citron			31		1.6	
Coconut	17–25	147–436	7–46	51–113	0.5–3.4	1.1
Fig	1–2	232–268	34–58	14–43	0.3–0.8	0.2–1.5
Grape	2	185–191	5–19	10–33	0.2–1.1	0.07
Grapefruit	0.1	127–150	11–34	7–18	0.05–2	0.07
Guava	2–4	284–417	17–33	15–40	0.1–0.7	0.2–0.23
Key lime	2	102	21–34	9–27	0.2–0.6	0.1
Kiwifruit	0.3–7	312–332	20–36	29–40	0.2–0.4	0.1–0.2
Lemon	3–6	145–163	21–107	21–29	0.4–0.7	0.1
Mamey	15	47	11–51	11–46	0.2–0.7	0.1
Mandarin	1–2	166–178	18–35	10–30	0.15–0.8	0.07
Mango	2–2.9	156–309	8–17	11–32	0.1–1.3	0–0.04
Muskmelon	9–17	182–309	9–23	5–32	0.2–1.4	0.07–0.2
Orange	0	169–181	17–43	12–51	0.09–0.8	0.06–0.08
Papaya	3	257	22–25	5–21	0.1–0.6	0–0.07
Passionfruit	28	348	9–12	21–68	1.6–1.7	0.1
Pineapple	1	108–125	10–221	5–13	0.25–0.4	0.08–0.12
Pomegranate	3–85	63–348	3–13	8–105	0.3–1.6	0.35
Pomelo	0	139	13–29	19–32	0.1–0.4	
Prickly pear	2–5	157–220	22–57	7–39	0.2–2.6	0.1–0.12
Soursop	14	278	14–38	27–43	0.3–0.7	0.1
Tamarind	28	628	74–118	86–113	0.8–2.8	0.1
Watermelon	1–8	73–116	4–12	3–283	0.2–0.7	0–0.1

Source: USDA, 2005

Most tropical fruit are good sources of iron (Fe) (Yahia, 2006). In humans, Fe is needed for the synthesis of proteins (i.e. hemoglobin and myoglobin) and Fe-containing enzymes (hemo enzymes, several enzymes required for energy production and those involved in immune defense and thyroid function). Deficiency in Fe can lead to anemia, which can decrease mental and psychomotor development in children, increase both morbidity and mortality of mother and child at childbirth, decrease work performance and decrease resistance to infection. In general, the content of Fe in tropical and subtropical fruits is moderate (Frossard *et al.*, 2000).

Some human diseases (Keshan and Kashin-Beck diseases) have been attributed to Selenium (Se) deficiencies. Selenium consumption is inversely correlated with the risk of developing cancer (Jackson and Combs, 2008). Hydrogen selenide, methylselenol and selenomethionine, the principal selenium metabolites, can regulate gene expression, protect DNA from damage, and stimulate the repair and regulation of cell cycles. Selenium deficiency has also been associated with an increased risk of suffering cardiovascular diseases. This mineral is involved in the kwashiorkor disease (protein-energy malnutrition disease), the susceptibility against malaria infection and the activity of some enzymes (Levander, 1987).

Zinc (Zn) is found abundantly in bones and skeletal muscle, and acts as a stabilizer of the structures of membranes and cellular components. It is a cofactor of several enzymes, including some involved in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. Zn is involved in gene expression and its deficiency causes a reduction in growth, sexual maturity and the immune response. It seems to be involved in a low incidence of diarrhea and respiratory infections. The content of Zn in tropical and subtropical fruits is moderate (Table 2.6) (Frossard *et al.*, 2000).

Magnesium (Mg) is required in many enzymatic reactions involved in the metabolism of macromolecules in humans. It is needed for contraction and relaxation of muscle and is an important component in bones and teeth (Shils, 1988). Mg can act as cofactor of superoxide dismutase enzyme that participates in the dismutation of peroxyl radical.

Phosphorus (P) is essential for cell division, involved in the formation and maintenance of bones and teeth, and required for proteins formation. Phosphodiester bonds link mononucleotide units forming long chains of DNA and RNA; the biosynthesis of all complex molecules of life is powered by energy released by the phosphate bond reversibly moving between adenosine diphosphate (ADP) and adenosine triphosphate (ATP). No life (including microbial life) is possible without it (Smil, 2000).

Ca has several functions in humans, but its involvement in the growth and development of bone is the most important (Frossard *et al.*, 2000).

Most tropical fruit are good sources of potassium (K) (Yahia, 2006). K influences many processes in the human body. It is the principal base in tissues and blood cells and plays an important role in the regulation of acid-base balance. It is also important in the transmission of nerve impulses to muscle fibers and in the contractility of the muscle itself. Sodium (Na) affects the osmotic pressure of

the plasma, the volume of plasma and interstitial fluid, acid-base balance, the electrical activity of body cells, and the responsiveness of the cardiovascular system to circulating endogenous pressor agents (Fregly, 1981).

2.3.5 Other compounds

Tropical and subtropical fruits are good sources of carbohydrates. Examples include bananas, plantains and breadfruit, which are widely used as sources of starch (Yahia, 2006). Avocado fruit and olives are high fat fruit, contain rare sugars of high carbon number and nitrogenous substances (Yahia, 2010). The oil content of avocado is high, ranging from as low as 3% to as high as 30%, while its sugar content is low (about 1%); hence it is recommended as a high energy food for diabetics (Yahia, 2010). A 100 g serving has about 177 calories, contains no cholesterol, and has about 17 g of fat, and the oil content is a key part of the sensory quality of the fruit. Oil quality in avocado fruit is very similar to that of olive oil. A high proportion of the oil (approximately 75%) is mono-unsaturated. Approximate proportions of saturated fatty acids and polyunsaturated fatty acids are 15% and 10% respectively. The major fatty acid in the edible portion of avocado fruit is oleic, followed by palmitic and linoleic acids, while the fatty acids present in trace amounts are myristic, stearic, linolenic and arachidonic (Mazliak, 1971; Tango *et al.*, 1972; Gutfinger and Letan, 1974; Itoh *et al.*, 1975; Swicher, 1984). Avocado and nuts also contain phytosterols, including β-sitosterol, which exert protective effects against cardiovascular diseases (Mazza, 1998). Some chemical components of *Carica papaya* fruit pulp have been reported by Oloyede (2005), suggesting that the astringent action of the plant, encountered in numerous therapeutic uses, is due to the presence of some phytochemicals such as saponins and cardenolides.

2.4 Bioactive compounds and antioxidant capacity

The human organism can produce high levels of free radicals under certain conditions. These compounds can damage several macromolecules (proteins, nucleic acids and membrane lipids), causing abnormal cellular activity and leading to the development of several degenerative diseases. Free radicals are involved in the pathology of 100 different diseases, including cancer, atherosclerosis, rheumatoid arthritis and cataracts. Tropical and subtropical fruits provide an optimal mixture of phytochemicals, such as vitamins C and E, carotenoids and flavonoids along with complex carbohydrates and fiber. These phytochemicals are able to scavenge or quench free radicals, diminishing the risk of suffering some diseases.

Overall, the ability of fruit phytochemicals to avoid oxidation of a substrate has been called their antioxidant activity. Individual phytochemicals contribute to different extents to the total antioxidant capacity of fruits and there are synergistic effects. Several methods have been used to determine the relative antioxidant

activity of fruits extracts; however, the values obtained by an individual assay reflect only the chemical reactivity under the specific conditions applied in that assay (pressure, temperature, reaction media, coreactants and reference points). Results of antioxidant activity assays can differ from method to method (Huang *et al.*, 2002). Existing methods for measuring the antioxidant activity seem to be considerably sensitive to hydrophilic antioxidants, especially to phenolic compounds. Wu *et al.* (2004) demonstrated that the lipophilic antioxidant activity of several carotenoids-rich tropical and subtropical fruits (cantaloupe, honeydew, mango and oranges) was 19–70 times lower than the hydrophilic antioxidant activity.

In a previous study of some American tropical fruits (acerola, acai, mangaba and uvaia) cultivated in Brazil, it was observed that the high antioxidant capacity of acerola fruit was positively related with its high vitamin C and phenol content (Rufino *et al.*, 2007). Similarly, Corral-Aguayo *et al.* (2008) found that the content of total phenols and vitamin C in several tropical and subtropical fruits were highly correlated with the antioxidant activity evaluated with six different assays. Corral-Aguayo *et al.* (2008) also reported that the antioxidant capacity of papaya (a carotenoids-rich fruit) extract was one of the lowest. Vitamin C content in juices from several citrus fruits (orange, and pink grapefruit) contributed 66–100% of the antioxidant activity (Gardner *et al.*, 2000). Banana has the lowest vitamin C, E and β-carotene levels compared with mango, papaya and pineapple. However, banana has a higher phenol content than pineapple and papaya. It appears that the high phenolic content of mango and banana contributes to a greater extent to their total antioxidant capacity than other bioactive compounds (Table 2.7). Vitamin C content in pineapple contributed only 0.8% of the antioxidant activity (Gardner *et al.*, 2000). Several studies have reported polyphenolic compounds in mango, papaya and pineapple flesh and peel, including various flavonoids, xanthones, phenolic acids and gallotannins (Berardini, 2005a; 2005b). Among these compounds, mirecitine, mangiferine, gallic acid and hydrolysable tannins, which are most likely gallotannins, are the major antioxidant polyphenolics found.

Tropical and subtropical fruits contain a wide range of antioxidant compounds at different levels, causing variations in antioxidant activity between these fruits. Some examples follow. Pineapple fiber showed higher (86.7%) antioxidant activity than orange peel fiber (34.6%) (Larrauri *et al.*, 1997). Orange (*Citrus sinensis*) was found to be more active than pink grapefruit (*Citrus paradisi*) in scavenging peroxyl radicals, while grapefruit juice was more active than orange juice, when the ORAC assay was used (Wang *et al.*, 1996). The total antioxidant activity in orange juice was thought to be accounted for by hesperidin and narirutin (Miller and Rice-Evans, 1997). Several nuts are among the edible plants with high total antioxidant content. Of the tree nuts, walnuts, pecans and chestnuts have the highest antioxidant content. Walnuts contain more than 20 mmol antioxidants per 100 g, mostly in the walnut pellicles (Blomhoff *et al.*, 2006; Yahia, 2010). Nine phenolic compounds have been identified in almonds by Sang *et al*. (2002), of which eight exhibited strong antioxidant activity. Almond skins were found to contain high levels of four different types of flavanol glycosides,

Table 2.7 Antioxidant activity of some tropical and subtropical fruits determined by three assays

Fruit	Method		
	Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) (mg EAA 100 g^{-1})	Oxygen Radical Absorbance Capacity (ORACFL) (μmol TEg^{-1})	Ferric Reducing Antioxidant Power (FRAP) (mmol $100g^{-1}$)
Avocado	143	10.9	
Banana	48.3	8.1	3.9
Coconut	11.5–45.8		
Grapefruit		15.5	
Guava	270		21.0
Kiwifruit	136	9.2	15.5
Lime	93.3		4.6
Mango	139	10.0	25.1
Melon	19.6	2.4–3.1	
Oranges	142	18.2	7.6
Papaya	141		
Pineapple	85.6	7.9	2.81
Watermelon	11.9	1.4	

Sources: Prior *et al.* (2003); Guo *et al.* (2003); Leong and Shui (2002); Wu *et al.* (2004); Corral-Aguayo *et al.* (2008)

which are thought to have powerful effects as antioxidants (Frison and Spons, 2002). Aqueous extracts of cactus pear fruit (*O. ficus-indica* L. Mill) were reported by Butera *et al.* (2002) to possess a high total antioxidant capacity, expressed as trolox equivalents, and exhibit a marked antioxidant capacity in several *in vitro* assays, including the oxidation of red blood cell membrane lipids and the oxidation of human LDLs induced by copper and 2,2′-azobis(2-amidinopropane-hydrochloride). Antioxidant components reported by these authors included vitamin C, negligible amounts of carotenoids and vitamin E. However, Corral-Aguayo *et al.* (2008) reported that the antioxidant capacity of extracts of prickly pear fruit ranked the lowest compared to seven other fruits. Some prickly pear fruits contain two betalain pigments, the purple-red betanin and the yellow indicaxanthin, both with radical-scavenging and reducing properties (Forni *et al.*, 1992; Fernandez-Lopez and Almela, 2001; Stinzing *et al.*, 2002; Castellanos-Santiago and Yahia, 2008).

There are also variations in antioxidant activity between different anatomical parts of the same fruit. Kondo *et al.* (2005) observed that total phenolics in guava and mango fruits were higher in the skin than in the flesh, being high at the immature stage and decreasing towards ripening. In contrast, total phenolic content of banana flesh was higher than in the skin, while those of papaya skin and flesh showed no significant differences. Recently, Rivera-Pastrana *et al.* (2010) reported that the exocarp of 'Maradol' papayas contains significantly higher phenols content than the mesocarp. Kanazawa and Sakakibara (2000) identified

higher levels of dopamine, a strong water-soluble antioxidant, in banana fruit (*Musa cavendishii*) peel than in pulp: 80–560 mg $100\,g^{-1}$ and 2.5–10 mg $100\,g^{-1}$ respectively. The concentration of ascorbic acid in the skin of bananas and papayas is higher than that in the flesh, while in guavas and mangoes the concentration of this bioactive compound is similar in the skin and the flesh (Kondo *et al.*, 2005).

Antioxidant activity is generally higher in the seeds than in the peel (Bocco *et al.*, 1998) due the high content of flavonols, tannins and other related compounds. However, it is important to consider the use of seeds as supplements in the diet since some seeds contain compounds with high antiphysiological potential harmful for the organisms. Seeds of lemons, sour orange, sweet orange, mandarins and limes had greater antioxidant activity than the peel (Bacco *et al.*, 1998).

2.5 Overview of health effects of some tropical and subtropical fruits

2.5.1 Cancer

Cancer is a chronic disease with public health relevance worldwide. In a simplistic fashion, cancer is caused by specific mutations in genes associated with cell cycle control, apoptosis and DNA repair enzymes. Epidemiological studies have revealed that the incidence of cancer is strongly related to geographical locations, inferring that it is a consequence of lifestyle rather than genetic differences. The consumption of five portions of fruits and vegetables per day has been proposed as a strategy to reduce the incidence of several chronic diseases, including cancer. Several studies have demonstrated that increased consumption of foods of plant origin reduces the incidence of some forms of cancer (Yahia, 2010). This effect has been attributed to the high content of fiber and some secondary metabolites in these types of foods. Several *in vitro* studies have demonstrated that these secondary metabolites (such as chlorophylls, phenolic compounds, carotenoids, betalains, vitamins, etc.) are responsible for the anticancer effects of fruits and vegetables (Table 2.8) through several possible mechanisms, such as exerting their antioxidant activity, modulating gene expression, preventing DNA damage, trapping or diluting carcinogens, alleviating the carcinogen-induced oxidative damage, altering the carcinogen metabolism, inducing apoptosis, killing cancer cells, etc. (Shih *et al.*, 2005). However, *in vitro* studies using pure secondary metabolites commonly found in fruits instead of whole fruits do not support the evidence provided by epidemiological studies (Temple and Gladwin, 2003). For example, epidemiological studies suggest an inverse correlation between apple consumption and colon cancer risk. However, under the cancer promoting condition of obesity, apple juice did not show cancer-preventive bioactivity (Koch *et al.*, 2009). Some intervention trials with pure carotenoids did not show any effect on cancer risk, and in fact other trials have shown increased risk (Stahl and Sies, 2005). Edenharder *et al.* (1994)

Table 2.8 Effect of some tropical and subtropical fruit extracts on human cancer cell lines in culture

Fruit	Human cancer cell line	Effect	Reference
Ethanolic extracts from longan fruit	SGC 7901 (stomach) A549 (lung) HepG2 (liver)	Reduction of proliferation	Prasad *et al*. (2009)
Delphinidin 3-O-glucoside and delphinidin 3-O-rutinoside from fruits of *Cornus alternifolia, Cornus controversa*, *Cornus kousa* and *Cornus florida*	AGS (stomach) HCT-116 (colon) MCF-7 (breast) NCI-H460 (lung) SF-268 (central nervous system)	Reduction of proliferation (50%)	Vareed *et al*. (2006)
Cornin from fruits of *C. kousa*	AGS (stomach) HCT-116 (colon) MCF-7 (breast) NCI-H460 (lung) SF-268 (central nervous system)	Reduction of proliferation (9–40%)	Vareed *et al*. (2007)
Ursolic acid and β-sitosterol from fruits of *C. kousa*	AGS (stomach) MCF-7 (breast) NCI-H460 (lung) SF-268 (central nervous system)	Reduction of proliferation (16–35%)	Vareed *et al*. (2007)
Aqueous extracts from fruits of *Rubus coreanum*	HT-29 (colon)	Viability reduction	Kim *et al*. (2005)
Chebulagic acid from fruits of *Terminalia chebula*	HCT-15 and COLO-205 (colon) MCF-7 and MDA-MB-231 (breast) DU-145 (prostate) K562 (erythromyeloblastoid leukemia)	Decreased viability and proliferation and induced cell death	Reddy *et al*. (2009)

Walnut methanolic extracts	Caco-2 (colon) A-498 and 769-P (kidney)	Reduction of proliferation	Carvalho *et al.* (2010)
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone from *Citrus fruits*	HT-29 (colon)	Reduction of proliferation	Hirata *et al.* (2009)
1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone from mangosteen pericarp	LOVO (colon) MCF-7 (breast)	Cytotoxicity (46–73%)	Yu *et al.* (2009)
Limonoids from citrus fruit of the Rutaceae and Maliaceae families	Breast cancer cells	Reduction of proliferation	Ejaz *et al.* (2006)
Methanolic extracts of *Terminalia chebula* fruits	PC-3 (prostate) HOS-1 (osteosarcoma)	Decreased viability, and proliferation	Saleem *et al.* (2002)
Extracts from fruits of *Fructus Bruceae*	Pancreatic adenocarcinoma cells	Cytotoxicity	Lau *et al.* (2008)
Hexane extracts from immature fruits of *Citrus grandis* Osbec	U937 (leukaemia cells)	Induction of cell death	Lim *et al.* (2009)
Polyphenols-rich extracts from litchi fruits	Liver cancer cells	Reduction of proliferation	Wang *et al.* (2006)
3-(8′(Z),11′(Z)-pentadecadienyl) catechol from whole fruits of *Semecarpus anacardium*	Leukaemia cells	Induction of cell death	Nair *et al.* (2009)
Extracts from barbados cherry	Oral cancer cells	Cytotoxicity	Motohashi *et al.* (2004)
Carotenoids-rich extracts from apples and oranges	Leukaemia cells	Cytotoxicity	Molnár *et al.* (2005)

classified some tropical and subtropical fruits according to their *in vitro* antimutagenic activity. Strong antimutagenic activities were detected in bananas, pineapple and watermelon while moderate activities were found in kiwifruit, mangoes and honeydew melons.

Several *in vitro* studies have shown that phenolic compounds in fruits and vegetables have an antiproliferative effect in different cancer cell lines (Sun *et al.*, 2002; Mertens-Talcott *et al.*, 2005; Percival *et al.*, 2006; Yahia, 2010). Quercetin, a flavonoid found in many tropical and subtropical fruits, has been shown to affect the metastatic potential of tumor cells in mice (Suzuki *et al.*, 1991). Mutagenicity of quercetin was examined by means of DNA fingerprint analysis using the Pc-1 probe that efficiently detects mutations due to recombination. Treatment of BMT-11 and FM3A tumor cells with quercetin resulted in gain and loss of bands in the fingerprints in both cell lines. The frequencies of the clones having undergone mutation were 3/11 and 6/26, respectively. This suggests that quercetin is mutagenic and induces recombination, and the results seem to provide a molecular basis for the phenotypic variations of BMT-11 tumor cells induced by quercetin. Chlorophyllin (CHL), a food-grade derivative of the ubiquitous tropical and subropical fruits pigment chlorophyll, has been shown to be a potent, dose-responsive inhibitor of aflatoxin B sub(1) DNA adduction and hepatocarcinogenesis in the rainbow trout model when fed with carcinogen (Breinholt *et al.*, 1995). Chlorophyllins are derivatives of chlorophyll in which the central magnesium atom is replaced by other metals, such as cobalt, copper or iron. The relative efficacy of chlorophyll and chlorophyllin has been shown to modify the genotoxic effects of various known toxicants (Sarkar *et al.*, 1994). CHL neither promoted nor suppressed carcinogenesis with chronic postinitiation feeding. By molecular dosimetry analysis, reduced aflatoxin B sub(1)-DNA adduction accounted quantitatively for reduced tumor response up to 2000 ppm dietary CHL, but an additional protective mechanism was operative at 4000 ppm CHL. The finding of potent inhibition (up to 77%) at CHL levels well within the chlorophyll content of some green tropical and subtropical fruits may have important implications in intervention and dietary management of human cancer risks. Monoterpenes, natural plant products found in the essential oils of several commonly consumed tropical and subtropical fruits which have been widely used as flavor and fragrance additives in food and beverages, have been shown to possess antitumorgenic properties (Kelloff *et al.*, 1996). Limonene, the simplest monocyclic monoterpene which is found in some citrus fruits, and perrillyl alcohol, a hydroxylated limonene analog, have demonstrated chemopreventive and chemotherapeutic activity against mammary, skin, lung, pancreas and colon tumors in rodent models (Wattenberg and Coccia, 1991; Crowell and Gould, 1994; Stark *et al.*, 1995).

Colon cancer

Consumption of tropical and subtropical fruits, and the associated vitamin C, carotene and fiber, have been reported to reduce the risk of colon cancer (Ziegler

et al., 1981). Diets containing citrus fiber have been reported to reduce the risk of intestinal cancer. Weanling rats were fed semi-purified diets containing 5% fat and 15% citrus fiber, at seven weeks of age, all animals except vehicle-treated controls received weekly injections of 8 mg azoxymethane (AOM) kg^{-1} body weight for ten weeks, and the AOM- or vehicle-treated groups were autopsied 20 weeks after the last injection of AOM. The animals fed the citrus fiber diet and treated with AOM had a lower incidence (number of animals with tumors) and multiplicity (number of tumors/tumor-bearing animal) of colon tumors and tumors of the small intestine than did those fed the control diet and treated with AOM. The number of adenomas but not the number of adenocarcinomas was reduced in rats fed on the citrus pulp diet. Research has suggested a possible effect of almond consumption on cancer, including colon cancer (Davis *et al.*, 2003).

Breast cancer

A meta-analysis of 26 prospective and retrospective studies (Gandini *et al.*, 2000) confirmed the reduction of the risk of breast cancer with enhanced intake of fruit and vegetables, in contrast to a pooled analysis of eight prospective studies that indicated that fruit and vegetable consumption did not significantly reduce the risk of breast cancer (Smith-Warner *et al.*, 2001). Garcia-Solis *et al.* (2008; 2009) studied the antineoplastic properties of some fruits and vegetables using *in vivo* and *in vitro* models. The effect of 'Ataulfo' mango fruit consumption was studied on chemically induced mammary carcinogenesis and plasma antioxidant capacity (AC) in rats treated with the carcinogen *N*-methyl-*N*-nitrosourea (MNU) (Garcia-Solis *et al.*, 2008). Mango was administered in the drinking water (0.02–0.06 gmL^{-1}) during both short-term and long-term periods to rats, and plasma antioxidant capacity was measured by ferric reducing/antioxidant power and total oxyradical scavenging capacity assays. Rats treated with MNU had no differences in mammary carcinogenesis (incidence, latency and number of tumors), and no differences in plasma antioxidant capacity. On the other hand, Garcia-Solis *et al.* (2009) screened (using methylthiazolydiphenyl-tetrazolium bromide assay) the antiproliferative activity of aqueous extracts of avocado, black sapote, guava, mango, cactus stems (cooked and raw), papaya, pineapple, four different prickly pear fruit, and grapes on the breast cancer cell line MCF-7. Only the papaya extract had a significant antiproliferative effect and there was no relationship between total phenolic content and AC with antiproliferative effect. These results suggested that each plant food has a unique combination in quantity and quality of phytochemicals which could determine its biological activity. The consumption of a mixture of phenolic compounds presented in purple grape juice inhibited mammary carcinogenesis in 7,12-dimethylbenzo[a]anthracene (DMBA) treated rats (Liu *et al.*, 2005; Jung *et al.*, 2006). However, the individual antioxidants studied in clinical trials, including β-carotene, vitamin C and vitamin E, do not appear to have consistent preventive effects comparable to the observed health benefits of diets rich in fruits and vegetables, suggesting that natural phytochemicals in fresh fruits and vegetables could be more effective than a dietary supplement.

Lung cancer
Goodman *et al.* (1992) concluded that some β-carotene rich fruits such as papaya, mango and yellow orange showed little influence on survival of lung cancer patients, and the intake of β-carotene before diagnosis of lung cancer did not affect the progression of the disease.

2.5.2 Cardiovascular disease (CVD)

Reduction of cardiovascular and coronary heart diseases by consumption of nuts is well known (Yahia, 2010). The Nurses Health Study indicated that frequent nut consumption was associated with a reduction in the risk of suffering cardiovascular diseases (Yahia, 2010). Clinical studies have evaluated the effect of nut consumption on blood lipids and lipoproteins as well as several factors of coronary heart disease (CHD) risk (oxidation, inflammation, and vascular reactivity), finding protective effects against CHD and associated risk factors. Jenkins *et al.* (2008) reported that nut consumption improves the blood lipid profile and causes a reduction in the risk of CHD. A pooled analysis of four US epidemiologic studies showed that subjects with the highest nut intake had a risk of CHD 35% lower than that of other experimental groups (Kris-Etherton *et al.*, 2008). The LDL cholesterol-lowering response caused by nut consumption is considerably high. Polyphenolic compounds and vitamin E in almonds are active in preventing the oxidation of LDL cholesterol (Millburry *et al.*, 2002). Individuals who replaced a half of their daily fat intake with almonds or almond oil for some weeks showed reduced total and LDL cholesterol 4% and 6%, respectively, while HDL cholesterol increased by 6% (Hyson *et al.*, 2002). Besides lipids, polyphenolic compounds and vitamin E, nuts contain other bioactive compounds that also could explain their protective effect on cardiovascular diseases (protein, fiber, K, Ca, Mg, phytosterols, resveratrol and arginine). All of these compounds also have relevance in human nutrition (Kris-Etherton *et al.*, 2008).

Avocados are considered as healthy food in terms of protection against heart disease due to their high content of mono- and polyunsaturated fat and low content of saturated lipids. Avocado fat has been shown to reduce blood cholesterol and preserve the level of high-density lipoproteins (Yahia, 2011a). Avocado enriched diet produced a significant reduction in low-density lipoproteins (LDL) and total cholesterol in patients with high cholesterol levels, while diets enriched with soy and sunflower did not change the total cholesterol concentrations (Carranza *et al.*, 1997). Avocados also contain other protective compounds against cardiovascular diseases (chlorophyll, carotenoids, α-tocopherol and β-sitosterol). Avocado fat is an important source of energy, also important in human nutrition.

The diverse classes and large amounts of phenolic compounds found in grapes were reported to play an important role in human health, lowering levels of low-density lipoprotein (Frankel *et al.*, 1993; Tussedre *et al.*, 1996). Extracts of fresh grapes and commercial grape juice inhibited human LDL oxidation from 22 to 60% and from 68 to 75% (Frankel *et al.*, 1998). The LDL antioxidant activity

correlated highly with the concentration of total phenolics for both grape extracts and commercial grape juices, and with the level of anthocyanins, flavonols, hydroxycinnamates and flavan-3-ols (Frankel *et al.*, 1993; 1998).

Cactus pear was also reported to exert protective effects against cardiovascular diseases, presumably as a consequence of its high content of antioxidants (Yahia, 2010b). Tesoriere *et al.* (2004) found that consumption of 500 g cactus pear (*O. ficus-indica*) pulp for two weeks reduced the levels markers of oxidative damage of plasma lipids, such as isoprostanes and malonaldehyde. The oxidative status of LDL, erythrocytes and the levels of plasma antioxidants increased (Tesoriere *et al.*, 2004).

2.5.3 Diabetes mellitus

Randomized controlled trials of patients with type 2 diabetes have confirmed the beneficial effects of nuts on blood lipids, also seen in non-diabetic subjects, but the trials have not reported improvement in A1c or other glycated proteins (Jenkins *et al.*, 2008). Therefore, Jenkins *et al.* (2008) concluded that it is justified to consider the inclusion of nuts in the diets of individuals with diabetes in view of their potential to reduce cardiovascular risk, even though their ability to influence overall glycemic control remains to be established. Nettleton *et al.* (2008) characterized dietary patterns and their relation to incident type 2 diabetes in 5011 participants from the Multi-Ethnic Study of Atherosclerosis and found that high intake of nuts was associated with a 15% lower risk of diabetes.

Some flavonoids, such as procyanidins, have antidiabetic properties because they improve altered glucose and oxidative metabolisms of diabetic states (Pinent *et al.*, 2004). Extract of grape seed procyanidins (PE) administered orally to streptozotocin-induced diabetic rats resulted in an antihyperglycemic effect, which was significantly increased if PE administration was accompanied by a low insulin dose (Pinent *et al.*, 2004). The antihyperglycemic effect of PE may be partially due to the insulinomimetic activity of procyanidins on insulin-sensitive cell lines.

2.5.4 Bone health

Antioxidants in fruits and vegetables including vitamin C and ß-carotene reduce oxidative stress on bone mineral density, in addition to the potential role of some nutrients such as vitamin C and vitamin K that can promote bone cell and structural formation (Lanham-New, 2006). Some tropical and subtropical fruits are rich in potassium citrate and generate basic metabolites to help buffer acids and thereby may offset the need for bone dissolution and potentially preserve bone. Potassium intake was significantly and linearly associated with markers of bone turnover and femoral bone mineral density (Macdonald *et al.*, 2005). Lin *et al.* (2003) indicated that high potassium, magnesium and calcium content in addition to antioxidants, phytochemicals and lower acidity of fruits could be important factors for bone health.

2.5.5 Arthritis

Dietary antioxidants and anti-inflammatory components in food are thought to be important in reducing the risk or improving the course of rheumatoid arthritis (RA), and therefore tropical and subtropical fruit can be associated with reduced risk of RA (Yahia, 2010). A study involving 29 368 married women from the US, predominantly white, average age 61.4 years, over 11 years, indicated that total fruit consumption (> 83 servings per month) was associated with reduced risk of RA (Cerhan *et al.*, 2003), oranges were the only individual fruit linked to reduced incidence of RA, and β-cryptoxanthin, a corotenoid found in this fruit, was consistently highly protective.

2.5.6 Birth defects

The effect of folic acid supplementation on reducing the risk of neural tube defects of the brain and spine, including spina bifida and anencephaly, is well documented (Eichholzer *et al.*, 2006). Tropical and subtropical fruits are an important source of dietary folate and their consumption has been associated with increased plasma levels of folate.

2.5.7 Diverticulosis

Studies have established an association between low fiber diets and the presence of diverticulosis (Aldoori and Rayan-Harschman, 2002). The intake of fruit fiber was inversely associated with risk of diverticulosis in a large prospective study of male health professionals, and therefore a high fiber diet including tropical and subtropical fruits is considered to be an important aspect of therapy for diverticulosis (American Dietetic Association, 2002).

2.5.8 Aging and cognition

Oxidative stress and inflammation are considered significant mediators in healthy aging of the brain and in age-related neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Shukitt-Hale *et al.*, 2006; Yahia, 2010). Animal and human studies have suggested that fruits and vegetables have the potential to mitigate some age-related processes, primarily due to the antioxidant and anti-inflammatory properties of several phytochemicals. An *in vitro* study has suggested that some classes of phytochemicals also act on cell signaling and thus may protect against aging by mechanisms other than oxidative and inflammatory processes (William *et al.*, 2004). Fruit extracts have been demonstrated to reverse or retard various age-related cognitive and motor deficits in rats (Lau *et al.*, 2005). Aging rats provided with 'Concord' grape juice at low concentration (10%) for nine weeks improved cognitive performance, while high (50%) concentration improved motor performance (Shukitt-Hale *et al.*, 2006). 'Concord' grape and juice contain a variety of flavonoids, and 10% grape juice supplementation was reported to be associated with the most effective increase in muscarinic receptor sensitivity in aging rats.

2.6 Preharvest factors affecting accumulation of nutritional and health components

The importance of fruit bioactive metabolites as protective compounds in human nutrition and health has been widely recognized. Interestingly, the same metabolites are also essential for the health of the fruit itself (Yahia, 2010). Tropical and subtropical fruits provide an optimal mixture of antioxidants; however, their qualitative and quantitative content can be affected by different conditions that could change negatively the nutraceutical value of these fruits. Many preharvest factors affect the nutritional and health components in tropical and subtropical fruits. The influence of climatic conditions, cultural practices, ripening stage at harvest, season, geographical origin and genotype on health-promoting components of several (especially temperate) fruit has been extensively studied during the past decade. However, cultural practices affecting nutritional/health quality of fruit such as soil and substrate properties, growing systems (field, greenhouse, organic farming), mulch and covering material, grafting and pruning, irrigation, fertilization, salinity, growth promoters, mechanical and pest injuries and maturity at harvest need to be studied in more detail in tropical and subtropical fruits.

Considering the impact of environment and crop management practices on fruit quality, light is often the major factor. Fruit from plants grown under high light intensity, especially those grown in the tropics, generally have high vitamin C, carotenoids such as lycopene and β-carotene, and phenols, while fruit from plants grown under low light have a lower content. This factor can be detrimental in the synthesis of some phytochemical compounds in some tropical fruits. Although light is not essential for the synthesis of ascorbic acid, its amount and intensity during the growing season influences the amount of ascorbic acid formed, because ascorbic acid is synthesized from sugars supplied through photosynthesis (Lee and Kader, 2000). Within the same plant, exterior fruits exposed to maximum sunlight usually contain higher amounts of vitamin C than interior and shaded fruits (Lee and Kader, 2000). Similarly, even though the formation of carotenoids in ripening fruit does not require induction by light, shaded fruits usually have lower carotenoid content than exposed fruits (Merzlyak *et al.*, 2002).

Content of bioactive compounds in tropical and subtropical fruits (predominantly climacteric) is markedly altered by ripening (Robles-Sánchez *et al.*, 2007). Ethylene increase in fruits leads to numerous chemical changes, including biosynthesis and degradation of bioactive compounds. Carotenoids accumulation in mangoes increases exponentially during ripening (Ornelas-Paz *et al.*, 2008a). Manthey and Perkins-Veazie (2009) demonstrated that the levels of mangiferin and ellagic acid in mangoes increased from 227 to 996 and from 25.7 to 187 μg $100\,g^{-1}$ FW as harvest season (as maturity) advanced, respectively. However, González-Aguilar *et al.* (2008) found that the antioxidant capacity of mangoes remains constant during ripening, suggesting that once mangoes reach maturity on the tree, their phytochemistry relating to radical scavenging activity

barely changes during ripening (González-Aguilar *et al.*, 2008). Similarly, Manthey and Perkins-Veazie (2009) did not report any effect of the stage of ripening on antioxidant activity of mango. Ripening also alters the content of bioactive compounds in papaya. Rivera-Pastrana *et al.* (2010) demonstrated that the major carotenoids in 'Maradol' papaya are lycopene (0.0015–0.012 g kg^{-1} fresh weight) and *β*-cryptoxanthin (0.0031–0.0080 g kg^{-1} fresh weight), which tend to increase during ripening. Gayosso-García *et al.* (2010) demonstrated that the stage of ripening of 'Maradol' papaya influenced *β*-carotene and phenolic content, and consequently the antioxidant capacity, measured by DPPH, ABTS and ORAC methods. The highest antioxidant capacity was observed in the mature-green stage, which coincided with the higher phenolic content of fruit (Gayosso-García *et al.*, 2010). Rivera-Pastrana *et al.* (2010) reported that the content of major phenols (ferulic acid, caffeic acid and rutin) in the exocarp of 'Maradol' papaya decreased during ripening. The concentration range for these compounds were 1.33–163, 0.46–0.68 and 0.10–0.16 g kg^{-1} dry weight for ferulic acid, caffeic acid and rutin, respectively (Rivera-Pastrana *et al.*, 2010). The decrease in phenolic compounds during fruit ripening was also reported in banana and mango (Abu-Goukh and Abu-Sarra, 1993; Ibrahim *et al.*, 1994). During longan fruit development, the content of total phenolics decreased rapidly and then increased while the content of (–)-epicatechin tended to decrease (Yu *et al.*, 2010). Recently, Qing-Yi *et al.* (2009) reported a significant increase in total carotenoid and fat content in avocados as the season progressed from January to September. The total content of carotenoids was highly correlated with the total fat content (Qing-Yi *et al.*, 2009). The content of ascorbic acid and phenolic compounds in pulp and peel of guavas progressively decreased during ripening (Bashir and Abu-Goukh, 2003). Similar findings have been reported for several other tropical and subtropical fruits.

The genotype influences the chemical composition in several fruits. White-fleshed guava contains more sugars than the pink-fleshed type (Bashir and Abu-Goukh, 2003). Ribeiro *et al.* (2007) reported that phenolic compounds, ascorbate and *β*-carotene of mangoes differ considerably between cultivars. 'Haden', 'Tommy Atkins', 'Palmer' and 'Uba' mangoes represent a potential source of natural antioxidants, but 'Uba' mango showed better results with the highest levels of antioxidants. Ornelas-Paz *et al.* (2007) compared the carotenoids profile in fruit of seven Mexican mango cultivars and found that the most abundant carotenoid in fruit of 'Ataulfo', 'Criollo' and 'Haden' was *all-trans-β*-carotene, while in the other cultivars *all-trans*-violaxanthin (as dibutyrate) was predominant. The content of *all-trans*-violaxanthin was higher than 9-*cis*-violaxanthin (as dibutyrates) in all cultivars. 'Haden' mangoes had high content of the three carotenoids, and its *all-trans-β*-carotene concentration was the highest compared to all other cultivars, while 'Ataulfo' mango showed the lowest amount of xanthophylls compared to the other cultivars (Ornelas-Paz *et al.*, 2007). In general, *α*-tocopherol content in mango fruit is independent of cultivar although 'Haden' and 'Tommy Atkins' contain a slightly higher content of this compound (Ornelas-Paz *et al.*, 2007). Gonzalez-Aguilar *et al.* (2007a;b) reported that 'Ataulfo'

mangoes contain higher amounts of vitamin C and carotenoids as compared with 'Keitt' and 'Kent' mangoes. Manthey and Perkins-Veazie (2009) studied the effect of cultivar on phytochemicals of different mango cultivars. They found that ascorbic acid ranged from 11 to 134 mg 100 g^{-1} of pulp puree, and β-carotene varied from 5 to 30 mg kg^{-1} among the five varieties. Total phenolic content ranged from 19.5 to 166.7 mg of gallic acid equivalents (GAE) 100 g^{-1} of puree. 'Tommy Atkins', 'Kent', 'Keitt' and 'Haden' mangoes had similar total phenolic contents, averaging 31.2 mg GAE 100 g^{-1} of puree, whereas 'Ataulfo' mango contained substantially higher values. Similar trends were observed in the DPPH radical scavenging activities among the five varieties (Manthey and Perkins-Veazie *et al.*, 2009).

Harvest season and geographical origin of fruit also alter the phytochemical content in fruits. For example, Gonzalez *et al.* (2005) observed that vitamin C content in of pineapple 'Cayena Lisa' varied with the harvest season. In contrast, Manthey and Perkins-Veazie (2009) did not report any effect of harvest location on ascorbic acid, total phenolic and DPPH radical scavenging in mango. Similarly, Qing-Yi *et al.* (2009) found that Hass avocados grown in different regions of southern California have a similar phytochemical profile.

2.7 Postharvest factors affecting nutritional and health components

Recently, due to health awareness campaigns, the general public has become more interested in foods that support and promote health. This trend has resulted in consumers wanting products with high health-promoting compounds rather than having superior external quality attributes as was previously the case. However, many postharvest factors, treatments and techniques can affect the nutritional and health components in tropical and subtropical fruits. Major research has been done focusing on the effect of postharvest treatments on preserving sensorial quality and assuring safety of fresh tropical and subtropical fruits, and some research has been done on the possible changes induced by postharvest treatments on nutraceutical value of tropical and subtropical fruits. The major factors that positively affect phytochemical content in postharvest fruit have been reported recently. Amarowicz *et al.* (2009) and González-Aguilar *et al.* (2010) reviewed the influence of postharvest processing and storage on the content bioactive compounds, including phenolic acids and flavonoids, β-carotene, vitamin C, vitamin E and antioxidant capacity, in different foods. However, they do not cover adequately the effects of these treatments on tropical and subtropical fruits. After harvest, tropical fruits are commonly transported to packinghouses, and delivered either to the fresh market or to processing plants. Fruits that are disinfected, cleaned and properly handled in the postharvest chain do not lose significant quantities of phytochemicals, but innapropiate handling can cause significant nutritional losses. Excessive sunlight and high temperature increase the activity of metabolic reactions and consequently enhance the senescence

processes, diminishing the amount of bioactive compounds. Processing inevitably induces qualitative and quantitative changes on phytochemical content of fruits. The content of total phenols and vitamin C decrease significantly with processing (Ninfali and Bacchiocca, 2004). The antioxidant capacity, when measured on each stock of processed fruits, describes the extent of quality loss. Many variables occur during processing, and producers wishing to provide consumers with high quality products should be aware of total antioxidant capacity and add this value to their packaging labels as proof of their attention to nutritional quality. Among the important postharvest factors affecting the content of bioactive compounds are storage temperature, controlled/modified atmospheres, edible coatings, heat treatments, cutting, use of natural compounds, irradiation and processing.

2.7.1 Storage temperature

Temperature has a profound effect on fruit metabolism and thus on its content of bioactive compounds. Lower temperatures slow respiration rates, as well as ripening and senescence processes, and therefore reduce the degradation of nutritional and health components, and prolong the postharvest life of fruits (González-Aguilar *et al.*, 2004). The metabolic effect of temperature can reflect on the phytochemical status of the tropical and subtropical fruits.

Many subtropical fruits and most tropical fruits are very susceptible to low temperature storage. These fruits develop different chilling symptoms that become apparent after transferring the fruit to higher temperatures. However, the changes in the phytochemical compounds that occur during these events are not well documented. Antioxidant content of 'Maradol' papaya was influenced by postharvest storage temperature with the exception of β-carotene and rutin (Rivera-Pastrana *et al.*, 2010). Ripe papaya stored at 25°C possessed a higher nutritional potential than those stored at 1°C. Low (chilling) temperature (1°C) negatively affected the content of major carotenoids, except β-carotene, and contributed to maintaining or increasing ferulic and caffeic acid levels, as compared to high (safe) temperature (25°C). Similarly, total phenolic content in banana decreased as the storage temperature increased (Aziz *et al.*, 1976). Storage of loquat fruit at 20–30°C caused an increase in sucrose and cryptoxanthin as compared with lower temperatures, and total phenolics did not change at low storage temperatures (≤10°C) (Ding *et al.*, 1998). It has been observed that during cold storage antioxidant activity and total phenolic content changes to a different extent depending on the type of the fruit and other factors (González-Aguilar *et al.*, 2008).

2.7.2 Controlled/modified atmospheres (CA/MA) and edible coatings

The effects of CA and MA technologies are well documented to extend the postharvest life of fruits including those of tropical and subtropical origin (Yahia, 2008). These effects include reduction of respiration rate, inhibition of ethylene production and action, retardation of ripening, and maintenance of nutritional and

sensory quality (Singh and Pal, 2008; Yahia, 2008). These techniques alter the O_2, CO_2 and/or C_2H_4 concentrations in the atmosphere surrounding the commodity to produce an atmosphere composition different from that of the normal air.

In general, atmospheric treatment within the ranges tolerated by the commodity reduces physiological and biochemical changes, including losses of vitamin C, chlorophylls, carotenoids of fruit and vegetables during storage. Studies on the effect of CA/MA on nutritional quality are limited. Kader (2008) reviewed the impact of this technique on the major nutrients of fruit and vegetables. For most commodities tested, 1–4 kPa O_2 generally slows AA degradation through prevention of oxidation. The effects of carbon dioxide on AA may be positive or negative depending upon the commodity, CO_2 concentration, duration of exposure, and temperature. CA conditions that delay ripening of fruits result in delayed synthesis of carotenoids, such as β-carotene in mango, but the synthesis of this compound was recovered upon transfer of the fruit to air at ripening temperatures (15–25°C). In general, high CO_2 can accelerate losses of AA and reduce the rate of postharvest synthesis of phenolic compounds, which can be desirable (no increase in browning potential and astringency) or undesirable (no increase in antioxidant activity) (Kader, 2009).

Litchi fruit stored in air had lower anthocyanin content throughout storage in MA and CA at 38°C. However, there are contrasting effects of MA and CA of fruit indicating that various plant matrices may behave differently even under the same or slightly modified storage conditions. Maintaining the optimal ranges of O_2, CO_2 and ethylene concentrations around the commodity extends its postharvest life by about 50%–100% relative to air control, including its nutritional quality. Montero-Calderon *et al.* (2010) studied the effect of low O_2 (12% O_2 in combination with 1% CO_2) and high O_2 (38% O_2) on fresh-cut pineapple fruit and found an increment of antioxidant capacity, total phenolics, volatile compounds and vitamin C content in low O_2 compared with high O_2. These results suggest that optimal conditions of CA/MA can improve the nutritional value of tropical fruits.

Antioxidant phytochemicals and quality of mango fruit are affected by hot water immersion and controlled atmosphere storage (Kim *et al.*, 2007). The concentration of gallic acid and hydrolysable tannins and their resultant antioxidant capacity were unaffected by the hot water treatment (46°C for 75 minutes), while total polyphenolics naturally decreased throughout fruit ripening, regardless of hot water treatment or storage atmosphere. However, the overall decline in polyphenolic concentration was inhibited by the CA treatments (3% O_2 + 97% N_2, or 3% O_2 + 10% CO_2 + 87% N_2) as a result of delayed ripening.

Litchi packed with the modified atmosphere system PropaFresh™ PFAM maintained proper levels of sugars and individual anthocyanins (Somboonkaew and Terry, 2010). Vitamin C content in longan fruit (cv. Shixia) decreased rapidly during CA storage under several O_2 levels (4–70%) (Tian *et al.*, 2002). Changes of ascorbic acid and total phenols in guava during storage were retarded by CA (2.5–1 kPa O_2 with 2.5–10 kPa CO_2, balance N_2) (Singh and Pal, 2008). Vigneault and Artes-Hernandez (2007) reviewed the literature on gas treatment for increasing

the phytochemical content of fruits and vegetables and concluded that much more research is needed before guidelines for choosing gas treatments to enhance the phytochemical content of fruit can be developed.

Some edible coatings, such as chitosan, have antibrowning characteristics and can maintain tissue firmness and reduce microbial decay of harvested fruits for extended periods. Gonzalez-Aguilar *et al.* (2009) found that chitosan coating is effective in the preservation of fresh-cut papaya. Liu *et al.* (2007) used chitosan to control gray mold and blue mold in tomato fruit stored at 25°C and 2°C. Chitosan has the potential for inducing defense-related enzymes such as polyphenol oxidase (PPO), peroxidase (POD) and polyphenol ammonia layase (PAL), but the possible mechanism of action is little understood. Robles-Sanchez *et al.* (2009) reported that dipping treatments containing ascorbic acid and calcium lactate affected total phenols, vitamin C, vitamin E, and gallic and *p*-OH-benzoic acids, whereas storage time affected all the parameters studied. The initial vitamin C values were of 11 and 44 mg 100 g^{-1} of fresh weight for control and treated fresh-cut mango cubes, respectively. As expected, a fourfold increase in vitamin C was observed immediately after the treatment with AA + CA + $CaCl_2$. However, in this study vitamin C losses during storage of fresh-cut mangoes were minimal in both control and treated tissue. A higher level of vitamin C in treated fresh-cut cubes was observed compared to controls. The use of vacuum impregnation and edible coatings containing antioxidants is another option to overcome the nutritional losses of fresh-cut fruits during storage.

Therefore, use of CA/MA and edible coatings for extending the shelf-life of fresh fruits, including those of tropical and subtropical origin, is widely recognized. However, more research needs to be carried out on the potential of these technologies to improve the nutrimental quality of fruits, especially their antioxidant status.

2.7.3 Heat treatments (HT)

Different types of heat treatments (hot air or hot water) are commercially used for different postharvest purposes in different tropical and subtropical fruits. Heat tolerance of different fruits depends on species, genotype, stage of fruit maturity, type and severity of HT applied, and whether postharvest conditioning treatments have been given before or after a HT (Jacobi *et al.*, 2001). Several studies have connected heat tolerance with the increase of heat shock proteins (HSP's), antioxidants enzymes and secondary metabolites such as carotenoids and phenolic compounds. Ghasemnezhad *et al.* (2008) found an increase in superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity after hot water treatments in mandarins. Overall, no significant differences in antioxidant capacity were found following the hot water treatment or low-temperature storage with values ranging from 41 to 51 TE g^{-1} dry weight. Only small differences were found during ripening, and no clear trend in radical scavenging properties was observed as the fruit progressed from green to a full ripe stage by day 16. The increases observed in hydrolysable tannins and carotenoids did not impact

the overall antioxidant properties as the fruit ripened. No structural information was obtained for the hydrolyzable tannins (that is, number of galloyl groups) to determine their antioxidant potential, but compounds such as carotenoids and *P*-hydroxybenzoic present in the ripe fruit are known to be poor peroxyl radical scavengers when using polar *in vitro* testing systems.

When mango fruit experience a thermal quarantine treatment and are exposed to low temperatures, enough to induce chill injury, the overall effect on antioxidant phytochemical constituents is relatively small despite adverse effects on aesthetic quality (Talcott *et al.*, 2005). These data indicated that hot water treatment may accelerate fruit ripening based on carotenoid biosynthesis and potentially limit shelf-life, whereas hot water treatments combined with extended low-temperature storage hindered carotenoid development that was attributed to either heat and/or chill injury. Talcott *et al.* (2006) found an increase in polyphenols and carotenoids and better antioxidant capacity in hot-water-treated compared with untreated mangoes. Similarly, Djioua *et al.* (2009) found an increase in total carotenoids and vitamin C content in mango fruit induced by the hot water dip treatment.

Heat treatment was also demonstrated to accelerate mango ripening, and carotenoid concentration was significantly increased due to hot water treatment (Vazquez-Salinas *et al.*, 1985; Jacobi and Giles, 1997). In mango fruit, hot water treatment (at 42°C for 24 h) inhibited polyphenol oxidase (PPO) and peroxidase (PE) activities leading to delayed anthocyanin synthesis, and thus protected color pigment changes by maintaining the anthocyanins in their red-pigmented form with high antioxidative activity in postharvest (Fallik, 2004). A possible activation of the antioxidative system is suggested by the further induced superoxide dismutase (SOD) activity and suppression of peroxidase activity following heat treatment of papaya (Huajaikaew *et al.*, 2005).

These results suggested that adequate HT's can prolong postharvest life of some fruits and can even promote an increase of some bioactive compounds. However, the potential effects of HTs on tropical and subtropical fruits still need to be investigated in more detail with regard to their effect on the antioxidant status.

2.7.4 Effect of cutting

Fresh-cut tropical and subtropical fruits are cut in a wide variety of shapes, and these could influence the degree of damage caused by the wound (Rivera-Lopez *et al.*, 2005). Minimal (fresh-cut) processing includes the unit operation of size reduction, and a given shape has to be provided to the product depending on its final use. Cutting is one of the most delicate steps, since it triggers degradative oxidation as a consequence of the decompartmentalization and contact of the phenolic substrates, present in the vacuoles, with the cytoplasmatic oxidases (Varoquaux and Mazollier, 2002). Therefore, phenolic concentration and antioxidant capacity may change significantly in comparison with the fresh, intact products. These parameters also seem to be relevant in determining the

bioavailability of phenolic compounds (Robles *et al.*, 2007). Different minimal processing techniques have been applied to tropical and subtropical fruit and its effect on nutritional and phytochemical contents in comparison to whole fruits has been reported by different authors. Gil *et al.* (2006) observed significant losses in ascorbic acid but an increase in dehydroascorbic acid throughout storage of fresh-cut pineapple, resulting in greater ascorbic acid content of fresh-cut versus whole fruit. Fresh-cut mango cubes had a decrease in ascorbic acid and an increase in dehydroascorbic acid during storage, maintaining the content of ascorbic acid close to the initial value for both fresh-cut and whole fruit, except at day nine when the high vitamin C contents of whole and fresh-cut fruit were rather different between them (Gil *et al.*, 2006). No significant changes in *β*-carotene were observed due to the effect of storage temperature and cutting shape. In another study, ascorbic acid content was not affected by cutting shape for both cubes and slices of fresh-cut papaya (Rivera-Lopez *et al.*, 2005). Ascorbic acid content of cubes and slices stored at 5°C decreased by 29.6 and 26.38% after 18 days of storage, respectively. Cubes and slices stored at 10°C decreased by 27.30% and 38.92%, respectively, from their initial ascorbic acid content after 14 days of storage (Rivera-Lopez *et al.*, 2005). Even though this study did not observe significant differences among the cutting shapes of papaya fruit, the cutting step *per se* could affect the ascorbic acid content. No significant effect of the cutting shape on the antioxidant capacity expressed as ORAC value was observed in fresh-cut papaya fruit (Rivera-Lopez *et al.*, 2005) but storage temperature significantly affected it. Fresh-cut papaya cubes and slices changed slightly during storage at 5°C. However, significant reductions in ORAC values were found in fresh-cut papaya at 10°C and 20°C (Rivera-Lopez *et al.*, 2005). The cutting shape of fresh-cut papaya fruit was not shown to have immediate effect on *β*-carotene content (Rivera-Lopez *et al.*, 2005). Fresh-cut papaya cubes and slices stored at 5°C did not present any change in *β*-carotene content during ten days of storage. No changes were observed in *β*-carotene content after six days of storage at 10°C; however, after 14 days of storage, there was a depletion of 62% for cubes and 63.4% for papaya slices. Fresh-cut papaya cubes and slices stored at 20°C showed the highest *β*-carotene losses. At this temperature and after six days of storage, *β*-carotene content of cubes and slices decreased by 57.4% and 60.63%, respectively.

Minimal processing steps are expected to induce a rapid enzymatic depletion of several natural antioxidants. Depletion of antioxidant capacity in cubed and sliced papaya fruit could be associated with depletion of both ascorbic acid and *β*-carotene, but in general, the highest loss of these important nutrients was reached before the product was unacceptable or spoiled (Rivera-Lopez *et al.*, 2005).

2.7.5 Use of natural compounds

There are several natural compounds that exhibit positive effects on fruit handling and quality. Among these the most studied are volatile compounds (e.g.

acetaldehyde, benzaldehyde, hexanal) acetic acid, jasmonates, glucosinolates, chitosan, active principles of some plants, and plant extracts, among others (Tripathi and Dubey, 2004).

It has been noticed that jasmonates are associated with defense response in plants (Turner *et al.*, 2002). Jasmonates play an important role as signal molecules in plant defence responses against pathogen attack, induce the synthesis of antioxidant molecules such as vitamin C, phenolic compounds and increase the activity of enzymatic antioxidant systems (Chanjirakul *et al.*, 2006). Gonzalez-Aguilar *et al.* (2004) found that methyl jasmonate (MJ, 10^{-5} and 10^{-4} M) retarded chilling injury in guava fruit after five, ten, and fifteen days at 5°C plus two days at 25°C, maintained better appearance, and increased the activity of phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) enzymes which improve the antioxidant status (Pinsky *et al.*, 2006). MJ at 10^{-5} M or 10^{-4} M treatment increased sugar concentrations in mangoes and guava fruit stored at 5°C that were still ripening, and the increase of sugar in MJ-treated fruit was concluded to be part of the plant defense response system (Gonzalez-Aguilar *et al.*, 2004). A positive correlation between MJ treatment and the increase of ascorbic acid and of PAL activity, which is associated with a plant's defense system, has been observed in guava fruit (Gonzalez-Aguilar *et al.*, 2004). These facts suggest a connection among jasmonates, ascorbic acid, and polyphenols in the inhibition of chilling injury. Changes in antioxidant capacity and the antioxidant enzymatic system in fruits treated with MJ have been reported. Cao *et al.* (2009) observed that MJ increase the content of organic acids, total phenolics, total flavonoids and the activity of SOD, CAT and APX in loquat fruit, and the treatment also maintained significantly higher antioxidant activity. The reaction of jasmonates to environmental stress has also been observed in drought conditions (Kondo *et al.*, 2005). SOD catalyzes the dismutation of O_2 to O_2^- and H_2O_2. SOD activities and endogenous jasmonate levels in the skin of fruit that were stored at 12°C were higher than those at 6°C. These results suggest a link between jasmonates, SOD activity, and O_2 scavenging activity. The possible relation of the antioxidant enzymes and bioactive compounds present in tropical and subtropical fruits with the antioxidant capacity was not widely reported.

It has been observed that the use of antioxidants to prevent browning and deterioration of fresh-cut fruits enhances the antioxidant status. Changes in ascorbic acid of pineapple slices treated with different antibrowning agents have been evaluated (González-Aguilar *et al.*, 2005). No significant changes in ascorbic acid levels were observed in the control or slices treated with acetylcysteine during the storage period at 10°C (Gonzalez-Aguilar *et al.*, 2005). In contrast, it has been found that dehydrated pineapple and guava pretreated with cysteine hydrochloride had high ascorbic acid retention and reduced color change during storage (Mohamed *et al.*, 1993). As is expected, ascorbic acid content was significantly increased after treatments with 0.05 M of ascorbic acid applied to fresh-cut pineapples (Gonzalez-Aguilar *et al.*, 2005). However, after three days, ascorbic acid content of fresh-cut pineapple treated with ascorbic acid decreased continuously from 45 to 18 mg 100 g^{-1} at the end of the storage period

(Gonzalez-Aguilar *et al.*, 2005). It has been reported that dipping treatments containing vitamin C, commonly used to prevent browning, increase the total antioxidant capacity of fresh-cut produce due to the addition of antioxidants as components of the dip solutions (Soliva-Fortuny and Martin-Belloso, 2003). Studies on the effects of these treatments in conjunction with other novel approaches, such as edible coating compounds, are scarce for tropical and subtropical fruits and need further investigation.

2.7.6 Use of ultraviolet (UV) light

UV-C irradiation (240nm–280nm) can be applied at lethal and sub-lethal doses. The detrimental effect of UV-C includes tissue structural damage, changes in cytomorphology and water permeability of inner epidermal cells (Lichtscheidl-Schultz, 1985). Nevertheless, low doses of UV-C irradiation stimulated beneficial reactions in biological organs, a phenomenon known as hormesis (Shama, 2007). It has been reported that hormetic doses of UV-C can prolong the postharvest life and maintain the quality of tropical fruits. These effects include delay of deterioration, senescence process and fruit ripening (Gonzalez-Aguilar *et al.*, 2001; 2007). These authors observed an increase in PAL enzymatic activity in guava tissue as a result of the UV-C treatments which in consequence can enhance flavonoid biosynthesis. Results obtained in this study reinforce the evidence of the efficacy of UV-C treatment to prevent deterioration and maintain quality of different crops, including some mango varieties (González-Aguilar *et al.*, 2001). However, the analysis of specific phytoalexins induced as a response to UV-C treatment in mango is needed to elucidate the possible mode of action of this treatment. The induction of the plant defense system can also trigger the accumulation of these compounds which exhibit antioxidant potential, improving the nutritional status of the fruit (Gonzalez-Aguilar *et al.*, 2007). On the other hand, the phenolic content of fresh-cut mangoes treated with UV-C irradiation increased, but significant losses of vitamin C were observed. These treatments could stimulate or inhibit the synthesis of bioactive compounds that contribute differentially to the antioxidant capacity of fruits (González-Aguilar *et al.*, 2007). Alothman *et al.* (2009) found an increase in phenols and flavonoids in guava and banana after 30 minutes exposure to UV-C light, contrary to the decrease found in pineapple and reduction in vitamin C in mango fruit (González-Aguilar *et al.*, 2007). Jiang *et al.* (2010) applied a dose of 4 kJ m^{-2} for 10 minutes and showed an increase in the antioxidant enzyme activity of CAT, SOD, APX and glutathione reductase (GR). Therefore, the accumulation of antioxidant compounds and nutraceutical quality of fruits treated by UV-C irradiation depends on the doses applied.

The biosynthesis of phenolic compounds is affected by gamma and ultraviolet irradiation due to effects on activity of PAL, an effect which was found in several fruits and vegetables such as citrus (Oufedjikh *et al.*, 2000). The increased PAL activity promotes the biosynthesis of phenolic compounds such as flavanones in citrus (Oufedjikh *et al*., 2000). In addition to PAL induction, ultraviolet irradiation increases the activity of other enzymes involved in flavonoid synthesis, such as

chalcone synthase, chalcone isomerase and dihydroflavonol-4-reductase (Tomas-Barberan and Espin, 2001). Flavonoids have various physiological functions such as antioxidative or antitumor activity as well as a UV protective effect (Higashio *et al.*, 2001). In previous work it was reported that UV-C irradiation processing of fresh-cut honey pineapple, banana 'Pisang mas', and guavas leads to an increase in antioxidants, polyphenols, and flavonoids. Hence, apart from the application of UV for microbial safety at industrial levels, this novel technology can also be exploited for enhancement of health promoting compounds. However, the responsiveness of harvested horticultural produce to UV-C treatment declines with increasing fruit ripening, and thus depends on the harvest time (Terry and Joyce, 2004). Similar results were reported for grapefruits treated with gamma irradiation (Patil *et al.*, 2004). Low doses (≤200 Gy) of irradiation are recommended for enhancing health-promoting compounds (e.g., flavanones, β-carotene, lycopene and ascorbic acid content) in early season grapefruit, while higher doses (400–700 Gy) showed detrimental effects.

Gamma irradiation can cause changes to both macro and micronutrients in foods, depending on the irradiation dose. The available research indicates, however, that carbohydrates, proteins, fatty acids, minerals and trace elements in tropical fruits undergo very minimal alteration during irradiation (www.foodstandards.gov.au). The safety of irradiating tropical fruits has been examined, and available studies on fruits indicate that there are no safety concerns (www.foodstandards.gov.au). There are no changes to the composition of the fruits following irradiation that are likely to cause public health and safety concerns. Irradiation of tropical fruits up to a maximum of 1 kGy (kiloGrey) and employing Good Manufacturing/Irradiation Practices is considered safe for Australian and New Zealand consumers (www.foodstandards.gov.au). Irradiation with 2 kGy had no significant effect on total vitamin C and total carotenoid content of pineapple samples. During the storage, however, vitamin C showed a significant decrease in both control and irradiated samples. Total carotenoids were stable to irradiation and did not show any decrease during the storage period of 12 days.

2.7.7 Effects of processing

Interest and demand for tropical fruits and vegetables is increasing worldwide, and therefore as a result, the tropical fruit industry continues searching for new processing methods to increase the diversity of available products. The emphasis of this industry is on the fresh market, which is currently much more profitable than the processing market. However, the fresh-cut industry for tropical fruits is increasing and demanded by different international markets. Also, a significant percentage of several important tropical fruit crops are unsuitable for fresh market sales, primarily due to various appearance defects and short postharvest life, and therefore different processed products are excellent alternatives. Several tropical fruits are processed as many different products.

The use of 'nonthermal sterilization techniques,' which include high-intensity pulsed electric fields (PEF), high hydrostatic pressures (HPP), and ionizing

(gamma) radiation (IR), has increased lately. Significant information is available on the modes of action of the different techniques against spoilage and pathogenic microorganisms or on their effectiveness on the microbial load reduction in model and real systems (Corbo *et al.*, 2009). However, studies assessing the effect of these emerging technologies on degradation of nutrients and bioactive compounds of food products are still not enough. González Aguilar *et al.* (2010) reported the most recent studies on this topic with emphasis on the effect on the most important bioactive compounds. In general, non-thermal treatments induce a slight loss of bioactive compounds in plant foods and may even enhance their recovery and retention throughout storage. Emerging technologies have different effects on the bioactive compounds. In processed juices, phenolic compounds were far more stable than vitamin C, showing retention/recovery values of 86–140%, anthocyanins showed the lowest recovery rates while carotenoids showed good retention rates (86–100%). Fermentation of noni fruit (*Morinda citrifolia* L.) for three months resulted in a loss of more than 90% free-radical-scavenging activity (RSA) while dehydration at 50°C reduced this variable by 20% (Yang *et al.*, 2007). Storage of noni juice or powder at −18°C and 4°C for three months decreased RSA by 10–55%. The reduction of RSA of noni juice or purée during heat treatment or dehydration was much greater than the reduction of total phenols (Yang *et al.*, 2007). The processed products (ready-to-drink juice, concentrated juice, frozen pulp) of acerola, cashew-apple and pitanga had appreciably lower flavonol levels than the unprocessed fruit, indicating losses during processing (Hoffmann-Ribani *et al.*, 2009). Antioxidant activity and vitamin C concentrations in melon were decreased by HPP and these losses were cultivar-dependent for vitamin C. Levels of β-carotene were significantly increased (Wolbang *et al.*, 2008). Carotenoids and anthocyanins decreased progressively in pineapple and papaya as the blanching temperature and time increased. Pretreatment with sodium metabisulphite prevented carotenoids from oxidation but caused bleaching of anthocyanins (Sian and Ishak, 1991). Kaya *et al.* (2010) studied the effect of drying temperature and moisture on vitamin C content in kiwifruit. They demonstrated that increasing drying temperature decreased vitamin C, its retention was improved by increasing moisture levels of drying air. Pasterurization of mango puree induced changes of *trans* to *cis* isomerisation of β-carotene, but vitamin A losses did not exceed 15.4% (Vásquez-Caicedo *et al.*, 2007).

There has been little research on the impact of HHP on the nutritional and health promoting properties of foods (McInerney *et al.*, 2007). It has been generally known that high pressure has very little effect on low molecular weight compounds such as flavour compounds, vitamins and pigments compared to thermal processes. This effect is particularly important in salads, as most vegetables are rich sources of antioxidant compounds, pigments and vitamins. Butz *et al.* (2002) studied the effect of 600 MPa pressure in combination with elevated temperatures on the pigment and vitamin content of three vegetables. Extractable carotenoids and lycopene contents were improved in pressurized citrus and tomato juice (500 MPa, 10 minutes) as compared with fresh juice, and they also retained more vitamin C content than was the case following thermal

processing (Polydera *et al.*, 2003). Model multivitamin systems containing varied levels of water-soluble vitamins such as ascorbic acid, thiamin and vitamin B6 (pyridoxal), and food systems containing naturally occurring levels of vitamin C were subjected to pressures ranging between 200 and 600 MPa for 30 minutes to determine the effect on vitamin retention. In the model systems observed, ascorbic acid losses were close to 12% while in food material, these losses were insignificant. Compared to conventional sterilization processes, high-pressure treatments retained the vitamins better. Thiamin and pyridoxal in the model system were unaffected by high-pressure processing (Sancho *et al.*, 1999). These findings confirm the fact that high pressure has minimal effect on nutrients in foods. High-pressure processed products are commercially available in the United States, European, and Japanese retail markets. Examples of high-pressure processed products commercially available in the United States include fruit smoothies, guacamole, ready meals with vegetables, fruit juices, among others. The case of guacamole has been a successful application, and volume of this product in the US market has been significantly increased in the past five years. However, more studies involving HHP treatments of tropical fruits will be necessary in order to develop an effective use of this technology, reducing waste, especially in developing countries, and maintaining original fruit characteristics.

The use of byproducts of tropical fruits for different purposes has increased considerably, and it is very important to bear in mind that some parts of the fruit may contain compounds that can cause different disorders in consumers. Mango allergens were shown to be very stable during technological processing. Irrespective of enzymatic matrix decomposition, mechanical tissue disintegration and heating during peeling, mash treatment, and pasteurization, significant loss of allergenicity could not be observed in the extracts of mango purees and nectars (Dube *et al.*, 2004).

2.8 Enhancement of nutritional and health components in tropical and subtropical fruits

In addition to increasing the consumption of tropical and subtropical fruits, the enhancement of their nutritional and health quality is an important goal in the effort to improve global health and nutrition. Erbersdobler (2003) and Boeing *et al.* (2004) advocated the intake of functional foods that are enriched with phytochemicals. Several crop production techniques have been reported to enhance phytochemical content in several fruits (Schreiner, 2005), and some postharvest practices and techniques can also affect the phytochemicals contents (Beuscher *et al.*, 1999; Goldmann *et al.*, 1999; Huyskens-Keil and Schreiner, 2004).

Genetic engineering can make a substantial contribution to improved nutrition in tropical and subtropical fruits (Fernie *et al.*, 2006). Biotechnology has been applied to improve the nutritional quality of a range of crops (Dalal *et al.*, 2006),

targeting protein quality and quantity, desirable fatty acids, vitamins and minerals and antioxidants. Plants, including fruits, naturally contain a large array of antioxidants, including carotenoids, vitamins C and E, and flavonoids, and efforts to increase their content have taken both breeding-based and transgenic approaches. Naturally occurring germplasm can provide a rich source of genetic variation and it is likely that new strategies for improving the nutritional value of tropical and subtropical fruits will result from screening for variation in nutritional and health composition in wild crops and the broadest possible spectrum of existing cultivars.

Vast variation in the amounts of phenolic antioxidants available via diet (Shetty *et al.*, 2003) coupled with reduced bioavailability and functionality has led to an urgent need to develop innovative strategies to enrich diets with phenolics and specifically phenolic antioxidants with consistent phytochemical profile for enhanced health functionality. Of the many strategies, two are important to enrich phenolic antioxidants. The first is genetic modification of cultivars to produce plants that will yield fruits with higher phenolic concentration. Currently, in terms of genetic improvement, breeding strategy coupled with micropropagation using tissue culture is being developed (McCue and Shetty, 2004). These strategies, along with genetic modification, could be directed toward phytochemical enrichment and quality improvement. However, this method presents important issues, such as regulation of key metabolites by multiple genes and biochemical pathways, acceptance of genetically modified foods, and the relative time and economic considerations involved (Kishida *et al.*, 2000). Another exciting strategy that can be used is the bioprocessing of botanicals using solid-state bioprocessing and synergies to generate phytochemical profiles with enhanced health functionality. This strategy can be used for juice and pulp as well as pomace that remain after the juice is extracted from the fruit. Fermentation of fruit juices, such as grape juice to wine, has already been shown to improve nutritive and health-promoting activities (McCue and Shetty, 2003; Randhir and Shetty, 2004). Solid-state bioprocessing done on the pulps using food grade fungi can result in enrichment of the pulp with phenolic antioxidants and functionally important phenolic phytochemicals, and also improve phytochemical profile consistency.

To date, the effectiveness of postharvest treatments has been assessed mainly by the quality maintenance of harvested fruit and vegetables. However, with rising consumer interest in foods that promote health, attention has shifted from quality maintenance to quality assurance with particular emphasis on the enhancement of health-promoting phytochemicals. Therefore, to obtain tropical fruit enriched with phytochemicals, postharvest treatments may be used either singularly or in combination to elicit the desired effect. To ensure an efficient and consumer- oriented supply chain, these postharvest treatments should be combined with crop management strategies. Such phytochemical-enriched fruit could be served as fresh products or used as raw material for functional foods and supplements and would act as a complementary or synergistic strategy to human nutrition programs and nutrition policy for enhancing the consumption of

phytochemicals. However, investigation of the beneficial effects of physical and chemical postharvest treatments on health promoting phytochemicals in fruit and vegetables needs to be extended.

2.9 Conclusions

Fruit consumption definitively has health-promoting properties. Tropical and subtropical fruits are rich sources of diverse nutritional and health components, and therefore their consumption is important for health. However, there are still needs for research on the characterization of the nutritional and health components of many tropical and subtropical fruits. In addition, there is a need for controlled, clinical intervention trials in order to investigate and to confirm the effect of the consumption of tropical and subtropical fruits on the different diseases, as well as studies to reveal the mechanisms behind the effect of the different phytochemical components in these fruits.

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3

Postharvest biology of tropical and subtropical fruits

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Abstract: Postharvest losses in quantity and quality of tropical and subtropical fruits are commonly very high, especially as many of these fruits are produced in developing countries and transported long distance to markets in developing and developed countries. Biological factors involved in deterioration of fresh fruit quality include the rates of respiration, ethylene production and transpiration and fruit sensitivity to ethylene and also compositional changes that affect color, texture, flavor (taste and aroma) and nutritional quality. Several physiological disorders also reduce fruit quality postharvest. These are the result of products' responses to various environmental and physical stresses, including temperature (the most significant factor), relative humidity, ethylene, atmospheric composition (concentrations of oxygen, carbon dioxide and other gases), and light. Lastly, other biological factors such as disease and insect infestation can reduce fruit quality. There are many opportunities to reduce quality deterioration associated with biological factors by selecting genotypes that have lower respiration and ethylene production rates, less sensitivity to ethylene, slower softening rate, improved flavor quality, enhanced nutritional quality (vitamins, minerals, dietary fiber, and phytonutrients including carotenoids and phenolic compounds), reduced browning potential, decreased susceptibility to chilling injury, and/or increased resistance to postharvest decay-causing pathogens. This chapter describes some of the important biological and environmental factors that influence deterioration rates, therefore causing losses of tropical and subtropical fruits. The potential uses of biotechnological approaches for improving quality and postharvest life are also briefly discussed.

Key words: biotechnology, composition, deterioration, ethylene, pathological breakdown, physiological disorders, respiration, ripening, senescence.

3.1 Introduction

Subtropical fruits include avocado, carob, cherimoya, citrus fruits (which are most important in international commerce), dates, figs, jujubes, kiwifruit, loquat,

litchi, olive, persimmon and pomegranate; some of these fruits are also grown in tropical areas. Tropical fruits include acerola, banana, breadfruit, carambola, durian, guava, jackfruit, longan, mamey sapote, mango, mangosteen, papaya, passion fruit, pineapple, prickly pear, rambutan, sapodilla, soursop and sweetsop; a few of these fruits are also grown in subtropical regions. Only four of these fruits, i.e., banana, mango, papaya and pineapple, are important in international commerce at this time, but it is possible that other tropical fruits will be added to this group in the future.

Tropical and subtropical fruits are very diverse in their morphological, compositional and physiological characteristics and therefore they can be classified into groups requiring different treatment to maintain their quality and extend their postharvest shelf life. However, all these fruits are characterized by high water content and potential for water loss resulting in shriveling, susceptibility to mechanical injury and susceptibility to attacks by microorganisms (bacteria, fungi) and insect infestation. Fresh tropical and subtropical fruits are living organs subject to continuous changes after harvest, and therefore attention to detail is required throughout the handling system between the sites of production and consumption. Some of the changes that occur after harvest in these fruits are desirable, and may require specific treatments and techniques to promote them, while many other changes are undesirable and require treatments and techniques to delay and minimize their incidence and severity. Although none of the changes in fresh fruits can be stopped, many can be retarded. Ripening and senescence, the last stages in the development of fresh tropical and subtropical fruits, are characterized by some irreversible processes that lead to breakdown and death of the fruit. Understanding the biological and environmental factors affecting postharvest deterioration rate is essential to developing treatments for maintaining quality and extending postharvest life. Several books on postharvest biology and technology of horticultural perishables including fruits have been published during the past two decades (Ben-Yehoshua, 2005; Chakraverty *et al.*, 2003; Florkowski *et al.*, 2009; Gross *et al.*, 2002; Kader, 2002a; Kays and Paull, 2004; Knee, 2002; Lamikanra *et al.*, 2005; Nunes, 2008; Paliyath *et al.*, 2008; Salunkhe and Kadam, 1995; Seymour *et al.*, 1993; Thompson, 2003; Wills *et al.*, 2007), but only one has focused on subtropical and tropical fruits (Mitra, 1997).

3.2 Diversity in fruit characteristics

Tropical and subtropical fruits are diverse in their morphological and compositional characteristics, in their postharvest physiology, and in their optimum postharvest requirements and recommendations. Subtropical fruit, for example, can be grouped into three subgroups, according to their relative perishability, as follows: (1) highly perishable such as fresh figs, loquat, litchi; (2) moderately perishable such as cherimoya, olive, persimmon; and (3) less perishable such as citrus fruits,

carob (dry), dried figs, date, jujube (Chinese date), kiwifruit, pomegranate (Kader and Arpaia, 2002). Both tropical and subtropical fruits are also of diverse sizes, shapes, and botanical structures (drupes, berries, aggregate fruits). Avocados are one-seeded berries which vary in size among cultivars and are usually pear-shaped, but can be round or oval in shape, while all citrus fruits are berry-like fruits classified as hesperidia, which have a separable rind. The pigmented part of the rind is called the flavedo (epidermis + several subepidermal layers), while the whitish part of the rind is called the albedo. The juicy part of the fruit consists of segments filled with juice sacs. The weight of subtropical and tropical fruit ranges from 5–7 g (acerola) to 8–20 kg (jackfruit). Many kinds of tropical fruits have large seeds, inedible skin, or tough rind, like the citrus fruits mentioned above. This contributes to a high waste index (inedible portion) relative to temperate-zone fruits (2–15% waste index). For example, the waste indices for papaya, banana and pineapple are 25, 30 and 41%, respectively. Some tropical fruits, such as guava and sapote, contain sclereids with heavily lignified walls (stone cells), which influence their textural quality (Kader *et al.*, 2002). Siriphanich (2002) concluded that many tropical fruits have peels that are physiologically distinct from the pulp and require special attention after harvest to satisfy consumers; an important requirement is to synchronize changes in the peel with those in the pulp.

3.3 Maturation and ripening

Maturity at harvest is the most important factor that determines storage life and final fruit quality. Immature fruits are more subject to shriveling and mechanical damage and are of inferior quality when ripe. Overripe fruits are likely to become soft and mealy with insipid flavor soon after harvest. Fruits picked either too early or too late in the season are more susceptible to physiological disorders and have a shorter storage life than those picked at the proper maturity.

All subtropical and tropical fruits, with a few exceptions such as avocados and bananas, reach their best eating quality when allowed to ripen on the tree or plant. However, some fruits are picked mature but unripe so that they can withstand the postharvest handling system when shipped long distances. Most currently used maturity indices are based on a compromise between those indices that would ensure the best eating quality to consumers and those that provide the needed flexibility in marketing.

Fruits can be divided into two groups: those that are incapable of continuing their ripening process once removed from the plant, known as non-climacteric fruits, and those that can be harvested mature and ripened off the plant, known as climacteric fruits. Citrus fruits (grapefruit, kumquat, lemon, lime, orange, mandarin and pomelo), pineapple and pomegranate are examples of those that cannot continue to ripen once picked. Example of fruits that can ripen off plant, however, include avocado, banana, cherimoya, kiwifruit, mango, papaya and

Table 3.1 Classification of some tropical and subtropical fruits into climacteric and non-climacteric respiratory patterns (modified from Kader, 2002c)

Climacteric fruit	Non-climacteric fruits
Avocado, banana, breadfruit, cherimoya, durian, feijoa, fig, guava, jackfruit, kiwifruit, mango, mangosteen, muskmelon, papaya, passion fruit, persimmon, plantain, rambutan, sapodilla, sapote, soursop, sweetsop	Carambola, cashew apple, coconut, date, grapefruit, jujube, kumquat, lemon, lime, longan, loquat, litchi, mandarin, olive, orange, pineapple, pomegranate, prickly (cactus) pear, pomelo, rose apple, star apple, tamarillo, tamarind

persimmon (see Table 3.1). Fruits of the first group produce very small quantities of ethylene and do not respond to ethylene treatment except in terms of degreening (removal of chlorophyll) in citrus fruits and pineapples. Fruits capable of off-plant ripening produce much larger quantities of ethylene in association with their ripening, and exposure to ethylene treatment will result in faster and more uniform ripening (see sections 3.5.1 and 3.5.2).

Maturity indices used commercially include fruit size, shape, external and/or internal color, firmness, juice content, total solids (dry matter content), and sugar/acid ratio. Some nondestructive methods for estimating internal quality factors are available, but more research and development efforts are needed to develop portable and less expensive instruments that can be used for determining harvest time based on optimal maturity and ripeness stage.

3.4 Quality attributes

Quality is defined as 'any of the features that make something what it is' or 'the degree of excellence or superiority'. Quality attributes include appearance, texture, flavor (taste and aroma), and nutritive value of fruits. Producers want their fruits to have good appearance and few visual defects, but for them a useful cultivar of a given fruit species must score high on yield, disease resistance, ease of harvest, and shipping quality. To receivers and market distributors, quality of appearance is most important; they are also keenly interested in firmness and long storage life. Consumers judge fruits as good quality if they look good, are firm, and offer good flavor and nutritive value. Although consumers buy on the basis of appearance and feel, their satisfaction and likelihood to buy that fruit again depends on their perception of good eating quality.

Various quality attributes are used in commodity evaluation in relation to specifications for grades and standards, selection in breeding programs, and evaluation of responses to various environmental factors and/or postharvest treatment, including storage conditions. The relative importance of each of these quality factors depends on the commodity and its intended use (fresh or processed). Numerous defects can influence appearance quality of horticultural crops. Morphological defects include seed germination inside fruits. Physical defects include shriveling of

all fruits; internal drying of some fruits; and mechanical damage such as punctures, cuts and deep scratches, splits and crushing, skin abrasions and scuffing, deformation (compression), and bruising. Temperature-related disorders (freezing, chilling, sunburn, sunscald) are examples of physiological defects (Kader, 2002a).

Textural quality of fruits is not only important for their eating and cooking quality but also for their shipping ability. Soft fruits cannot be shipped long distances without extensive losses owing to physical injuries. This necessitates harvesting fruits at less than ideal maturity from the flavor quality standpoint in many cases.

Flavor quality involves perception of the tastes and aromas of many compounds. Objective analytical determination of critical components must be coupled with subjective evaluations by a taste panel to yield useful and meaningful information about flavor quality of fresh fruits. This approach can be used to define a minimum level of acceptability. To find out consumer preferences for flavor of a given commodity, large-scale testing by a representative sample of consumers is required. Postharvest life of fruits based on flavor is generally shorter than postharvest life based on appearance and textural quality. To increase consumer satisfaction, fruits should be sold before the end of their flavor life.

Fresh fruits play a very significant role in human nutrition, especially as sources of vitamins (vitamin C, vitamin A, vitamin B_6, thiamin, niacin), minerals and dietary fiber. They also contain many phytochemicals (such as antioxidant phenolic compounds and carotenoids) that have been associated with reduced risk of some forms of cancer, heart disease, stroke, and other chronic diseases (Vicente *et al.*, 2009; Yahia, 2009b; Yahia, 2010; Yahia and Ornelas-Paz, 2010). Tropical fruits have always been and are still an important source of nutrients for people in tropical areas. Bananas and breadfruits are widely used as starch sources. Acerola fruits contain the highest known ascorbic acid content among all fruits (1000–3300 mg 100 gm^{-1} fresh weight). Other good sources of vitamin C include guava, litchi, papaya and passion fruit. Mango, papaya and the recently introduced yellow-flesh pineapple cultivars are good sources of vitamin A. Breadfruit and cherimoya contain relatively high amounts of niacin and thiamin. Most tropical fruits are good sources of minerals, especially potassium and iron. Citrus fruits are very good sources of vitamin C and rank as number 1 in their contribution of vitamin C to human nutrition in the US.

Safety factors include levels of naturally occurring toxicants in certain crops that vary according to genotypes and are routinely monitored by plant breeders to ensure that they do not exceed their safe levels in new cultivars. Contaminants, such as chemical residues and heavy metals in fresh fruits are also monitored by various agencies to ensure compliance with established maximum residue tolerance levels. Sanitation procedures throughout the harvesting and postharvest handling operations are essential to minimizing microbial contamination. Proper preharvest and postharvest handling procedures must be enforced to reduce the potential for growth and development of mycotoxin-producing fungi.

Successful postharvest handling depends on preharvest factors (Arpaia, 1994) that influence the initial quality of the crop at harvest, including the degree of maturity, and on careful handling to minimize mechanical damage, ensure proper management

of the environmental conditions, and good sanitation. Applying adequate postharvest techniques and treatments is important to maintain quality in terms of appearance, texture, flavor and nutritive value, to maintain food safety and to reduce losses along the supply chain between harvest and consumption and to prolong postharvest life.

3.5 Biological factors affecting deterioration

The tropics possess an astonishingly vast and diverse number of species of edible fruits, but the climatic conditions also tend to hasten spoilage. Most tropical and subtropical fruits have a limited postharvest life due to their high perishability, sensitivity to low (chilling) temperatures and to several decay organisms. The following biological factors affect fresh tropical and subtropical fruits and therefore influence their postharvest life and quality.

3.5.1 Respiration

Aerobic respiration is the process by which stored organic materials (carbohydrates, proteins, fats) are broken down into simple end products with the release of energy. Oxygen (O_2) is used in this process, and carbon dioxide (CO_2) is produced (Fig. 3.1).

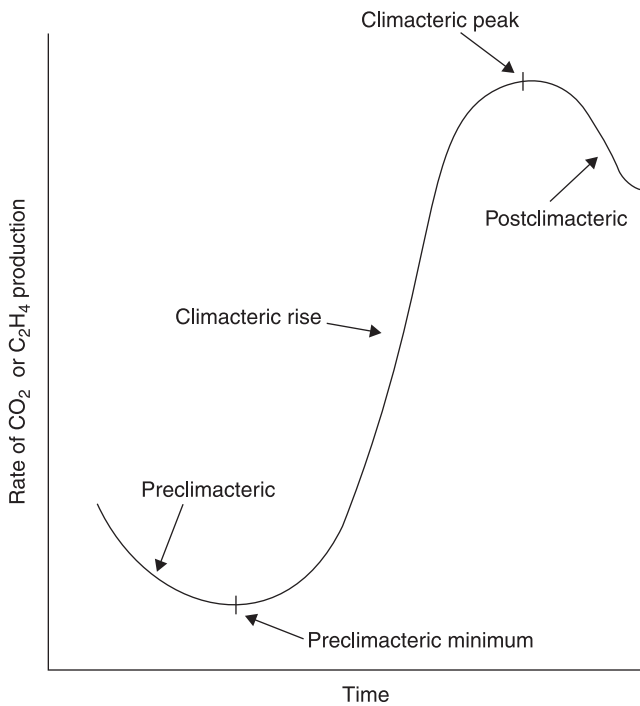

Fig. 3.1 Phases of the respiratory climacteric in a ripening climacteric fruit.

The loss of stored food reserves in the commodity during respiration hastens senescence as the reserves that provide energy to maintain the commodity's living status are exhausted. This reduces the food value (energy value) for the consumer, causes loss of flavor quality, especially sweetness, and causes loss of salable dry weight (especially important for commodities destined for dehydration). The energy released as heat, known as vital heat, affects postharvest technology considerations such as estimations of refrigeration and ventilation requirement (Kader, 2002c).

Saltveit (2002) provides a clear description of aerobic respiration:

> Maintaining a supply of high-energy compounds like adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH) and pyrophosphate (PPi) is a primary function of respiration. The overall process of aerobic respiration involves regeneration of ATP from ADP (adenosine diphosphate) and Pi (inorganic phosphate) with release of CO_2 and H_2O. If glucose is used as substrate, the overall equation for respiration can be written as follows:
>
> $$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6\ H_2O + 686\ \text{kcal mole}^{-1}$$
>
> The components of this reaction have various sources and destinations. The one mole of glucose (180 g) can come from stored simple sugars like glucose and sucrose or complex polysaccharides like starch. Fats and proteins can also provide substrates for respiration, but their derivatives, i.e., fatty acids, glycerol and amino acids, enter at later stages in the overall process and as smaller, partially metabolized molecules (Fig. 3.2). The 192 g of O_2 (6 moles × 32 g mol^{-1}) used to oxidize the

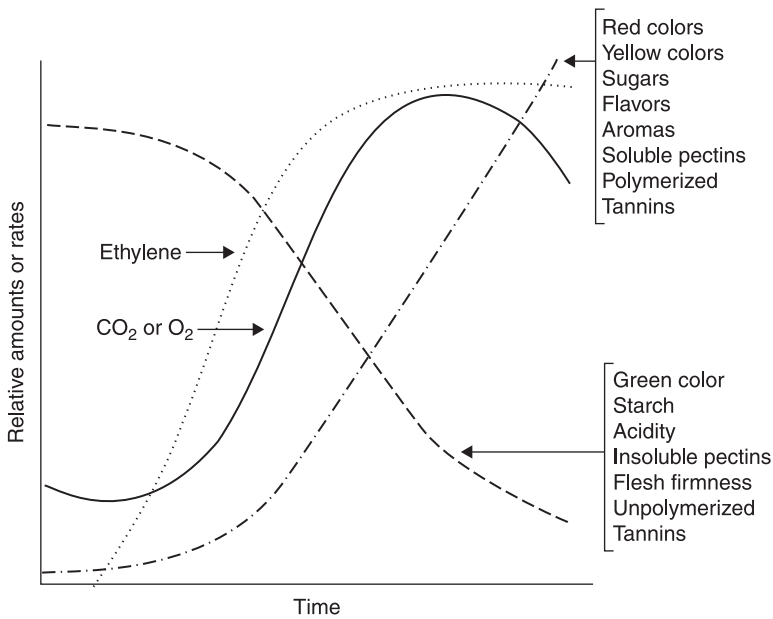

Fig. 3.2 A schematic of compositional changes associated with fruit ripening.

1 mole of glucose diffuses into the tissue from the surrounding atmosphere, while the 6 moles of CO_2 (264 g) diffuses out of the tissue. The 6 moles of H_2O (108 g) that are produced are simply incorporated into the aqueous solution of the cell.

Aerobic respiration involves a series of three complex reactions, each of which is catalyzed by a number of specific enzymes which either: (1) add an energy containing phosphate group to the substrate molecule; (2) rearrange the molecule; or (3) breakdown the molecule to a simpler one. The three interconnected metabolic pathways are glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport system.

1. Glycolysis, i.e., the breakdown or lyse of glucose, occurs in the cytoplasm of the cell. It involves the production of two molecules of pyruvate from each molecule of glucose. Each of the ten distinct, sequential reactions in glycolysis is catalyzed by one enzyme. Two key enzymes in glycolysis are phosphofructokinase (PFK) and pyruvate kinase (PK). Cells can control their rate of energy production by altering the rate of glycolysis, primarily through controlling PFK and PK activity. One of the products of respiration, ATP, is used as a negative feed-back inhibitor to control the activity of PFK. Glycolysis produces two molecules of ATP and two molecules of NADH from the breakdown of each molecule of glucose.
2. Tricarboxylic acid (TCA) cycle, which occurs in the mitochondrial matrix, involves the breakdown of pyruvate into CO_2 in nine sequential, enzymatic reactions. Pyruvate is decarboxylated (loses CO_2) to form acetate that condenses with a co-enzyme to form acetyl CoA. This compound then enters the cycle by condensation with oxaloacetate to form citric acid. Citric acid has three carboxy groups from which the cycle derives its name. Through a series of seven successive rearrangements, oxidations and decarboxylations, citric acid is converted back into oxaloacetate that is then ready to accept another acetyl CoA molecule. Besides producing the many small molecules that are used in the synthetic reactions of the cell, the TCA cycle also produces one molecule of flavin adenine dinucleotide (FADH2) and four molecules of NADH for each molecule of pyruvate metabolized.
3. Electron transport system, which occurs on membranes in the mitochondria, involves the production of ATP from the high energy intermediates FADH2 and NADH. The energy contained in a molecule of NADH or FADH2 is more than is needed for most cellular processes. In a series of reactions, one NADH molecule produces three ATP molecules, while one FADH molecule produces two ATP molecules. The production of ATP is not only dependent on the energy contained in NADH and FADH2, but also on the chemical environment (i.e., pH and ion concentrations) within the cell and mitochondria (Saltveit, 2002).

Fermentation, or anaerobic respiration, occurs when the O_2 concentration in the fruit falls below a certain level and can reduce fruit quality. Saltveit (2002) describes the process as follows:

fermentation, or anaerobic respiration involves the conversion of hexose sugars into alcohol and CO_2 in the absence of O_2. Pyruvate produced through glycolysis via a series of reactions that do not require O_2 can be converted to lactic acid, malic acid, acetyl-CoA, or acetaldehyde. The pathway chosen depends on cellular pH, prior stresses, and the current metabolic needs of the cell. Acidification of the cytoplasm

enhances the activity of pyruvic decarboxylase that then shunts pyruvate to form CO_2 and acetaldehyde. The acetaldehyde is converted by the enzyme alcohol dehydrogenase to ethanol with the regeneration of NAD^+. Two molecules of ATP and 21 kcal of heat energy are produced in anaerobic respiration (alcoholic fermentation) from each molecule of glucose.

For more details about the biochemistry of respiratory metabolism, see the book by Kays and Paull (2004), among several others.

As mentioned in section 3.3, tropical and subtropical fruits are commonly classified according to their respiration patterns into 'climacteric' and 'non-climacteric' fruits (Table 3.1). Climacteric fruits are those that show a sudden increase in aerobic respiration, coincident with their maturation and ripening, while non-climacteric fruits do not show such a pattern in their respiration and ethylene production rates. Deterioration rates are inversely related to aerobic respiration rates of subtropical and tropical fruits (Table 3.2). Both respiration and deterioration rates are accelerated by an increase in temperature, decrease in relative humidity, exposure to ethylene, wounding and other mechanical injuries and exposure to other abiotic and biotic stresses.

The oxygen concentration at which a shift from predominantly aerobic to predominantly anaerobic respiration occurs is known as the extinction point, the anaerobic compensation point, or the fermentative threshold (Saltveit, 2002). The shift from aerobic to anaerobic respiration depends on fruit maturity and ripeness stage (gas diffusion characteristics), temperature, and duration of exposure to stress-inducing concentrations of O_2 and/or CO_2 (Kader, 1986a). Since the oxygen concentration at any point in a fruit varies due to rates of gas diffusion and respiration, some parts of the commodity may also become anaerobic while others remain aerobic (Saltveit, 2002).

Elevated carbon dioxide concentrations are highly effective in altering primary and secondary metabolism of harvested fruits and may have a marked impact on many biochemical processes, thus affecting quality parameters. The response to

Table 3.2 Classification of some tropical and subtropical fruits according to their rate of respiration (modified from Kader, 2002c)

Class	Respiration rate (mg CO_2 kg^{-1} hr^{-1} at 20°C)	Fruit
Very low	< 35	Dates, dried tropical and subtropical fruits
Low	35–70	Avocado (mature), banana (mature-green), carambola, citrus fruits, jackfruit, kiwifruit, litchi, longan, mangosteen, papaya, pineapple, pomegranate
Moderate	70–150	Durian, fig, guava, lanzone, mango, olive, rambutan
High	150–300	Avocado (ripe), banana (ripe)
Very high	>300	Cherimoya, passion fruit, sapote, soursop

high carbon dioxide concentrations may be beneficial or detrimental depending upon the nature of the product, the concentration of carbon dioxide outside and inside the tissue, the duration of exposure and the concentration of oxygen (Kader, 1986a). Since carbon dioxide is toxic, many tissues undergo damage following exposure to high carbon dioxide concentrations, and high variability is present among and within species in terms of susceptibility to carbon dioxide injury, resulting in internal and external physiological disorders and the appearance of off-flavors often related to the products of fermentation (Kanellis *et al.*, 2009). Severe atmospheric stress (very low O_2 and/or very high CO_2 atmospheres) conditions decrease cytoplasmic pH and ATP levels, and reduce pyruvate dehydrogenase activity. Alcohol dehydrogenase, lactate dehydrogenase, cytosolic glyceraldehyde dehydrogenase, aldolase, glucose phosphate isomerase, pyruvate decarboxylase, sucrose synthase, and alanine aminotransferase are induced or activated by anaerobiosis (Kanellis *et al.*, 2009), causing accumulation of acetaldehyde, ethanol, ethyl acetate, and/or lacate, which may be detrimental to the commodities if they are exposed to atmospheric stress conditions beyond their tolerance limits (Kanellis *et al.*, 2009). Gene expression is strongly altered by anoxia, and protein synthesis is redirected to the production of a new set of anaerobic polypeptides (ANP); several ANPs have been identified, and have been shown to be enzymes engaged with the glycolytic and fermentative pathway (Kanellis *et al.*, 2009).

Limits of tolerance to low O_2 concentration and elevated CO_2 concentrations may vary depending on storage temperature and duration of exposure. The duration for which a commodity is tolerant to low O_2 levels becomes shorter as storage temperature rises, because a tissue's O_2 requirements for aerobic respiration increase with higher temperatures (Kader, 1986a; Beaudry, 2000). Depending on the commodity, damage associated with CO_2 may increase or decrease with an increase in temperature. A rise in temperature may cause an increase or decrease of CO_2 in the tissue and, furthermore, the physiological effect of CO_2 can be temperature dependent. Tolerance limits to elevated CO_2 decrease with a reduction in O_2 level, and similarly the tolerance limits to reduced O_2 increase with the increase in CO_2 level.

Up to a point, fruits are able to recover from the detrimental effects of low O_2 and/or high CO_2 stresses (fermentative metabolism) and resume normal respiratory metabolism. Plant tissues have the capacity for recovery from the stresses caused by brief exposures to fungistatic atmospheres (>10 kPa CO_2) or insecticidal atmospheres (40 to 80 kPa CO_2). Postclimacteric fruits are less tolerant to atmospheric stress and have a lower capacity for recovery following exposure to reduced O_2 and/or elevated CO_2 levels than preclimacteric fruits. The speed and extent of recovery depend upon duration and levels of stresses, and underlying, metabolically driven cellular repair (Kader, 1986a).

3.5.2 Ethylene production and response

Ethylene, the simplest of the organic compounds affecting the physiological processes of plants, is a natural product of plant metabolism and is produced by all tissues of higher plants and by some microorganisms. As a plant hormone,

ethylene regulates many aspects of growth, development and senescence and is physiologically active in trace amounts (less than 0.1 ppm) (Abeles *et al.*, 1992). Ethylene induces fruit abscission, softening and some physiological disorders (Abeles *et al.*, 1992). Ethylene may increase decay development of some fruits by accelerating their senescence and softening, and by inhibiting the formation of antifungal compounds in the host tissue (Kader, 1985). The detrimental effects of ethylene on quality center on altering or accelerating the natural processes of development, ripening and senescence, while the beneficial effects of ethylene on quality center on roughly the same attributes as the detrimental effects, but differ in both degree and direction (Saltveit, 1999). Low temperatures, controlled or modified atmospheres (CA or MA), and ethylene avoidance and/or scrubbing techniques are used to reduce ethylene damage.

Several factors affect ethylene production rates, including species (Table 3.3), cultivar, maturity and ripening (generally increases due to maturity and ripening, especially in climacteric fruits), temperatures (increases at temperatures of up to 30°C), physical/mechanical injury, different types of stress such as water stress and microbial attack. Other factors governing plant responses to ethylene exposure include ethylene concentration (linear response with logarithmic increases in ethylene concentration), atmospheric composition (more than 10% oxygen and less than 1% carbon dioxide), and duration of exposure (Saltveit, 1999).

Generally, ethylene production rates increase with maturity at harvest, physical injures, disease incidence, increased temperatures up to 30°C, and water stress. On the other hand, ethylene production rates by fresh fruits are reduced by storage at low temperature, and by reduced O_2 (less than 8%) and/or elevated CO_2 (above 1%) levels around the commodity (Kader, 2002c). Elevated-CO_2 atmospheres inhibit activity of ACC synthase (key regulatory site of ethylene biosynthesis), while ACC oxidase activity is stimulated at low CO_2 and inhibited at high CO_2 concentrations and/or low O_2 levels. Ethylene action is inhibited by elevated-CO_2 atmospheres. These interactions among ethylene, O_2, and CO_2 provide the

Table 3.3 Classification of some tropical and subtropical fruits according to their ethylene production rate (modified from Kader, 2002c)

Class	Ethylene production rate ($\mu L\ C_2H_4\ kg^{-1}\ hr^{-1}$) at 20°C	Fruits
Very low	Less than 0.1 ppm	Citrus fruits, jujube, pomegranate
Low	0.1–1.0	Carambola, olive, persimmon, pineapple, tamarillo
Moderate	1.0–10.0	Banana, breadfruit, durian, fig, guava, litchi, mango, mangosteen, plantain, rambutan
High	10.0–100.0	Avocado (ripe), feijoa, kiwifruit (ripe), papaya
Very high	> 100.0	Cherimoya, mamey apple, passion fruit, sapote, soursop

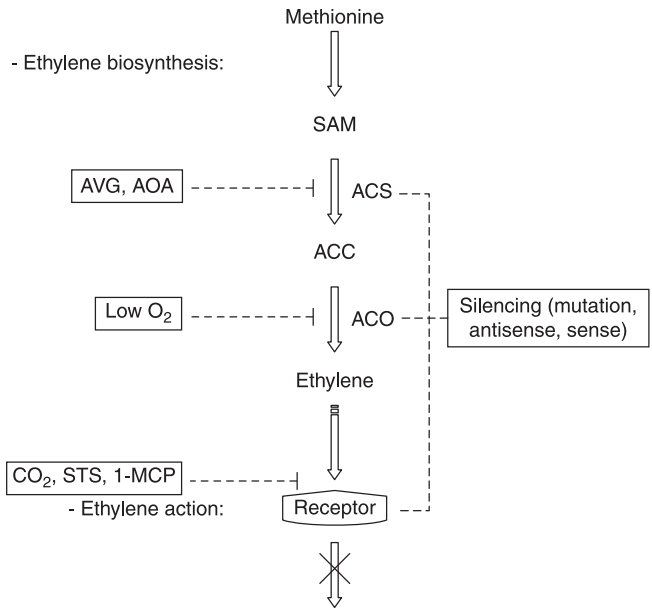

Fig. 3.3 An overview of factors affecting ethylene biosynthesis and action.

biological basis of modified (MA) and controlled atmospheres (CA) effects on delaying ripening and senescence of fruits (Yahia, 2009a).

Ethylene is synthesized from the amino acid methionine which is converted to S-adenosylmethionine (AdoMet = SAM) by the enzyme S-AdoMet synthase (ADS) (Fig. 3.3). AdoMet is converted by the enzyme 1-aminocyclopropane-1-carboxylate synthase (ACC synthase = ACS) to 5-methylthioadenosine (MTA), which is converted back to methionine via the Yang-cycle and to 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene in the presence of O_2. ACC is finally oxidized by ACC oxidase (ACO) to form ethylene, cyanide and carbon dioxide. The conversion of AdoMet to ACC by ACS is the first committed and generally considered as the rate-limiting step in ethylene biosynthesis (Yang and Hoffman, 1984; Kende, 1993; Kays and Paull, 2004; Broekaert *et al.*, 2006).

Saltveit *et al.* (1998) reviewed the history of the discovery of ethylene as a plant growth substance and concluded that 'while great advances had been made with the traditional techniques of physiology and biochemistry, further elucidation of ethylene biosynthesis and action hinged on using the modern techniques of molecular biology and genetic engineering'. Breakthroughs in understanding ethylene signal transduction came from pursuing a genetic approach in *Arabidopsis thaliana* (Bleeker, 1999). A family of ETR1-like receptors interact with CTR1 to express ethylene response pathways while ethylene binding inhibits this activity. Ethylene signaling exists in climacteric and non-climacteric fruits.

Molecular and genetic analysis of fruit development, and especially ripening of fleshy fruits, has resulted in significant gains in knowledge over recent years

about ethylene biosynthesis and response, cell wall metabolism, and environmental factors that impact ripening (Seymour *et al.*, 1993; Giovannoni, 2001). The isolation of fruit ripening-related genes has resulted not only in tools for studying the direct effects of specific gene products on ripening but also in opportunities to isolate and study gene regulatory elements that may illuminate regulatory mechanisms (Giovannoni, 2001; Klee and Clark, 2002). Biotechnology is a tool that can be utilized, in an interdisciplinary approach, to address some of the concerns about quality attributes and the biological causes of deterioration of harvested produce (King and O'Donoghue, 1995; Baldwin, 2002; Klee and Clark, 2002; Pech *et al.*, 2005).

3.5.3 Compositional changes

Tropical and subtropical fruits contain all classes of pigments (chlorophylls, carotenoids, flavonoids, betalaines), and these are important in diverse processes that contribute to color, flavor, resistance to microbial and pests attack, human nutrition and health, among others (Yahia and Ornelas, 2010). Many changes in pigments take place during development and maturation of the fruit on the plant. Some may continue after harvest and can be desirable or undesirable. Loss of chlorophyll (green color) is desirable in fruits. Development of carotenoids (yellow and orange colors) is desirable in fruits such as citrus and papaya. The desired red color development in blood oranges is due to anthocyanins and in pink grapefruit it is due to a specific carotenoid (lycopene). Beta-carotene is provitamin A and is important in nutritional quality. Development of anthocyanins (red and blue colors) is desirable in fruits such as pomegranates, litchi, and rambutan; these water-soluble pigments are much less stable than carotenoids. Changes in anthocyanins and other phenolic compounds, however, are undesirable because they may result in tissue browning (Kader, 2002c).

Changes in carbohydrates include starch-to-sugar conversion (desirable in banana, kiwifruit, mango, and other fruits), sugar-to-starch conversion and conversion of starch and sugars to CO_2 and water through respiration. Breakdown of pectins and other polysaccharides results in softening of fruits and a consequent increase in susceptibility to mechanical injures. Changes in organic acids, proteins, amino acids, and lipids can influence flavor quality of the fruit. Loss in vitamin content, especially ascorbic acid (vitamin C), is detrimental to nutritional quality. Production of flavor volatiles associated with ripening of fruits is very important to their eating quality. Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage, higher temperature, low relative humidity, and chilling injury of chilling-sensitive commodities (Kader, 2002c).

3.5.4 Growth and development

Some growth and developmental changes may continue to take place after harvest, such as periderm formation associated with wound healing (a desirable change)

and seed germination inside fruits such as tomatoes, peppers and lemons (an undesirable change).

3.5.5 Transpiration and water loss

All fresh horticultural commodities, including tropical and subtropical fruits, are characterized by high water content (up to 90–95% in some fruits), which is readily lost. Water loss is a main cause of deterioration because it results not only in direct quantitative losses (loss of salable weight) but also in losses in appearance (wilting and shriveling), textural quality (softening, flaccidity, limpness, loss of crispness and juiciness), and nutritional quality. The dermal system (outer protective coverings) governs the regulation of water loss by the commodity. It includes the cuticle, epidermal cells, stomata, lenticels, and trichomes (hairs). The cuticle is composed of surface waxes, cutin embedded in wax, and a layer of mixtures of cutin, wax, and carbohydrate polymers. The thickness, structure and chemical composition of the cuticle vary greatly among commodities and among developmental stages of a given commodity (Kader, 2002c).

The rate of transpiration (evaporation of water from the plant tissues) is influenced by internal or commodity factors (morphological and anatomical characteristics, surface-to-volume ratio, surface injuries, and maturity stage) and external or environmental factors (temperature, relative humidity, air movement, and atmospheric pressure). High temperatures, low RH and high air velocity promote high water loss, for example. Transpiration is a physical process that can be controlled by applying treatments to the commodity (e.g., waxes and other surface coatings and wrapping with plastic films) or by manipulating the environment (e.g., refrigeration, maintenance of high relative humidity and control of air circulation) (Kader, 2002c).

3.6 Environmental factors affecting deterioration

Several external environmental and physical factors affect the rate of deterioration and postharvest life of tropical and subtropical fruits via their effects on the internal, biological factors listed in the previous section. Stresses related to environmental factors such as excessive heat, cold, or improper mixtures of environmental gases, such as oxygen, carbon dioxide, and ethylene, can also cause certain physiological disorders, which negatively affect fruit quality and reduce its postharvest life. Several of these disorders can be initiated before harvest and expressed either before or after harvest. Some disorders may also be caused by mechanical damage. All of these disorders are abiotic in origin (i.e. not caused by disease organisms); however, abiotic disorders often weaken the natural defenses of fresh produce, making it more susceptible to biotic diseases that are caused by disease organisms. Furthermore, in many cases injuries caused by chilling, bruising, sunburn, senescence, poor nutrition and other factors can mimic

biotic diseases. The effects of external factors are cumulative during the time between harvest and consumption. The different environmental and physical factors affecting fruit quality and the physiological disorders they cause are described below.

3.6.1 Temperature

Temperature is the single most important environmental factor that affects tropical and subtropical fruit deterioration (Fig. 3.4). Low temperatures (above those that cause chilling injury) decrease metabolic activity, reduce the incidence of postharvest disease and slow its development, reduce insect activity and lessen water loss, while high temperatures (up to a certain limit) accelerate all these deterioration factors. For each increase of 10°C above optimum, the rate of deterioration increases twofold to fourfold. Temperature also influences the effects of ethylene and MA/CA storage. Spore germination and growth rate of pathogens are greatly influenced by temperature; for instance, cooling commodities below 5°C immediately after harvest can greatly reduce the incidence of *Rhizopus* rot (Kader, 2002c; Sommer *et al.*, 2002). The best environmental conditions for ripening are 20–24°C, and 90–95% relative humidity. Temperatures above 27°C accelerate softening and may cause tissue discoloration, excessive decay, and

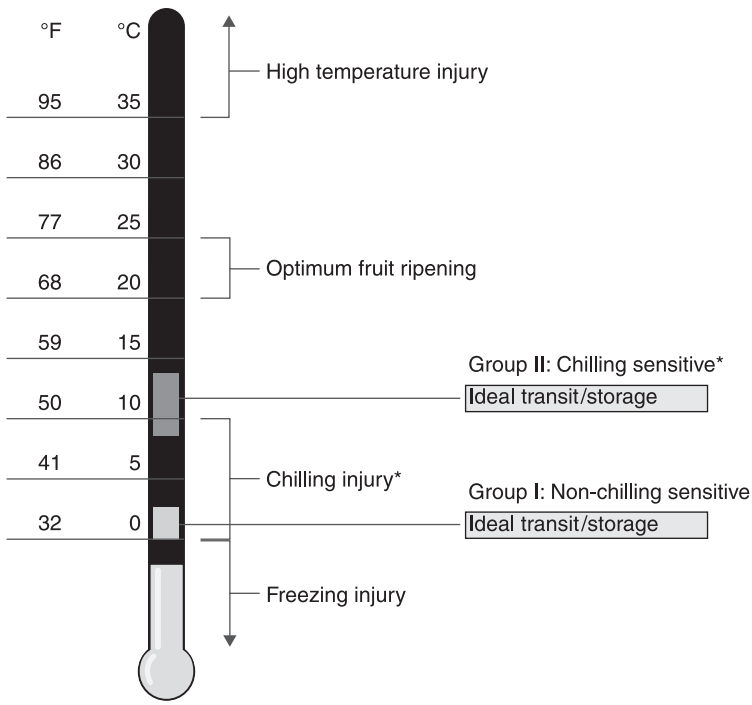

Fig. 3.4 Effect of temperature on postharvest quality of fruits.

off-flavors.Temperatures above 35°C inhibit ethylene production and action and, consequently, inhibit ripening. The ripening rate can be manipulated by temperature management between 14 and 24°C. Exposure to undesirable temperatures results in many physiological disorders, which are reviewed in the following sections.

Chilling injury

Chilling injury (CI) occurs in most fruits of tropical and subtropical origin if held at temperatures above their freezing point and below 5° to 15°C, depending on the commodity (Table 3.4). This physiological disorder is the most important obstacle to the expansion of trade in tropical fruits in the world market (Siriphanich, 2002) and is the major cause of their typically short postharvest life (7–40 days). CI symptoms become more noticeable upon transfer to higher (non-chilling) temperatures. The most common symptoms are surface and internal discoloration (browning), pitting, water soaked areas, uneven ripening or failure to ripen, off-flavor development, and accelerated incidence of surface molds and decay (especially organisms not usually found growing on healthy tissue) (Table 3.5). Ripening is also impaired as a result of exposure to temperatures that cause CI. The ideal control of CI is avoidance of exposure at chilling temperature. Approaches to lessen chilling injury incidence and severity include temperature conditioning, intermittent warming, CA storage, chemical treatments, and growth regulator application (Wang, 1994). These techniques reduce CI by either increasing the tolerance of commodities to chilling temperature or retarding the development of CI symptoms.

Subtropical fruits vary in their relative susceptibility to chilling injury among species and cultivars within a species. For example, grapefruit, lemon, lime and pomelo are much more susceptible to chilling injury than orange, kumquat and mandarin. Dates, figs, kiwifruits and 'Hachiya' persimmons are not sensitive to chilling injury. 'Fuyu' persimmons and other subtropical fruits are chilling-sensitive. Some tropical fruit are highly sensitive such as banana, breadfruit, jackfruit, mamey, mango, mangosteen, papaya, pineapple, rambutan and soursop, and others are moderately sensitive such as carambola, durian, guava, sugar apples and tamarillo (see Table 3.4).

Table 3.4 Some chilling sensitive and non-sensitive tropical and subtropical fruits

Chilling sensitive fruits	Non-chilling sensitive fruits
Avocado, banana, breadfruit, carambola, cherimoya, citrus fruits, durian, feijoa, guava, jackfruit, jujube, lanzone, longan, litchi, mango, mangosteen, olive, papaya, passion fruit, pepino, pineapple, plantain, persimmon (Fuyu), pomegranate, prickly (cactus) pear, rambutan, sapodilla, sapote, tamarillo	Date, fig, kiwifruit, loquat, persimmon (Hachiya)

Source: Modified from Kader, 2002c

Table 3.5 Chilling injury symptoms of selected subtropical and tropical fruits

Fruit	Minimum[1] safe temperature°C	Symptoms
Avocado	5–10	Grayish brown discoloration of flesh, softening, pitting, development of off-flavors
Banana	13–15	Surface discoloration, dull color, failure to ripen, browning of flesh
Grapefruit	10–13	Pitting, scald, watery breakdown
Lemon	10–13	Pitting, membranous stain, red blotch
Lime	10–13	Pitting, skin browning, accelerated decay
Mango	10–12	Grayish scald-like discoloration of skin, uneven ripening, poor flavor, increased decay
Olive	5–8	Internal browning, pitting
Orange	3–5	Pitting, brown stain
Papaya	10–12	Pitting, failure to ripen, off-flavor development, accelerated decay (especially Alternaria Spp.)
Pineapple	8–10	Uneven ripening, dull color, water-soaked flesh, off-flavor, increased acidity, loss in ascorbic acid content, wilting of crown leaves, endogenous brown spot

Note: [1] Varies with cultivar, maturity stage, and duration of exposure

Freezing injury

Just like all other horticulture commodities, fresh tropical and subtropical fruits are injured at temperatures below their freezing point (about – 2°C) (Fig. 3.4). The tissues of frozen fruit break down immediately upon thawing. Freezing is usually the result of inadequate refrigerator design or poor setting or failure of thermostats. In winter conditions, freezing can occur if produce is allowed to remain for even short periods of time on unprotected transportation docks.

Heat injury

High temperatures, either before or after harvest, can result in injuries to several tropical and subtropical fruits. This injury can be induced by exposure to direct sunlight or excessive high temperatures, such as during the use of heat (hot air or hot water) treatments. Preharvest exposure to the sun can result in sunburn (such as in figs) and sunscald (such as in pomegranates). Transpiration in growing plants maintains temperatures in the optimal range, but once removed from the plant, fruits lack the protective effects of transpiration, and direct sources of heat such as full sunlight can rapidly heat tissues to above the thermal death point of their cells. Heat injury is usually expressed as bleaching, scalding, uneven ripening, excessive softening, and desiccation. Tropical and subtropical fruits vary significantly in their tolerance to heat injury (Paull, 1994). Temperatures above 25°C cause uneven softening, skin discoloration, flesh darkening, and off-flavors development in some

fruits, such as avocados. Impaired ripening of other fruits occurs at temperatures above 35°C (reduced ethylene production and action). Heat treatments (hot water or hot air) have been commercially applied to some tolerant fruits such as mango, papaya and citrus, especially for the control of decay and insects (Lurie, 1998).

3.6.2 Relative humidity (RH)

As discussed in section 3.5.5, water loss from fruits is affected by both commodity factors (e.g. morphological and anatomical characteristics) and external or environmental factors. Focussing on the environmental factors, the rate of water loss depends on the vapor pressure deficit (VPD) between the fruit and the surrounding ambient air, which is influenced by temperature and relative humidity (RH). At a given temperature and rate of air movement, the rate of water loss from the fruit depends on the RH. At a given relative humidity, water loss increases with the increase in temperature (Kader, 2002c). The optimum RH during storage ranges from 85 to 95% for subtropical and tropical fruits. Lower RH (55 to 65%) is recommended for tree nuts and dried fruits. Very high RH (above 95%) and water condensation on the fruits can accelerate pathogen attack and decay development.

3.6.3 Ethylene

As discussed in section 3.5.2, ethylene plays a vital role in the postharvest life of many horticultural crops. This section describes how the quantity of ethylene in the environment surrounding a commodity can be adjusted to achieve the desired effect.

The effect of the exposure of fruits to ethylene can be either desirable (such as in the case of the acceleration of uniform ripening and color development) or undesirable (acceleration of ripening, undesirable breakdown of colors, several physiological disorders), and depend on the type of product, physiological age, ethylene concentration, and temperature (Kader, 1985; Saltveit, 1999).

The stage of maturity at harvest influences ripening rate and storage life. Picking bananas and mangos at the mature-green stage hastens their ripening by lowering their response threshold to endogenous ethylene concentration. Bananas take 1–2 weeks vs. 6–7 weeks to ripen off vs. on the plant, respectively. Avocados do not ripen on the tree. Factors which prevent avocado ripening on the tree continue to exert their effects for about 24 hours after harvest. Ripening of avocados can be hastened by exposure to 10 ppm C_2H_4 at 15–17°C for two days. CO_2 produced by ripening avocados must be removed to prevent its accumulation to a level that negates C_2H_4 effects. Cold nights followed by warm days are necessary for green color (chlorophyll) loss and yellow or orange color (carotenoids) development in citrus fruits. This is the reason citrus fruits remain green after attaining full maturity and good eating quality in tropical areas. Some regreening of Valencia oranges may occur after the fruit has reached full orange color (Kader and Arpaia, 2002).

The optimum temperature range for ripening is 15° to 25°C (Fig. 3.4); the higher the temperature the faster the ripening. The optimum relative humidity range is 90–95%. Ethylene can be added to the atmosphere surrounding certain

fruits to promote their ripening. Ethylene at 100 ppm is more than adequate and can be supplied in a continuous flow system using a compressed cylinder of ethylene diluted with an inert gas such as nitrogen. An alternative is to use ethylene generators where ethylene is produced by heating ethanol plus a catalyst. To ensure uniform distribution of ethylene within the room, the air containing ethylene can be forced through the pallets containing the commodity. Carbon dioxide accumulation should be avoided (via introduction of fresh air) or corrected (by use of CO_2 absorbers such as hydrated lime or a molecular sieve) if needed.

The presence of ethylene, however, may also promote undesired accelerated softening and ripening of fruits during transport and storage, resulting in shorter postharvest life and faster deterioration. For example, the presence of ethylene in cold storage facilities of kiwifruits and avocados kept in air or in a CA can significantly hasten softening and reduce storage life of these fruits. The incidence and severity of ethylene damage depend on its concentration, duration of exposure, and storage temperature. Ethylene may be excluded from storage rooms and transport vehicles by using electric fork-lifts and by avoiding mixing ethylene-producing commodities with those sensitive to ethylene. Ethylene may be removed from storage rooms and transport vehicles by using adequate air exchange (ventilation) (e.g. one air change per hour provided that outside air is not polluted with ethylene) and by using ethylene absorbers such as potassium permanganate. The air within the room or transit vehicle must be circulated past the absorber for effective ethylene removal. It is also very important to replace the used absorbing material with a fresh supply as needed. Catalytic combustion of ethylene on a catalyst at high temperatures (greater than 200°C) and use of ultraviolet radiation can also be used to remove ethylene (Reid, 2002). Ethylene damage can also be greatly reduced by holding the commodity at its lowest safe temperature and by keeping it under MA or CA (reduced oxygen and/or elevated carbon dioxide: 2–5 kPa O_2 and/or 5–10 kPa CO_2 is recommended) or in hypobaric storage. Under such conditions, both ethylene production by the commodity and ethylene action on the commodity are significantly reduced. Gamma irradiation has been shown to delay ripening by reducing ethylene production and its effects in several tropical fruits, but its potential detrimental effects on the fruits (at doses above those needed for insect control) exceed the benefits (Kader, 1986b).

The discovery of the ethylene action inhibitor, 1-methylcyclopropene (1-MCP), in the early 1990s was a major breakthrough. In July 2002, 1-MCP (under the trade name 'SmartFresh'), at concentrations up to 1ppm was approved by the US Environmental Protection Agency for use on apples, apricots, avocados, kiwifruit, mangoes, nectarines, papayas, peaches, pears, persimmons, plums and tomatoes. The first commercial application has been on apples to retard their softening and scald development and extend their postharvest life. As more research is completed, the use of 1-MCP will no doubt be extended to several other commodities in the future (Blankenship and Dole, 2003; Watkins, 2006; Sozzi and Beaudry, 2007; Watkins, 2008). Also, 1-MCP has been used and continues to be used to differentiate between ethylene-dependent and ethylene-independent processes in plant tissues (Defilippi *et al.*, 2005; Pech *et al.*, 2005; Huber, 2008).

3.6.4 Atmospheric gases

As already described in section 3.5.1, reduction of oxygen and elevation of carbon dioxide, whether intentional through MA or CA, or unintentional (restricted ventilation within a shipping container, a transport vehicle, or both), can either delay or accelerate deterioration of fresh fruits (Yahia, 2009a). Very low oxygen (less than 1%) and high carbon dioxide (greater than 20%) atmospheres can cause physiological breakdown (fermentative metabolism) in most fresh horticultural commodities (Figs. 3.5 and 3.6). The interactions between O_2, CO_2, and ethylene concentrations, temperature and duration of storage influence the incidence and

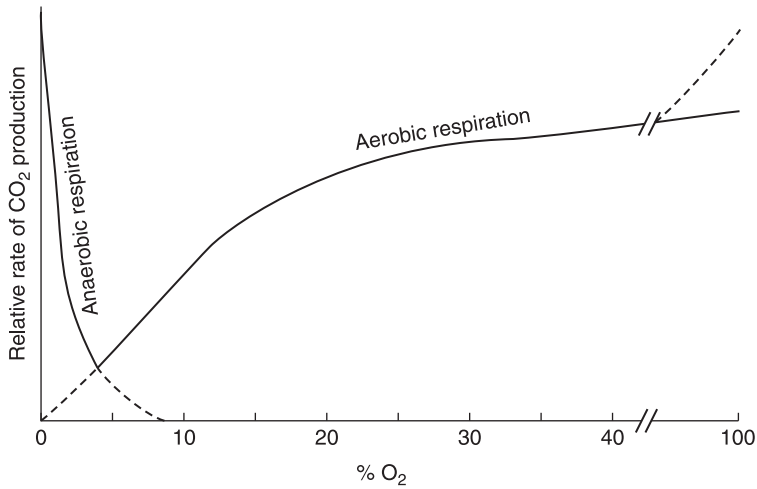

Fig. 3.5 Effect of O_2 level on respiration rate.

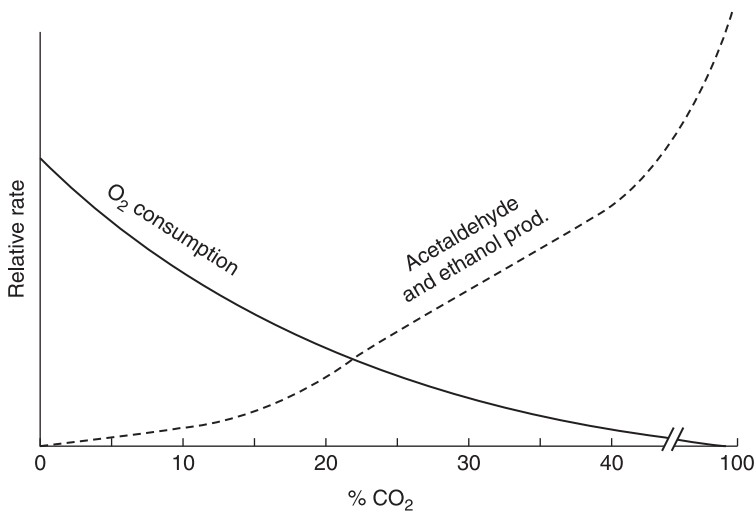

Fig. 3.6 Effect of CO_2 level on respiration rate.

severity of physiological disorders related to atmospheric composition (Kader, 1986a; Singh *et al.*, 2009; Yahia and Singh, 2009). The magnitude of these effects also depend on the species, cultivar and physiological age (Kader, 1986a; Yahia, 1998; Yahia, 2009a).

During the past 50 years, the use of CAs and MAs to supplement temperature management has increased steadily and has helped to extend the postharvest life and maintain the quality of several subtropical (Singh *et al.*, 2009) and tropical (Yahia and Singh, 2009; Yahia, 1998) fruits. However, the overall extent of commercial use of CA and MA on subtropical and tropical fruits is still limited (Kader, 2003b; Yahia, 2009a). Applications of these technologies are expected to increase as international market demand for year-round availability of various commodities grows and as technological advances are made in attaining and maintaining CA and MA during transport, storage and marketing. The use on nuts and dried fruits (for insect control and quality maintenance including prevention of rancidity) is increasing and will likely continue to increase because it is an excellent alternative to chemical fumigants (such as methyl bromide) used for insect control. Several refinements in CA storage have been made in recent years to improve quality maintenance; these include low O_2 (1.0–1.5%) storage, low ethylene CA storage, rapid CA (rapid establishment of the optimum levels of O_2 and CO_2), and programmed (or sequential) CA storage (e.g., storage in 1 kPa O_2 for two to six weeks followed by storage in 2–3 kPa O_2 for the remainder of the storage period).

Other developments, which may expand use of MA during transport and distribution, include using edible coatings or polymeric films to create a desired MA within the commodity (Yahia, 2009a). The use of polymeric films for packaging produce and their application in modified atmosphere packaging (MAP) systems at the pallet, shipping container (plastic liner), and consumer package levels continues to increase (Yahia, 2009a). MAP (usually to maintain 2–4 kPa O_2 and 8–12 kPa CO_2) is widely used in extending the shelf life of fresh-cut fruit products. Use of absorbers of ethylene, carbon dioxide, oxygen, and/or water vapor as part of MAP is increasing. Although much research has been done on the use of surface coatings to modify the internal atmosphere within the commodity, commercial applications are still very limited due to the inherent biological variability of the commodity.

Potential benefits of MA and CA for subtropical and tropical fruits are species specific (Singh *et al.*, 2009; Yahia and Singh, 2009). The extension of storage life in avocado, litchi, guava, kiwifruit, loquat, longan, persimmon, pomegranate and rambutan has been achieved through CA/MA application (Kader, 2003b; Singh *et al.*, 2009). Most research conducted on MA/CA of tropical crops has been on banana, mango and papaya, some research on cherimoya, durian and pineapple, and little research on feijoa, lanzone, mangosteen, passion fruit, sapodilla, sugar apples and wax apples (Yahia, 1998; Yahia and Singh, 2009). Adequate handling systems, including temperature management, humidity control, avoidance of mechanical damage, sanitation, and ethylene removal treatment (for some crops), is essential for the successful application of MA/CA (Yahia and Singh, 2009).

3.6.5 Light

The effects of light conditions in storage facilities on tropical and subtropical fruit quality should also be considered. Radiant heat from high-intensity light sources can raise commodity temperatures. Exposure of some commodities to light might result in some changes, especially in the development of pigments.

3.6.6 Nutrition

Certain types of physiological disorders originate from preharvest nutritional imbalances. For example, soft nose of mangoes results from calcium deficiency. Increasing calcium content via preharvest or postharvest treatments can reduce the susceptibility to physiological disorders. The calcium content also influences the textural quality and senescence rate of fruits and vegetables: increased calcium content has been associated with improved firmness retention, reduced CO_2 and ethylene production rates, and decreased decay incidence.

3.6.7 Mechanical damage

Fresh tropical and subtropical fruits are very prone to mechanical damage throughout the postharvest chain, e.g. during harvest, packing, packaging, transport, marketing, and even at home. These injuries are very common, are major contributors to deterioration and can cause major qualitative and quantitative losses. Types of physical damage include surface injuries, impact bruising, vibration bruising and cuts. Browning of damaged tissues results from membrane disruption, which exposes phenolic compounds to the polyphenol oxidase enzyme. Mechanical injuries not only are unsightly but also accelerate water loss, provide sites for fungal infection, and stimulate CO_2 and ethylene production by the commodity (Kader, 2002c).

3.7 Pathological disorders and insect infestation

Other biological factors such as disease and insect infestation can reduce fruit quality. These are summarised below. Futher information on the control of postharvest decay caused by fungi can be found in Chapter 6. The control of quarantine pests is reviewed in detail in Chapter 7.

3.7.1 Pathological disorders

Some of the most common and obvious symptoms of deterioration result from the activity of bacteria and fungi (Snowden, 1990; Sommer *et al.*, 2002) and diseases are, in fact, the major cause of postharvest losses in tropical and subtropical fruits. Fungi are more common than bacteria in fruits. Viruses seldom cause postharvest diseases, although they, like postharvest disorders, may weaken the produce. Disease can be initiated either before or after harvest, but attack by most organisms follows physical injury or physiological breakdown of the commodity. In a few

cases, pathogens can infect apparently healthy tissues and become the primary cause of deterioration. In general, fruits exhibit considerable resistance to potential pathogens during most of their postharvest life. The onset of ripening in fruits, and senescence in all commodities, renders them susceptible to infection by pathogens (Sommer *et al.*, 2002). This is due to several factors including the fact that maturing and ripening fruit is usually softer, contains more sugar, and has less acidity and phenolic compounds. Many factors preharvest and postharvest affect the severity of pathogen attack and the development of decay, such as temperature, relative humidity, free water and ethylene (Sommer *et al.*, 2002; Broekaert *et al.*, 2006). Stresses such as mechanical injuries, chilling, and sunscald lower the resistance to pathogens (Kader, 2002c).

The most important decay-causing organisms are *Colletotrichum gloeosprioides* (causing anthracnosis), *Diplodia natalensis* (causing stem end rot), *Ceratocystis paradoxa* (causing black rot in banana and pineapple), and *Penicillium* and *Fusarium* (causing brown rot on pineapple). The disease anthracnosis is particularly noteworthy as it is the major postharvest problem in several tropical fruits, and latent infection occurs in green fruit before harvest.

Preharvest treatments and methods of disease control include the use of healthy seedlings, as well as proper field sanitation practices (Korsten, 2006). Pesticides are still used to control decay before and after harvest, but postharvest heat treatments are effective and safer for consumers. The control of biotic postharvest diseases depends on understanding the nature of disease organisms, the conditions that promote their occurrence, and the factors that affect their capacity to cause losses. Treatments with sulfur dioxide, fungicides and antioxidants help to reduce losses from postharvest diseases, as does careful attention to humidity and temperature. Hot water treatments (48–55°C for 5–15 minutes) can be used for mango and papaya. Most important, however, is careful handling to minimize injuries. Bruised fruit, or fruit with a damaged skin, is more vulnerable to diseases, spoils more quickly and sells more slowly.

Biological control of postharvest diseases is emerging as an effective alternative to chemical methods of control. Antagonists to wound-invading necrotrophic pathogens can be applied directly to fruit wounds. They are applied using delivery systems such as drenches, line sprayers and on-line dips, and a single use can significantly reduce fruit decay. BioSave® is one such pioneering product that was registered by EPA in 1995 for biocontrol of postharvest rots of pome fruit, respectively, and is commercially available. The effectiveness of biocontrol products has some limitations, but their effectiveness can be improved through the control of their formulation and the environment in which they are used or physiological and genetic enhancement of the biocontrol mechanisms. The use of mixtures of beneficial organisms, and the integration of biocontrol with other alternative methods that alone do not provide adequate protection but in combination with biocontrol provide additive or synergistic effects, are other options (Droby *et al.*, 2009).

Anthracnose is the most serious postharvest disease of tropical fruits and several subtropical fruits. There are no sure methods for its control, but the

following procedures can help greatly to reduce its development: (1) an effective preharvest disease control program; (2) careful handling to minimize mechanical injury; (3) avoiding chilling injury and exposure to high temperatures; (4) postharvest treatment with vapor heat or hot water, or hot air for 20 minutes or longer, depending on the temperature; and (5) a five-minute hot water (51.5°C) dip that may be combined with a fungicide (Sommer *et al.*, 2002; Korsten, 2006).

3.7.2 Insect infestation

A large number of insects can be carried by fresh fruits during postharvest handling. Many of these insect species, especially the fruit flies of the family Tephritidae (e.g. Mediterranean fruit fly, Oriental fruit fly, Mexican fruit fly, Caribbean fruit fly), can seriously disrupt trade among countries. Continuing globalization of the fresh produce market will be facilitated by use of acceptable disinfestation treatments. Selection of the best treatment for each commodity will depend upon the comparative cost and the efficacy of that treatment against the insects of concern with the least potential for damaging the host (Paull, 1994; Paull and Armstrong, 1994). Much of the research during the past 20 years has been focused on finding alternatives to methyl bromide fumigation (Yahia, 2006). For example, new fumigants such as carbonyl sulfide, methyl iodide and sulfuryl fluoride, insecticidal atmospheres (<0.5 kPa O_2 and/or 40–60 kPa CO_2 employed alone or in combination with heat treatments) and ultraviolet radiation are under investigation. Each of these treatments is usable on a limited number of commodities but causes phytotoxic effects on others (Neven, 2010).

Most insects are sterilized when subjected to irradiation doses ranging between 50 and 750 Gy. The actual dosage required varies in accordance with the species and its stage of development. An irradiation dose of 250 Gy has been approved for certain fresh fruits, such as litchis, mangoes, and papaya by US quarantine authorities in light of its efficacy in preventing the reproduction of tropical fruit flies. Most fresh fruits will tolerate an irradiation dose of 250 Gy with minimal detrimental effects on quality. At doses above 250 Gy and up to 1000 Gy (the maximum allowed as of 2010), damage can be sustained by some commodities (Kader, 1986b). Fruits, in general, are tolerant to the expected dose range (250 to 500 Gy absorbed by fruits on the inside *vs.* those on the outside of the pallet). Detrimental effects on fresh fruits may include loss of green color (yellowing), tissue discoloration, and uneven ripening (Kader, 1986b).

3.8 Biotechnological approaches for improving quality and postharvest life

Fresh subtropical and tropical fruits vary greatly in their storage potential, which is related to their degree of perishability. Genotypic differences have been found in the storage potential of many commodities. These differences may be related to

variation among cultivars in morphological structure, turgidity, composition (such as content of nitrogen, calcium, phenolic compounds, polyphenol oxidase activity, and organic acids), and softening rate. The relative susceptibility to pathogens can also be an important factor in determining storage life.

Biotechnology is a tool that can be utilized, in an interdisciplinary approach, to address some of the concerns about quality attributes and the biological causes of deterioration of harvested produce (King and O'Donoghue, 1995; Baldwin, 2002; Kader, 2002b; Kader, 2003a; Pech *et al.*, 2005). Dandekar (2003) stated that microarrays can be developed to study expression and detect single nucleotide polymorphisms. Plant transformation can then be effectively used to evaluate and authenticate newly discovered endogenous genes to characterize their function in fruit as well as to genetically manipulate fruit quality and productivity. Bonghi and Trainotti (2006) suggested that comparative proteomics is an efficient strategy that could be used to provide further information on regulation of fruit ripening.

Three approaches are being utilized to extend postharvest life and maintain quality: selecting for slower ripening lines, modification of ethylene responses or reducing softening rate. For example, papaya varieties having slow ripening characteristics have been selected, delayed ripening by the down-regulation of ethylene synthesis enzymes (ACS and ACO) is being tested for banana and papaya, and the modification of fruit softening related enzymes is being examined (Paull and Chen, 2004).

Combination of metabolite and transcriptomic data revealed that transcript abundance is less strictly coordinated by functional group than metabolite abundance, and this suggests that post-translational mechanisms dominate metabolic regulation (Tonutti and Bonghi, 2009). Nevertheless, there were some correlations between specific transcripts and metabolites, and several novel associations were identified that could provide potential targets for manipulation of fruit compositional traits. A strong relationship between ripening-associated transcripts and specific metabolite groups, such as TCA-cycle organic acids and sugar phosphates, was observed. These examples make it clear that our understanding of plant metabolic networks will rely upon integrative (targeted and non-targeted) analyses (Tonutti and Bonghi, 2009).

Following are some examples of the opportunities and limitations in using biotechnology to maintain postharvest quality and safety of fresh fruits.

3.8.1 Composition and appearance quality

Color is a very important appearance quality factor that is related to biosynthesis and degradation of pigments, including chlorophylls, carotenoids, flavonoids and betalains. Biotechnology can be used to improve color uniformity and intensity and to minimize undesirable colors, such as browning. Tissue browning is dependent upon the concentration of phenolic compounds, the activity of polyphenol oxidase (PPO), and the concentration of antioxidants. These factors can be manipulated to produce genotypes with low browning potential, which is a

very useful trait in many commodities when marketed intact or as fresh-cut products.

3.8.2 Composition and textural quality

Genetic manipulations to reduce the rate of fruit softening can be very useful in maintaining their textural quality. Another use for biotechnology is to reduce the rate of fruit softening to maintain their firmness and minimize physical damage throughout the postharvest handling system. This can be achieved by altering cell wall metabolism in all fruits and/or ethylene biosynthesis and action in climacteric fruits, as has been demonstrated in papaya. The genetic engineering of fruits to delay softening via down-regulation of individual gene encoding wall-modifying enzymes has given limited success in some cases (Bennett, 2002). However, approaches yielding the modified expression of combinations of genes could be useful for altering cell wall disassembly more dramatically or overcoming functional redundancy (Vincente *et al.*, 2006). An alternative approach that could have value in modifying wall behavior would be a focus on modifying cell wall synthesis in order to generate custom-designed cell walls that could have the desired functional properties (Vincente *et al.*, 2006).

3.8.3 Composition and flavor quality

Flavor quality factors include sweetness (kinds and quantity of sugars), sourness or acidity (kinds and quantity of acids), astringency (phenolic compounds), and aroma or odor (volatile compounds). The relative importance of each of these factors and their interactions depends upon the commodity. The greatest need is to produce new fruit genotypes with better flavor, which means high sugars and moderate to high acids (with balance between them), low phenolics, and enough of the organoleptically important volatiles for good aroma. Since flavor quality involves perception of the tastes and aromas of many compounds, it is much more challenging to manipulate than other quality factors (Scott, 2002). This has been true for plant breeders in the past and it will continue to be so with biotechnology approaches. This may be the reason that improvement of flavor quality has received much less attention from biotechnologists so far than textural quality of fruits. However, there is an increasing interest in investigating the genetic control of biosynthesis of plant volatiles (Aharoni *et al.*, 2005). Goff and Klee (2006) pointed out that the predominance of volatiles derived from essential nutrients and health-promoting compounds suggests that these volatiles provide important information about the nutritional makeup of foods.

In a recent review, Nookaraju *et al.* (2010) noted that modifying the activity of enzymes in carbohydrate metabolism such as sucrose synthase, acid invertase, ADP-glucose pyrophosphorylase, sucrose phosphatesynthase, and sucrose transporters was found to influence carbohydrate partitioning and sucrose accumulation in sink tissues of several fruits. Plant based taste-modifying sweet proteins such as brazzein, cucurmin, mabinlin, monellin, miraculin, neoculin and

thaumatin have potential application for developing transgenic plants to improve the sweetness and flavor quality of fruits (Nookaraju *et al.*, 2010).

3.8.4 Composition and nutritional quality

Plant breeders have been successful in selecting genotypes with a much higher content of ascorbic acid (vitamin C) in guava and tomatoes, β-carotene (provitamin A) in carrots and tomatoes, and flavonoids in berries (Yahia, 2009b; 2010). Biotechnology approaches can be utilized to improve the content of vitamins, minerals, dietary fiber and phytonutrients in fruits and vegetables, especially those with high per capita consumption rates. Phytonutrients that can lower the risk of heart disease, cancer and other diseases include carotenoids, flavonoids (anthocyanins, phenolic acids, polyphenols), isoflavones, phytosterols and organosulfur compounds (Yahia, 2010). The antioxidant capacity of fruits is related to their contents of anthocyanins, phenolic compounds, carotenoids, ascorbic acid and vitamin E (Corral-Aguyao *et al.*, 2008). Large genotypic variations in total antioxidant capacity have been shown in many commodities, indicating the potential for further improvements using biotechnology (Giovannoni, 2002).

3.8.5 Rates of respiration, ethylene production and ripening

In many commodities there is an association between their postharvest life (rate of deterioration) and their rates of respiration and ethylene production. Selecting genotypes with lower rates of respiration and ethylene production is likely to result in lower rates of deterioration and longer postharvest life potential. However, in cases when ethylene production is largely inhibited in fruits, their volatile production is also inhibited, which has a negative impact on their aroma quality (Defilippi *et al.*, 2005; El-Sharkawy *et al.*, 2005). The challenge is to separate the effects of genetic manipulation on ethylene biosynthesis from those on biosynthesis of esters and other desirable aroma volatiles.

Using molecular tools, there have been demonstrations of effective control of fruit ripening in several species. However, unintended side effects of interfering with ethylene signalsing have proven to be major challenges to bringing biotechnology-based products to the market (Klee, 2005). Studies applying integrative approaches have revealed new regulators, molecular connections and mechanisms in ethylene signaling and unexpected links to other plant hormones, such as auxin and gibberellins (Yoo *et al.*, 2009). In a recent review article, Bapat *et al.* (2010) concluded that ethylene is perceived by a set of ethylene receptors which transduce signals through a cascade of factors such as ctr, ein2, ein3/EIL and finally to ethylene response factors (ERFs), transcriptional regulatory proteins. These ERF proteins bind to ethylene-responsive elements such as GCC box and others present in the upstream region of the ethylene-responsive target genes. These target genes in turn are either expressed or repressed to finally affect biochemical reactions which collectively initiate, promote or suppress ripening (Bapat *et al.*, 2010).

3.8.6 Susceptibility to physiological disorders

Many physiological disorders have been identified and associated with exposure to undesirable temperatures, with low calcium levels, with O_2, CO_2, and/or C_2H_4 concentrations beyond those tolerated by the fruit, or with other factors. However, the physiological and biochemical basis of most of these disorders remains largely unknown. Thus, physiologists and biochemists need to identify the specific targets for biotechnological manipulation before biotechnology can be used to address these problems. Genotypic differences in susceptibility to chilling injury have been shown in most chilling-sensitive commodities. Thus, it should be possible to use biotechnology to produce cultivars with lower chilling sensitivity to allow their handling at lower temperatures to extend their postharvest life.

3.8.7 Susceptibility to pathological breakdown

One of the most common and obvious symptoms of deterioration results from the activity of fungi. Attack by most organisms follows physical injury or physiological breakdown of the commodity. In a few cases, pathogens can infect apparently healthy tissues and become the primary cause of deterioration. In general, harvested fruits exhibit considerable resistance to potential pathogens during most of their postharvest life. The onset of ripening in fruits results in their becoming susceptible to infection by pathogens (McCollum, 2002). There is an association between phenolic content in many commodities and their susceptibility to decay-causing pathogens. However, the challenge to biotechnological approaches is to maintain a balance between the desirable concentrations of phenolic compounds for resistance to pathogens and the undesirable levels in terms of astringency and/or browning potential. Another approach is to introduce polygalacturonase inhibitors and/or to increase the level of endogenous antifungal compounds without negative effects on quality and safety of the commodity.

Broekaert *et al.* (2006) concluded that induced ethylene biosynthesis and subsequent intracelular signaling through a single conserved pathway lead to a cascade of transcription factors consisting of primary EIN3-like regulators and downstream ERF-like transcription factors. The latter control the expression of various effector genes involved in various aspects of systemic induced defense responses. Moreover, at this level significant cross-talk occurs with other defense response pathways controlled by salicylic acid and jasmonate, eventually resulting in a differentiated disease response (Broekaert *et al.*, 2006).

3.8.8 Safety considerations

Minimizing chemical and microbial contamination (Sapers *et al.*, 2006) during production, harvesting and postharvest handling of fruits is essential to assuring their safety to the consumer. Research and development efforts must continue to define optimum procedures for avoiding contamination with mycotoxins, heavy metals and microorganisms during handling of fresh fruits and their products. It may be possible to use biotechnology to alter the morphological structure of the

surface of some commodities to minimize the areas in which human pathogens can be protected from washing and disinfection treatments.

3.8.9 Concluding remarks

It is clear that there are numerous opportunities in using biotechnology to maintain postharvest quality and safety of fresh tropical and subtropical fruits. Thus, priorities for each commodity should be established on the basis of the relative importance of its postharvest deterioration causes and what is needed to encourage increased consumption. Overall, priority should be given to the following three goals (Kader, 2002b):

1. To attain and maintain good flavor and nutritional quality to meet consumer demands and encourage greater consumption of fresh fruits.
2. To introduce resistance to physiological disorders and/or decay-causing pathogens to reduce use of chemicals.
3. To modify surface structure and/or composition of some commodities to reduce their microbial contamination potential.

The challenge to molecular biologists is that many of the desired improvements require manipulation of more than one gene, and in some cases target genes have not yet been identified.

3.9 Conclusions

The use of cold temperatures to extend the postharvest life of subtropical and tropical fruits is limited by their susceptibility to chilling injury. Heat treatments and other stresses needed to satisfy quarantine requirements of importing countries often reduce the postharvest life of tropical and subtropical fruits. Climacteric tropical and subtropical fruits ripen faster than temperate fruits, and thus require greater efforts to delay ripening and expedited handling so that they reach the consumer before becoming more susceptible to decay-causing pathogens. Whether or not a fruit has biotechnologically conferred traits, the keys to successful maintenance of quality and safety postharvest are the same: harvesting at the optimum maturity stage, careful and expedited handling, maintenance of optimum ranges of temperature and relative humidity, and minimizing chemical and microbiological contamination throughout the handling system. It is possible that new genotypes will void the need for some supplementary treatments, but it is not likely that the need to pay attention to the four key factors mentioned above will reduce.

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4

Preharvest and harvest factors influencing the postharvest quality of tropical and subtropical fruits

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Abstract: Growing conditions, time of harvest and harvesting methods are of great importance for the postharvest quality attributes of fresh produce. Genetic factors also have a significant effect. This chapter identifies the preharvest factors that can affect the quality of harvested fruit and ways in which to produce fruit with less predisposition to postharvest disorders. Cultivar and variety selection, environmental factors, mineral nutrition and chemical and biochemical treatments are among the factors discussed.

Key words: fruit quality, preharvest factors, postharvest behavior.

4.1 Introduction

The postharvest quality attributes of fresh produce are highly influenced by preharvest factors. Postharvest technologies can only aim to maintain produce quality, not to improve it. Although genetic material drives external and internal quality attributes, the postharvest physiology and pathology of fresh fruits can be affected markedly by the climatic and agronomic factors which influence the development of the crop in the field, such as mineral nutrition, environmental conditions, chemical and biochemical treatments, and pest management (Sams, 1999). As an example, standard fruit concentrations of N, P, K, Ca and Mg are now used as a basis for assessing the storage potential of particular apples under specified conditions. This chapter reviews the preharvest factors that can affect the quality of harvested fruit and cultivation practices, and preharvest technologies that can assist in the production of fruit with less predisposition to postharvest

disorders. The effects of preharvest factors on postharvest fungal decay are reviewed in more detail in Chapter 6.

4.2 Genetic factors

4.2.1 Cultivars

The first step in defining the quality and productivity of a fruit crop lies in the selection of the cultivar. Even though quality traits apply to later postharvest stages, the choice and combination of genes controlling these traits is regarded as a preharvest factor (Wehling, 2000). Fruit quality attributes, the products of physiological processes during the ripening period, are determined by a number of characteristics. Textural quality, for example, is determined by fruit firmness or softness, succulence, and sensory qualities, while aroma is determined by a number of chemicals; various organic acids and sugars that contribute to fruit taste (Mattheis and Fellman, 1999; Sams, 1999). Each quality attribute is the result of highly regulated, multiple processes inherent in the individual fruit.

For any given crop, the range of genotypic variation affecting composition, quality, and post-harvest-life potential has resulted in a tremendous number of species and cultivars with different quality attributes. The selection of the right crop variety for the particular cultivation conditions and market channel can greatly influence the subsequent postharvest quality. Certain varieties are more suitable for longer-term storage (Shewfelt and Prussia, 1993).

The range of genotypic variation and the number of cultivars with different quality attributes is also the result of breeders' efforts to develop fruit cultivars of interest to the consumer and the grower. Although the importance of individual traits varies with the region and country, generally size, appearance (color and shape), flavor (sweet:tart ratio and aromatics), texture, firmness, juiciness and nutrition are attributes that appeal to the consumer. As for the grower, characteristics relating to yield, favorable cultural characteristics, namely annual flowering, ease of thinning, absence of physiological problems or nutrient disorders and desirable tree growth in particular environments, and disease and pest resistance influence the selection of one cultivar over another (Green, 2003). The final selection of a cultivar is often a compromise between the target market, proposed use of the fruit commodity, namely fresh market use *vs* fresh-cut *vs* processing, and the production conditions.

Some examples of cultivars with different properties follow. There are banana cultivars with high levels of carotenoid and vitamin A (Englberger *et al.*, 2006), melon cultivars with extended shelf lives (Aggelis *et al.*, 1997), watermelon with higher sugar content and firmer flesh or lower fruit sugar with firm flesh (Davis and King, 2007), pineapple cultivars with higher levels of carotenoids and sugars (Janick and Goldman, 2003; Grieg, 2004) and jackfruit cultivars with firmer flesh (Campbell and El-Sawa *et al.*, 1998), to name but a few. In addition to variation between species and cultivars, variation exists in the expression levels of the quality attributes within the fruit component. Manthey and Perkins-Veazie (2009) reported levels of L-ascorbic acid ranging from 11 to 134 mg/100 g in five mango

cultivars from Mexico, Peru, Brazil and Ecuador. Flavedo tissues of citrus contained four times higher L-ascorbic acid content than the juice (Nagy, 1980). Similarly, the enzymatic antioxidants in orange-flesh honeydew melon, namely ascorbate peroxidase, catalase, and superoxide dismutase, were higher in the inner mesocarp tissues near the seed cavity than in those from the outer sub-peel (Lester, 2008).

Furthermore, in some fruit crops, the rootstock exerts significant influence on a number of fruit quality attributes (Castle, 1995), such as fruit size, soluble solids concentrations, firmness, shelf-life, as well as crop load and tree yield. Castle (1995) further indicated that effects of the rootstock can be either direct or indirect. Direct effects influence juice quality through plant water relations, whereas indirect effects operate through crop load and tree yield or growth efficiencies. Jawaharlal *et al.* (1991) reported an increase in shelf life and lower weight loss during storage in fruits of acid lime grown on 'Rangpur' lime rootstock than fruits from trees with 'Troyer' or trifoliate rootstocks. Wutscher and Bistline (1988) reported high color score (using a HunterLab® colorimeter) of juice of 'Hamlin' oranges on *Poncirus trifoliate* and mandarin (juice color number 34.2) than in 'Hamlin' grafted on sour orange (juice color number 32.9). Reddy *et al.* (2003) recorded more vigorous growth of 'Alphonso' mango grafted on 'Muvandan' and 'Bappakai' than of 'Vellaikulamban'. The canopy volume of 'Alphonso' was 65.2 and 55.5% more on the 'Muvandan' and 'Bappakai' rootstocks, respectively. The vigorous rootstocks were found to produce higher fruit yield per tree. Similar effects were reported with 'Gomera 1' and 'Gomera 3' rootstocks onto which the mango scion cultivars 'Osteen' or 'Keitt' were grafted. Duran Zuazo *et al.* (2006) observed significantly higher fruit yield with 'Osteen'–'Gomera 1' or 'Keitt'–'Gomera 3' combinations, but not the 'Osteen'–'Gomera 3' or 'Keitt'–'Gomera 1' combinations. However, higher fruit weights were obtained with 'Keitt' grafted on 'Gomera 3', whereas smaller fruits were harvested from 'Keitt'–'Gomera 1' combinations. Both 'Keitt' and 'Osteen' on the two rootstocks exhibited the greatest height and canopy volume.

4.2.2 Conventionally bred and genetically modified cultivars

The majority of the cultivars available today have been generated through the classical method of hybridization (Scorza, 2003). Crossing closely related varieties with sufficient genetic diversity and selecting progeny with desirable traits from either parent has been successful in generating improvements in traits such as yield, but does not generally result in improvements in quality, composition and adaptation. The generation of novel characteristics, especially where genetic diversity is low, involves genetic hybridization between closely related species or mutants (Zamir, 2001; McCouch, 2004; Fernie *et al.*, 2006). However, the efficient selection of favorable alleles in some instances is difficult as most fruit quality traits are complex and controlled by several processes. Further, many rounds of back crossing may be required to obtain an improved commercial product with the desired horticultural traits. In melon, for example, the key traits associated with domestication and cultivar improvement are sweet, non-bitter and low acid levels. The three genes underlying these characteristics, *suc/suc, so/so*, and *bif/bif*, are in the recessive form

(Burger *et al.*, 2003). Invariably dominance will cause the effects of these genes to be manifest in hybrids generated from intraspecific crosses between melon land races and commercial melon cultivars (Nunez-Palenius *et al.*, 2008).

Mutation breeding is another technique that has proven effective in expanding the repertoire of fruit cultivars with novel characteristics. A number of spontaneous mutations have been recorded in various fruit crops and successfully used as new cultivars or in breeding programs (Muggleston, 1995). 'Ruby Red' grapefruit is an example of a mutant fruit cultivar derived from a spontaneous mutation in the early 1920s. 'Ruby Red' was the first commercial grapefruit cultivar with red flesh and blushed peel. Cultivars of mandarin (*Citrus clementina*), satsuma mandarin (*C. unshiu*) and sweet orange (*C. sinensis*) are other examples of mutations (Asins *et al.*, 1999) derived from naturally occurring bud mutants. The underlying mechanism behind bud mutations is not well understood and is presumed to be the result of transposon activity, gene mutation, and DNA methylation (Breto *et al.*, 2001). Using suppression subtractive hybridization and microarray analysis, Liu *et al.* (2009) generated a collection of differentially expressed genes involved in various biological processes, such as organic acid metabolism, lipid metabolism, transport, and pyruvate metabolism as well as signal transduction or transcription factors in the red-flesh bud mutant, 'Hong Anliu' of 'Anliu' sweet orange (*Citrus sinensis* (L.) Osbeck). Further studies should reveal the functions of the genes and their role in this mutation.

Induced mutation breeding has also contributed significantly to the improvement of crop cultivars and is regarded as an important complement to conventional breeding programs. The primary aim of induced mutation breeding has been to improve one or two major traits of a well-adapted plant cultivar (Ahloowalia *et al.*, 2004). The technique is applicable to vegetatively propagated plants, especially fruit trees that are difficult to breed through sexual hybridization, where this is time consuming or inefficient due to heterozygosity and long juvenility (Asins *et al.*, 1999; Aradhya *et al.*, 2003; Kenis and Keulemans, 2005). More than 2 250 mutant cultivars have been released into commerce (Maluszynski, 2001). However, a limited number of mutant fruit cultivars have also been released. The FAO/IAEA Mutant Varieties Database lists five mutant cultivars for orange/mandarin, two for grapefruit, one for papaya and a few temperate fruit crops (Maluszynski *et al.*, 2000). Factors including the choice of well adapted, widely grown cultivars, improvement of easily recognizable traits that are monogenetically inherited and careful testing and selection, significantly affect the development of mutant fruit cultivars (Broertjest and van Harten, 1988).

The *in vitro* culture techniques have also proven useful for the selection of valuable genotypes. The techniques offer plant breeders new possibilities not only for micropropagation, but for an extension of genetic diversity and means of overcoming incompatibility barriers (Maluszynski and Kasha, 2001; Dunwell, 2009). In some cases the main hurdle is the inability to regenerate fertile plants from tissue explants or cells. Progress in cultivar improvement programs (e.g. pineapple and banana) has been achieved by coupling *in vitro* culture techniques with those of mutagenesis (Lapade *et al.*, 1995; Rao *et al.*, 1995).

More recent methodologies have facilitated improved efficiency of genotype selection through the identification of quantitative trait loci (QTLs) and diagnostic molecular markers associated with these QTLs (Collard *et al.*, 2005). Luby and Shaw (2001) suggested that marker assisted selection in fruit crop breeding programs is applicable in instances where the trait is (or traits are) simply inherited, expressed in the mature phase and can be easily tested early in the juvenile phase. Work with segregating melon populations derived from crosses between Spanish and Japanese accessions revealed genomic regions controlling the quality traits fruit length, diameter and shape and soluble solids content (SSC, Eduardo *et al.*, 2007). Similarly with guava, 21 QTLs were identified for the quality traits, fruit width, weight, pulp thickness, SSC, acidity and vitamin C content (Rodriguez *et al.*, 2007). Some of the QTLs were later reported to co-locate or were closely linked to SSR markers (Ritter *et al.*, 2010). More recently, work was initiated with litchi for the construction of a high density molecular genetic linkage map with AFLP and RAPD molecular markers (Liu *et al.*, 2010). Invariably these findings and those with other tropical fruits provide the starting point for further characterization of the genetic factors involved in fruit quality and form the basis for an efficient selection in marker assisted breeding together with information on the stability of QTLs across different environments and within different genetic backgrounds (Bryne, 2007). Nonetheless, advances in genetics have segued into whole genome sequencing. Whole genome sequences of a number of fruit crops such as papaya will further reveal a tremendous amount of information on the genotypic basis of phenotypic variation and foster targeting candidate genes in selection and genetic transformation for improved fruit quality (Ming *et al.*, 2008a, 2008b; Moore and Ming, 2008; Yu *et al.*, 2009; Allan *et al.*, 2009).

Genetic transformation or genetic engineering (GE) – in particular GE breeding – offers the plant breeder another tool for the development of germplasm as it represents an additional way for generating genetic diversity in fruit cultivars (Wehling, 2000; Manshardt, 2007). Additionally, the limitations generally associated with classical breeding methods, such as loss of valuable trait complexes through crossing or transfer of undesirable genes, are not a major concern in this approach. These restrictions are circumvented through the transfer of transgenes or cisgenes to agronomically adapted cultivars that have been highbred through classical methods (Gepts, 2002). Transgenes and their regulatory sequences generally originate from donors other than plants, that is, microorganisms or animals. A more recent term, cisgenes, has been coined to differentiate these genes from those of plant origin that are flanked by their native promoter and terminator sequences (Rommens, 2004). Marker genes are not used in cisgenesis (Jacobsen and Schouten, 2009).

The potential of GE breeding for improving both the productivity and quality of major fruit crops is great. Attractive traits that can be introduced into fruit crops include: increased resistance to pests and diseases and improved productivity and quality traits, such as increased levels of phytonutrients and the manipulation of ethylene biosynthesis and sensitivity to delayed ripening and senescence (Khachaturians *et al.*, 2002; Hewitt, 2006). Early studies targeted the repression of genes involved in ripening (1-aminocyclopropane-1-carboxylic acid (ACC)

synthase and ACC oxidase) (Matas *et al.*, 2009). More recent investigations, for example with melon (Nunez-Palenius *et al.*, 2008) and papaya (Tecson Mendoza *et al.*, 2008), have developed the technology for investigating fruit ripening in addition to transgenic insect resistance and resistance to pathogen infections. Other studies have examined possibilities of improved fruit quality through modifying the expression of invertase (Yu *et al.*, 2008) through introducing the expression of a magainin analogue (a broad spectrum antimicrobial compound, Mhatr *et al.*, 2009), or cold hardy genes (*CBF1, CBF2, CBF3*, Chandra and Mishra, 2007).

Although considerable research is in progress and many opportunities exist for the application of GE breeding to improve productivity, fruit attributes and postharvest quality, few horticultural GE crops are currently available in commerce and to farmers (James, 2008). The transgenic papaya cultivar with virus resistance is a notable example that is commercially available now. This lag in commercialization of GE fruit crops is not due to a lack of potential genes, but rather to unique issues inherent in horticultural crops. First, for any given fruit crop the diversity in cultivars and the turnover of new cultivars invariably slows the application of GE breeding. Add to this the difficulty in breeding many perennial trees through sexual hybridization, as well as the requirements of processors, distributors and retailers. The strategy often adopted with crops involves the transformation and deregulation of one cultivar, including obtaining licenses for intellectual property, followed by backcrossing to other cultivars. Given the nature of many fruit crops, this is not a viable or economical option. Thus, regulatory and intellectual-property costs need to be resolved before significant progress can be made toward the release and commercialization of transgenic fruit crop products. Another impediment to the commercialization of transgenic fruit crops, and perhaps the most important, is reticence in market acceptance and promotion of GE products (Oelck, 2001; Clarke *et al.*, 2004; Kahn, 2007).

4.3 Environmental factors

Environmental factors refer to prevailing climatic conditions during the growing season and include temperature, light and air humidity. These factors affect important processes during fruit development, such as cell cycle duration, photosynthesis and respiration, transpiration, phloemic transport, metabolism which invariably modify fruit size, and external and internal fruit qualities and storage ability (Ladaniya, 2008).

4.3.1 Temperature

Atmospheric temperature is a major environmental factor influencing growth and productivity. The effects on fruit growth are at the sink level, i.e. fruit demand and growth rate (Léchaudel and Joas, 2007). In some instances, the effects of this factor are manifested in changes to fruit shape and size. For example, trees of the avocado cultivar ‘Harvest N4–5’ grown in cooler environments of southern California

produced more rounded fruits compared to the more elongated fruit produced in the warmer environments of the San Joaquin Valley, California (Arpaia *et al.*, 2004). Adverse effects on ovule formation or fertilization, resulting in less fruit set, were demonstrated with purple passion fruit at high temperatures (Utsunomiya, 1992). Fruit weight, sucrose accumulation, SSC and titratable acids were also reported to be affected. Higher levels were observed at lower temperatures. In citrus, sugar accumulation is a function of temperature and light (Kimball, 1984).

High preharvest temperatures are also known to influence tolerance to high postharvest temperature treatments or low postharvest storage temperatures. Ferguson *et al.* (1999a) monitored the temperatures of avocado fruit in the field and subsequent postharvest response. Flesh temperatures ranged between 35 and 40°C and were associated with elevated levels of mRNA transcripts of heat shock proteins (hsp). Lower temperatures of 25°C were observed at night, but the hsp mRNA levels remained the same. Avocado fruits with greater resistance to low temperature injury, slower ripening rates and higher thermotolerance were obtained.

Preharvest temperatures can predispose fruit to certain postharvest diseases (Woolf and Ferguson, 2000). For example, translucence in pineapple. The disorder, similar to watercore in apples, occurs before harvest and increases upon storage of the fruit (Ferugson *et al.*, 1999b). High temperatures, radiation and rainfall are contributing factors (Soler, 1994; Paull and Reyes, 1996). Affected fruits exhibit water soaking and increased porosity (Paull and Reyes, 1996; Chen and Paull, 2001).

Sunburn or solar injury is a more common disorder resulting from high fruit surface temperatures. The disorder is less affected by postharvest conditions. Early signs include yellowing or bleaching of the skin and the development of a rough, corky fruit surface. In severe instances, tissue failure and inactivation of the photosynthetic system result. The disorder is often found in avocado (Schroeder and Kay, 1961; Woolf *et al.*, 1999). Stylar-end breakdown is a common disorder of 'Tahiti' lime that is exacerbated by increasing temperatures but it can also be induced by postharvest hot water treatments or exposure to the sun before or after harvest (Davenport and Campbell, 1977). As the name of the disorder suggests, stylar-end breakdown begins with the collapse of tissues at the stylar end of the fruit. Essentially juices from ruptured vesicles invade the rind of the stylar end, resulting in a wet appearance of the region (Davenport and Campbell, 1977). It is also suggested that high temperatures and elevated calcium levels contribute to the physiological disorder of papaya referred to as skin freckles. Increased cell wall rigidity presumably facilitates increased turgor pressure which leads to rupturing of latex vessels, latex leaking and the development of freckle-like blemishes on the fruit surface (Campostrini *et al.*, 2010). Low temperatures contribute to navel rind stain and preharvest peel pitting in citrus (Agusti *et al.*, 2002).

4.3.2 Light

The intensity and quality of light are important for optimum plant productivity and harvest index (Jifon and Syvertsen, 2001). The effects are either direct or indirect, affecting the photosynthetic photon flux on the rate of electron flow or

leaf photosynthetic capacity, respectively. Another direct response to sunlight is fruit color (Giovannoni, 2001). In horticultural plants, fruit color is an important consideration in consumer choice, is often associated with better flavor and allows for product discrimination. As a result, there is growing interest in the development of cultivars with fruits of altered color, hues, patterns, or total anthocyanins content. Many tropical fruits are characterized by bright red or purple skin pigments suggesting the presence of anthocyanins. Multiple flavonoid structural genes are co-regulated for anthocyanin synthesis. The requirement for light varies and has been inferred in some fruits, such as mango and pomegranate, from the absence of the pigment in the skin of shaded fruit (Gil *et al.*, 1995; Hetherington, 1997; Simmons *et al.*, 1998).

Carotenoid accumulation in a number of species is also affected by light. Citrus fruits contain the largest number of reported carotenoids, and differences in carotenoid composition are used to classify citrus fruits into various groups (Fanciullino *et al.*, 2006). However, the accumulation of carotenoids in chromoplasts depends not only on genetic factors but also on temperature and humidity in addition to light (Dhuique-Mayer *et al.*, 2009). Sweet oranges from tropical and subtropical growing conditions are reported to contain marked reduction in the total carotenoid contents (up to a 10-fold difference) compared with fruits grown in the Mediterranean environment. The difference was less marked in mandarins and clementines and limited to the carotene content. The converse was observed with 'Star Ruby' grapefruit, in that higher carotenoid and lycopene levels were obtained with fruits grown in subtropical and tropical areas than in Mediterranean areas (Dhuique-Mayer *et al.*, 2009). It was speculated that environmental conditions appear to influence the first step of the carotenoid biosynthesis pathway which is catalyzed by phytoene synthase or the synthesis of carotenoid precursors.

Tao *et al.* (2003) further emphasized the importance of light in stimulating carotenoid synthesis, especially the accumulation of beta-cryptoxanthin, in citrus fruit peel. The effects of bagging 'Hongshigan' citrus fruits at the later stages of fruit enlargement on the levels of chlorophyll and carotenoid in the fruit peel were examined. Decreased chlorophyll levels in the peel were accompanied by decreases in total carotenoid content. As a result, the fruits displayed an earlier color shift and were lighter in color than their unshaded counterparts. Decreases in sugar accumulation were also noted. However, the effects were reversed on removal of the enclosing opaque paper bags. Bagging of fruits, a cultural practice used to reduce disease in mango, diminishes light received on the skin and reduces the area of red color on the peel and its intensity (Hofman *et al.*, 1997).

4.3.3 Carbon dioxide

Carbon dioxide is one of the rate limiting factors in photosynthesis. Elevated atmospheric CO_2 generally enhances leaf and canopy photosynthesis (Kimball and Mauney, 1993; Amthor, 2001; Kimball *et al.*, 2002) especially in C_3 crops. This response comes about because the levels of atmospheric CO_2 do not saturate Rubisco and because high CO_2 inhibits the competing process of photorespiration

(Lawlor and Mitchell, 2000). As regards fruit productivity, fruit growth is dependent on the partitioning of carbohydrates between the fruit bearing branches, fruit growth and storage in leaves and stems. Decreased carbon supply invariably results in decreased fruit growth in dry mass (Léchaudel and Joas, 2007). It has been demonstrated that mango fruit size increased with an increasing leaf-to-fruit ratio (Spreer *et al.*, 2007). Similarly, the link between a reduction in net CO_2 assimilation and lower sugar production and fruit set is supported in citrus and guava (Iglesias *et al.*, 2007; Normand and Michels, 2007).

4.3.4 Other environmental factors

Thaipong and Boonprakob (2005) estimated the genetic and environmental variances with eight white and pink flesh guava cultivars over two seasons in Thailand. Most of the chemical traits, such as total soluble acids and titrable acid, were higher in the winter season than in the summer. However, the physical traits including fruit weight, fruit firmness and seed cavity weight, were reported higher in the summer season.

Apart from climatic factors, tree characteristics as well as some cultivation practices affect environmental factors that influence the quality of fruit at harvest and during storage. Microclimatic gradients (Léchaudel and Joas, 2007), leaf area near the fruit and vegetative vigor of shoots bearing fruit contribute to within-plant variation in quality (Ketsa *et al.*, 1992; Wolstenholme and Whiley, 1992; Whiley and Schaffer, 1994; Syvertsen and Lloyd, 1994; Iwagaki 1997). A balance between fruit size and yield requires effective management of fruit set and canopy manipulations. Fruit thinning to one and two fruits per panicle in mango resulted in increased fruit number, weight and yield per tree at harvest (Yeshitela *et al.*, 2004). In addition, thinning to 50% of the panicles resulted in increased fruit size, quality and fruit retention. Likewise, positive effects are noted in citrus only when thinning is higher than 50–60% of total fruits and conducted during the early stages of fruit development (Zaragoza *et al.*, 1992). Improved fruit weight, as well as a heavier off-season crop, was observed following fruit thinning in guava (Michels and Normand, 2004).

Pruning and the removal of leaves around fruits facilitates increased light penetration and thus increases fruit color, fruit size and quality (Singh *et al.*, 2007; Singh and Dhaliwal, 2007). Girdling or ringing, involving the removal of a ring of bark from scaffold branches or a single cut around the circumference of the branch without the removal of bark, respectively, also positively affect fruit size in fruit trees (Agusti *et al.*, 2002). However timing is very important, since negative effects, namely increased fruit set and reduced fruit size, occur if conducted during the early stages of fruit development.

4.4 Physico-chemical factors

The quality of fresh sub-tropical and tropical fruits, as well as other fresh crops, is determined at the harvesting stage. Because the harvested product has been

removed from its source of water and nutrients, there is no further improvement of the quality and the components that would contribute to quality attributes of the crops. With the very best of preharvest factors, the best that can be achieved during the postharvest handling is the reduction of the deterioration rate through their handling from harvesting to the consumer (Hewett, 2006). Therefore, it is crucial to understand which preharvest factors influence the quality attributes of the crops, and how these affect the rate of postharvest deterioration, and, subsequently, consumer behavior in the marketplace.

These factors encompass production and management decisions concerning soil fertility, variety selection, irrigation, and pest management (Ladaniya, 2008), thus, many of the decisions made during crop production can greatly influence the postharvest quality of fruits.

4.4.1 Water quality and irrigation

Adequate soil moisture during the preharvest period is essential for the maintenance of postharvest quality. Water stress during the growing season can affect the size of the harvested plant organ, and lead to soft or dehydrated fruit that is more prone to damage and decay during storage. On the other hand, crops experiencing an excess of water during the growing season can show a dilution of soluble solids and acids, affecting flavor and nutritional quality (Ladaniya, 2008; Shewfelt and Prussia, 1993), while soil moisture would affect the postharvest texture of crops (Sams, 1999). Excess moisture on the harvested fruits can also increase the incidence of postharvest diseases. To minimize the amount of water on harvested fruits brought into storage, it may be beneficial to choose surface or subsurface irrigation rather than overhead irrigation. Fruits harvested in the early morning, during rainy periods, and from poorly ventilated areas can also experience increased postharvest decay (Silva, 2010).

Water stress during growing directly affected the activity of the browning enzyme polyphenol oxidase (PPO) in ripe avocado during storage. This implies that postharvest cell damage occurs. The likelihood and intensity of browning as a result of increased PPO activity will be higher in fruit from trees with a history of water stress (Bower, 1988).

4.4.2 Soil quality, fertilization and mineral nutrition

Plant performance depends on a balanced availability of mineral nutrients that may be limiting in soils, and mineral nutrition has a great impact on the quality of horticultural crops, particularly physiological fruit disorders (Ferguson and Boyd, 2002). Therefore, management practices have been developed to apply appropriate fertilizers to the crop at times when benefits of yield or quality can be achieved (Hartz, 1997). Maintaining optimal levels of plant nutrients throughout the growing season will allow for optimal quality of fresh fruits throughout the packing and distribution processes, and deficiencies or overabundance of certain plant nutrients can positively or negatively affect crops' susceptibility to

physiological disorders, disease, and composition and textural changes (Kays, 1999). When optimizing soil fertility to improve postharvest quality, it is important to remember that these may not be the same soil nutrient levels that produce the highest yields (Ladaniya, 2008; Sams, 1999).

Nitrogen

Nitrogen is an important mineral element that is used by almost all crops. Nitrogen is a key component of plant proteins that plays an important role in plant growth and development (Novoa and Loomis, 1981). Because of nitrogen's involvement in protein synthesis, soil nitrogen deficiencies may lead to lower protein concentrations in fruits, thereby affecting the nutritional composition of the crop. Adequate soil nitrogen supplies allow for the optimal development of color, flavor, texture and nutritional quality. Excess soil nitrogen can be problematic as well (Silva, 2010). Excess nitrogen may lower fruit sugar content and acidity, but tends to decrease the vitamin C content (Lee and Kader, 2000).

Phosphorus and potassium

Potassium is important in plant water balance and enzyme activation. High levels of soil phosphorus have been shown to increase sugar concentrations of fruits while decreasing acidity. High levels of soil potassium often have a positive effect on the quality. Increased soil potassium concentrations have been shown to increase the vitamin C and titrable acidity concentrations and color. Soares *et al.* (2005) reported that soil applications of potassium to growing pineapple plants could improve fruit quality during postharvest life. They reported that preharvest soil application of potassium reduced internal browning (IB) of pineapple fruit (*Ananas comusus* L.) during storage by reducing phenylaalanince amonialyase (PAL) and peroxidase (POD) activities which were lower in fruit from plants treated with potassium as was the incidence of IB.

Calcium

It is well known that calcium plays a role in the maintenance of cell stability and is important to plant cell walls, particularly membranes, under stress conditions such as postharvest, low temperature storage (Roux and Slocum, 1982). The more stable the cell membranes are, the less likely it is that cell damage resulting in browning will occur. Deficiencies in soil calcium have been associated with a number of postharvest disorders, including blossom-end rot, brown heart; blackheart and tipburn (Bangerth, 1979; Ferguson, 1984; Millaway and Wiersholme, 1979). Poovaiah (1979) attributed the slower rate of senescence in tomatoes having higher calcium content. High soil calcium concentrations reduce these disorders and are associated with other postharvest benefits, including increased vitamin C content, extended storage life, delayed ripening, increased firmness, and reduced respiration and ethylene production. In papaya, low mesocarp calcium concentrations have been linked to fruit softening (Qui *et al.*, 1995), while Chaplin and Scott (1980) found that the higher is the level of calcium the lower are the cold-induced disorders in avocados during storage.

4.4.3 Chemical treatments

Calcium dips may be used to reduce postharvest physiological disorders of mangoes (Singh *et al.*, 1993). Bangerth (1976) observed an increase in vitamin C content of apples and tomatoes treated with calcium chloride. Preharvest treatment with $CaCl_2$ also improved storability of apples and cherries (Brown *et al.*, 1996) and kiwi fruit (Gerasopoulos *et al.*, 1996). Dehydrated pineapples and guava pretreated with cysteine hydrochloride had increased ascorbic acid (AA) retention and reduced color change during storage (Mohamed *et al.*, 1993). Kiwifruit slices stored in ethylene-free air contained three times more AA than controls. When dipped in one percent $CaCl_2$ after cutting and kept in an ethylene-free atmosphere, slices had a slightly higher AA content than those treated with one per cent $CaCl_2$ only (Agar *et al.*, 1999). Nigro *et al.* (2006) reported that salts could be used to control postharvest diseases of grapes. They noted that preharvest application of calcium chloride (CC), potassium carbonate (PC), sodium bicarbonate (SB) and sodium carbonate (SC) significantly reduced the incidence of grey mold caused by *Botrytis cinerea* on table grape bunches during storage. According to Zheng *et al.* (2006) nickel, considered as having an inhibitory effect on 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, could be exploited as an effective new reagent to prolong persimmon shelf-life via pre-harvest treatments. Some fungicides such as flusilazol, pyrazophos as well as monthly pre-harvest applications of copper-oxychloride were also tested for their effectiveness in controlling soft brown rot and stem-end rot of mango fruit during postharvest storage and resulted in significantly improved disease control (Saaiman, 1997).

Torrigiani *et al.* (2004) reported that preharvest application of natural or synthetic polyamines decreased ethylene production and modulated postharvest ripening of nectarines, and application of putrescine reduced suppressed ethylene biosynthesis and retarded softening of plums during storage (Khan *et al.*, 2007).

Preharvest application of fungicides can also be an effective approach for control of some diseases. Five potential fungicides were applied to control postharvest anthracnose in citrus and results showed that two were effective (Zhang and Timmer, 2007). Franck *et al.* (2005) also reported that preharvest fungicide applications effectively reduced decay of table grapes caused by *Penicillium expansum* and *Botrytis cinerea* in long term cold storage. Moreover, endogenous and exogenous (applied) plant growth regulators (PGRs) and pesticide sprays are also major factors that determine or modify fruit internal qualities and storage ability of citrus (Ladaniya, 2008), and grapes (Zoffoli *et al.*, 2009).

4.5 Diseases

Postharvest diseases of fruits cause heavy losses during storage and in the supply chain. Most pathogens causing decay are controlled by various methods such as refrigeration, controlled atmosphere, chemicals (fungicides, ethanol, etc), and physical methods (hot water, irradiation, etc). However, during the past two decades, biological control strategies have emerged as promising alternatives to

the use of synthetic fungicides preharvest to reduce postharvest disease, especially when combined with the classic methods, such as as refrigeration. Thus, several antagonistic microorganisms have been found that can effectively inhibit postharvest diseases of subtropical and tropical fruits.

Preharvest application of *Candida sake* controlled blue mold in apples during postharvest storage (Teixido *et al.*. 1998). A yeast, *Candida oleophila*, was also evaluated for the potential biocontrol of gray mold caused by *Botrytis cinerea* on grape, and results showed that treatment maintained some of the fruit quality characteristics under cold storage conditions (El-Neshawy and El-Morsy, 2003). Ippolito *et al.* (2005) reported that postharvest rotting of sweet cherries would be controlled by preharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. Application of chitosan to control diseases during postharvest life was also suggested. BhaskaraReddy *et al.* (2000) reported that chitosan pre-harvest sprays of strawberry fruit were effective during the postharvest storage and protected the fruits against infection and deterioration by *Botrytis cinerea*.

4.6 Other factors

Development of disorders during postharvest ripening and storage of fruit depends also on a number of other factors such as the fruit position on the tree and fruit temperature history during cropping. For example, incidence of postharvest disorders such as bitter pit of apples is influenced by fruit mineral content which is strongly related to the fruits' position on the tree (Ferguson *et al.*, 1999). High temperatures experienced by apples and avocado on the tree can influence their response to low and high postharvest temperatures. Specific disorders such as watercore in apples and chilling injury in avocado can also be related to fruit exposure to sunlight and high temperatures; disorders such as scald in apples may be related to frequency of low temperature exposure over the season (Ferguson *et al.*, 1999; Woolf and Ferguson, 2000).

4.7 Harvesting factors

4.7.1 Maturity at harvest

In general terms, fruit can be divided into two groups depending on their respiration behavior: (1) those that are incapable of continuing their ripening process once removed from the plant, and (2) those that can be harvested at the mature stage and allowed to ripen off the plant. Group 1 includes fruits such as citrus fruits, grape, litchi, pineapple and pomegranate. Group 2, on the other hand, includes fruits such as avocado, banana, cherimoya, guava, kiwifruit, mango, papaya, passion fruit, sapodilla and sapota. Fruits in Group 1 produce very small quantities of ethylene and do not respond to ethylene treatment except in terms of degreening (removal of chlorophyll); these should be picked when fully ripe, if good flavor quality is to be

ensured. Fruit in Group 2, on the other hand, produce comparably larger quantities of ethylene when they ripen and undergo more rapid and uniform ripening upon postharvest exposure to ethylene. These can be picked before they are fully ripe.

Maturity indices indicate the ideal harvest time for a fruit and are therefore important for the formulation of market strategies, trade regulation and efficient use of labor and resources. Fruits harvested at an improper maturity stage tend to exhibit high physiological weight loss and poor internal quality and are prone to physiological disorders. Various methods used to determine harvest maturity of fruits are reviewed below.

Computation method

This involves computing the number of days from full bloom (DFFB) to harvest or measuring heat units. DFFB to harvest can provide a good maturity index provided the number of days used has been derived in the region where it is being used as it differs largely from year to year and location to location (Filder, 1973; Ryall *et al.*, 1941). Typically, the measurements for a particular crop in a particular area are standardized over the years, taking into consideration time of full bloom, weather conditions, cultivar and rootstock. DFFB have been known to be used as reliable index with several fruit crops such as banana, (Israeli and Lohav, 1986) Chinese gooseberry (Pratt and Reid, 1974), and fig (Turk, 1989). Similarly, in some fruits accumulated heat units are related to optimum harvest maturity of fruits. Accumulated heat units are computed by calculating the time in relation to temperature above a certain minimum base temperature. This mean base temperature varies with type of fruit and cultivar.

Physical methods

Some physical, destructive and non-destructive methods can be used to determine the maturity of fruits. They can involve judging or measuring fruit size, weight, surface texture or skin morphology, acoustic properties, peel color, firmness, abscission layer, electrical properties, magnetic properties or optical properties. However, fruit size and weight are not considered reliable maturity indices since a number of factors such as crop load, nutrition, locality and seasonal variation have profound influence on final fruit size. Fruit skin morphology can indicate the stage of harvest in some fruits like banana, litchi and pineapple. Fruit firmness is recognized as a maturity index and is measured with a penetrometer. The change in skin color during fruit development is widely used as a maturity index as shown by Plate III (in the color section between p. 238 and p. 239) where skin color of mango is used to determine readiness of fruit for harvesting. In several fruits, the force required to separate the fruit from the tree is also used as, in some crops, a special band of cells (the abscission zone) develops on the pedicel of the fruit.

Chemical methods

Certain sensory organoleptic characteristics are widely accepted as maturity indices. During fruit maturation in most fruits there is a decrease in acid content (except in banana) and total soluble solids (TSS) content increases. The TSS:acid ratio is

commonly related to fruit maturity. TSS is also regarded as a reliable index of maturity, as it mainly indicates sugar content. It is measured with a refractometer. Starch content of fruit also sometimes determines the time of picking. Starch content generally increases during the developmental stage of fruits and declines significantly during the ripening process. Reaction of iodine with starch on the cut surface of fruit gives a dark bluish stain indicating the starch content in fruit. In some fruits, increase in oil content can be used as an index of the onset of maturity.

Physiological methods

Fruit maturation is accompanied by several metabolic changes that can be assessed by measuring changing patterns of respiration and ethylene production. Measurements of these physiological changes have been used successfully as reliable methods to determine the optimum physiological maturity stage of fruits (Sharma and Chopra, 1997).

4.7.2 Mode of harvesting

Mode of harvesting is an important factor which can significantly affect fruit postharvest quality and shelf life. Improper harvesting and handling causes mechanical injuries (bruising, surface abrasions, etc.) which lead to rapid decomposition of fruit by pathogens. Bruised fruit lacks consumer acceptability and fetches poor market prices. The method of harvesting depends upon several factors including the maturity of fruits, delicacy of crop, climatic conditions, packing material, transportation facilities, availability of skilled labor and economy of operation. Most tropical and subtropical fruits are harvested manually: mechanical harvesting is not especially popular.

Manual harvesting

Manual harvesting is a commonly practiced method of fruit harvesting in many parts of the world. It is more feasible for produce to be eaten fresh and is particularly important for fruits that have a wider range of maturity and need several pickings during a season. This method involves harvesting of fruit by hand, using fruit clippers and knives etc. Properly trained workers harvest and handle the fruits with minimum damage. The picked fruit is placed in small containers/baskets of different shapes and sizes or in a specially designed picking apron, depending upon the size of fruit. Good harvesting practices ensure that there is little mechanical injury to the produce. The force needed to detach fruit from the tree usually determines the method of harvesting to be employed. Manual harvesting may be done either by hand using harvesting tools or by pole mounted 'cut and hold' picking shears (see Plate IV in the color section between p. 238 and p. 239). The fruits that can easily be detached from tree such as guava, ber and phalsa should be harvested by grasping the fruit firmly and gently, and pulling upwards.

Mechanical harvesting

Depending upon the type of fruit, mechanical harvesters employ various methods such as cutting, pulling, shaking, snapping, twisting, stripping, combing and

compacting (Sudheer and Indira, 2007). The main disadvantage of mechanical harvesting is the higher risk of mechanical injury to the fruit trees, e.g. bark damage by tree shaker. The machinery is also costly and can be employed only if the orchard is relatively large.

Harvesting guidelines for quality produce

- Specialized trained persons should be employed to harvest fruit carefully and to avoid damage to the tree. Training on the maturity indices such as color, size, and firmness should also be imparted to the harvesters.
- Workers should have trimmed fingernails, wear proper clothing, cotton gloves and hats.
- Harvesting containers should be of proper size, free from sharp edges, smooth and ventilated.
- The angle of harvesting ladders should be carefully set or checked before use.
- Fruit should be picked from the tree from bottom to top.
- Fallen fruit should never be mixed with picked fruits.
- Fruit should be harvested during the early morning when the weather conditions are optimal. Wet fruit should be avoided, as their rinds are turgid and can be readily bruised, leading to decay during subsequent handling.
- After harvesting, fruits should be collected in picking bags and pooled in the shade for subsequent grading.

4.8 Phytohormones and other growth regulators

Plant hormones and plant growth regulators applied to preharvest fruit affect not only fruit development before harvest but also fruit quality and shelf life after harvest. Plant hormones are natural substances produced by the plant that regulate many behavioral patterns and functions. Plant growth regulators (PGRs) are a wider group of organic compounds, natural and synthetic, but not including nutrients, that when applied to plants in small amounts promote, inhibit or otherwise modify plants' physiological processes. There are five major recognized groups of natural plant hormones and growth regulators: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. Recently several other compounds that can regulate plant growth and development process such as jasmonates, salicylates, polyamines, brassinosteriods etc. have been described and recognized as PGRs. The role of some plant growth regulators in tropical and subtropical fruit maturity, ripening and postharvest handling is discussed below.

4.8.1 Auxins

Fruit ripening is greatly influenced by endogenous levels as well as exogenous applications of auxins. Exogenous treatments of auxins, depending upon the concentration, either delay or enhance the ripening process. During the series of endogenous reactions, auxins are degraded by enzymes like indole acetic acid

(IAA) oxidase, and the degraded fruit tissue becomes sensitive to ethylene. IAA decreases to low levels in all parts of avocadoes with the onset of maturity (Wolstenhome *et al.*, 1985). Treatment with 2,4-D (Dichlorophenoxyacetic acid), a synthetic auxin, lowered ABA concentration, prolonged storage and reduced fruit rotting in 'Valencia' oranges (Liu and Xu, 1998; Tatli and Ozgyuven, 1999). Spraying with 2, 4-D (20 ppm) and naphtalen acetic acide (NAA) (10–100 ppm) one month prior to harvest increased juice content in 'Coorg' mandarin. In monsoon crop fruit, sprays of 2,4,5-Trichlorophenoxyacetic acid (25–50 ppm) and CLPA (chlorophenoxy acetic acid) at 25 ppm before harvest resulted in 28–35% less weight loss than control during 35 days storage at 5–6°C and 85–90% RH. Treated fruit had better marketability although respiration was unchanged (Rodrigues and Subramanayan, 1966). Preharvest spraying of pineapple fruits with alpha-NAA or ethrel along with K_2SO_4 solution (5% w/v) four weeks before harvesting significantly increased the potassium ion-concentration of the fruits and decreased internal browning during cold storage (Rao, 2005).

4.8.2 Gibberellins

Gibberellins are known as senescence-delaying agents. Exogenous application of gibberellic acid delays ripening changes such as degreening and synthesis of carotenoids. Gibberellic acid treatments minimized fruit drop and puffiness, delayed color development and fruit softening and reduced weight loss in 'Nagpur' mandarin during storage (Ladaniya, 1997; Ladaniya and Sonkar, 1999). Gibberellic acid applications maintained fruit peel quality on the tree and in storage and reduced postharvest decay (El-Otmani and Coggins, 1991). Preharvest spray of gibberellic acid and carbendazim on mango trees delayed ripening by 22 days after harvest when they were stored at 10–12°C (Sudhavani and Shanker, 2002). In sapota, preharvest treatment of 200 ppm GA_3 extended the shelf life by reducing the Physiological Loss in Weight (PLW) and rotting (Choudhury *et al.*, 2003). GA_3 treatment maintained fruit firmness, inhibited the increase in ethanol content, reduced alcohol dehydrogenase and polyphenol oxidase activities, reduced respiration rate and rate of ethylene production, delayed the flesh browning of 'Cui Jujube' (*Z. Jujube*) in cold storage (Xue *et al.*, 2003), reduced amylase activity and retarded the degradation of peel chlorophyll in mango fruit (Khader *et al.*, 1988; Khader, 1992). This growth regulator has also proved to be highly effective in reducing the activity of peptic substances degrading enzymes in cape gooseberry (Majumder and Majumdar, 2001). Persimmon fruits sprayed with 50 ppm gibberellic acid can be stored up to 70 days at 0–2°C (Testoni, 1979). In 'Kinnow' mandarin, preharvest spray of gibberellic acid (GA_3) (75 ppm) reduced weight loss during storage (Ahlawat *et al.*, 1984) and delayed color development (Sandhu, 1992).

4.8.3 Cytokinins

Cytokinins also influence the senescence phenomenon by delaying color changes. In many fruits, cytokinin content is high during the initial fruit development stage,

but it declines at maturity. Tsantili *et al.* (2002) indicated that cytokinins may have only a regulatory role in some ripening processes, such as color development. Ramteke *et al.* (2002) observed that treatments with growth regulators, i.e. 4-chlorophenoxyacetic acid (CPA), *N*6 benzoyladenine (6BA) and N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) in grapes increased the pedicel thickness by 19.4 % and would enhance the shelf life compared with the application of GA_3. Treatment of mature unripe 'Alphonso' mango fruits with kinetin delayed ripening as indicated by the lower levels of peel carotenoids during storage at 30 ± 2°C (Majumdar *et al.*, 1981). The highest palatability rating in ber (*Zizyphus mauritiana* Lamk) fruits was obtained when treated with 10 ppm benzyladenine (BA) solution and stored at ambient conditions for seven days after packing in 150 gauge polyethylene bags (Sandhbhor and Desai, 1991). Kinetin (100–150 ppm) application at pre-bloom and second rapid growth stage of berry development remarkably reduced berry shatter and other losses in stored 'Himrod' grapes (Dhillon *et al.*, 1975).

4.8.4 Abscisic acid (ABA)

ABA is generally known as a gibberellin antagonist. It plays a vital role in ripening and senescence of fruits. In fruits, ABA content increases late during fruit development and ripening. It is an important ripening promoter which stimulates ethylene production in pre-climacteric fruits. Fruit ripening depends on the synergistic effect of ABA and ethylene (Tian *et al.*, 1994). In avocado fruits, ABA increased PPO activity, reduced phenolic substrate levels and fruit quality by promoting internal discoloration (Cutting *et al.*, 1990). During storage ABA content increased to a peak, then declined. Preharvest application of ABA (300 µl/L) was found an effective alternative to ethephon for enhancing the color and maintaining post harvest quality of 'Crimson seedless' grapes (Cantin *et al.*, 2007). ABA decreased the chilling injury of banana fruit during storage (Wang *et al.*, 2004; Peerasak *et al.*, 2002).

4.8.5 Ethylene

Ethylene, unlike other plant hormone compounds, is a gaseous hormone. It is produced in all higher plants and is usually associated with fruit ripening. The coloration in mandarin can be advanced by three weeks with two pre-harvest sprays of ethephon (Sonkar and Ladanyia, 1999). The higher dose of ethephon increased carotenoids content in ber fruit (Bal *et al.*, 1986). Metizidakis and Sfakiotakis (1993) reported that the cellulase activity was inhibited in avocado fruits stored in a low oxygen atmosphere at 20°C, the inhibition being mediated via an effect on ethylene action. Ethephon (400 ppm) at color break stage advances ripening by two weeks in 'Umran' variety of ber and produces attractive, uniform, better quality fruits that are deep golden yellow with a chocolate tinge (Anon., 2008a). Watada *et al.* (1976) reported that AA content was slightly higher in mature-green tomatoes treated with ethylene than in those ripened without added

ethylene. Kader *et al.* (1978) found a significant difference in ethylene-treated versus control mature-green tomato fruit.

4.8.6 Polyamines

Polyamines, a group of polyvalent compounds containing two or more amine groups, are ubiquitous bioactive compounds found in all organisms. Plant polyamines are involved in many growth and developmental processes. Polyamines and ethylene show opposite effects in relation to fruit ripening; polyamines retard the fruit senescence and prolong the shelflife (Kakkar and Rai, 1993). Polyamines may compete directly with the synthesis of ethylene as they share a common precursor S-adenosylmethionine (Bagni and Torrigiani, 1992). Several workers reported that polyamines extend the shelf life of fruits. In litchi fruits, putrescine, spermidine and spermine polyamines delayed the changes associated with senescence such as browning, peroxide level, ethylene production and cell leakage (Jiang and Chen, 1995). Putrescine application inhibited the production of ethylene during storage (Qiu *et al.*, 2002). Preharvest spray of putrescine on 'Kensington Pride' mango reduced ethylene production, increased fruit firmness and sugars as compared to the control. After 20 days storage, the putrescine sprayed fruits exhibited higher firmness, TSS and lower fruit rot (Malik *et al.*, 2003).

4.8.7 Jasmonate

Jasmonic acid (JA) and related compounds were initially isolated as inhibitors of growth. Jasmonate plays a role together with ethylene in regulating the early steps of climacteric fruit ripening (Fan *et al.*, 1998). Exposure of mango fruits to methyl jasmonate vapor (10–4 M) reduced chilling injury during storage at 7°C (Gonzalez *et al.*, 2000). The chilling tolerance induced by methyl jasmonates was positively correlated with the reduction in the percentage ion leakage of mango tissue. Methyl jasmonates delayed the increase in respiration, but promoted ethylene production during the later part of storage. Exposure of papaya fruit to methyl jasmonates vapor inhibited fungal decay and reduced chilling injury development and loss of firmness during storage at 10°C (Gonzalez *et al.*, 2003).

4.8.8 Brassinosteriods

Brassinosteriods are steroidal hormones, essential for normal plant growth and development. The application of Brassinosteriods to grape berries significantly promoted ripening while brassinazole, an inhibitor of Brassinosteriods biosynthesis, delayed fruit ripening (Symons *et al.*, 2006).

4.8.9 Salicylic acid (SA)

Salicylic acid can maintain fruit anti-oxidant activity and nutrition. Pre-harvest treatment with SA (1.0 and 2.0 mmol/L) significantly increased the carotenoid

content (lycopene and alpha-carotene), ascorbic acid, glutathione, total phenolics and total flavonoids in the pulp and peel of of 'Cara cara' navel orange (*Citrus sinenisis* L. Osbeck) during storage (Renhua *et al.*, 2008). SA treatment has been found to delay the ripening of banana fruit (*Musa acuminate*) by decreasing fruit softening, pulp:peel ratio, reducing sugar content, invertase and respiration rate (Srivastava and Dwivedi, 2000). Pre-harvest sprays of SA (2000 mg/L) significantly slowed down changes in fruit skin color, firmness and ripening of 'Kensington Pride' mango (Zainuri *et al.*, 2001).

4.8.10 Paclobutrazol

Preharvest treatment of mango cv. 'Alphonso' fruit with paclobutrazol (2000 ppm) reduced the incidence of spongy tissue (Ravindra and Shivshanker, 2004). Similarly in peach, pachlobutrazol lowered and delayed the peaks in the rates of ethylene production (Hu and Ding, 1993).

4.8.11 Diaminozide

Diaminozide inhibits ethylene production by preventing the conversion of methionine to ACC (Gussman *et al.*, 1993). Alar® at 2000 ppm decreased the incidence of Jonathan apple breakdown and retarded the loss of vitamin C during storage (Ben and Kroop, 1986). Preharvest application of Alar (500 or 1000 ppm) on guava fruits significantly retarded the activity of cellulase during storage (Singh and Chauhan, 1981).

4.9 Conclusions

From the points developed above, it is obvious that the quality of fruit at harvest and during postharvest shelf life is affected by decisions made at all stages of the cultivation process. Often, the effects of preharvest factors on postharvest quality are overlooked and underestimated, and the losses are observed immediately at harvesting. These could reach up to 20% at this stage. Moreover, it is critical to remember that fruit quality is only maintained and not improved after harvest. Thus, it is of the utmost importance to consider each preharvest factor that can contribute to maximizing the quality of the fruit destined for the fresh market, storage or processing. These factors encompass production and management decisions concerning soil fertility, variety selection, irrigation, and pest management, and other physical and chemical treatments prior to harvesting.

4.10 References

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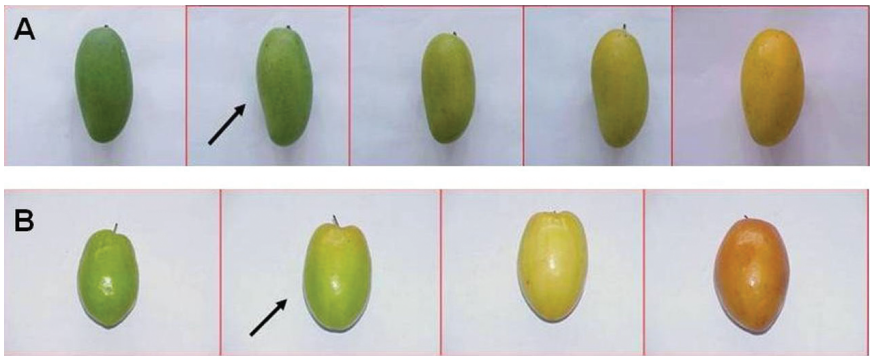

Plate III (Chapter 4) Judging maturity of mango (A) and ber (B) fruits by color (Mahajan *et al.*, 2007).

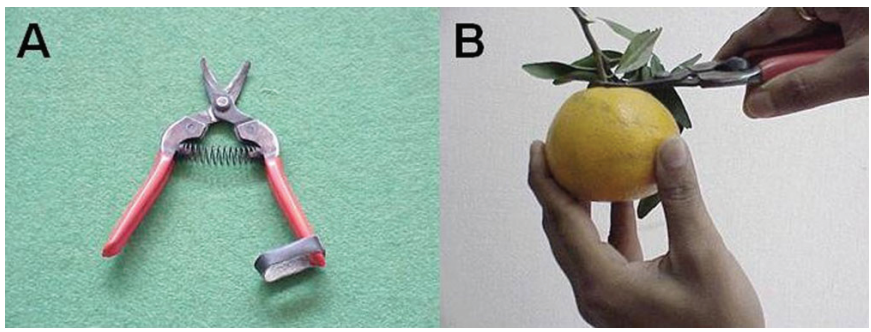

Plate IV (Chapter 4) Fruit harvesting clipper (A) and method of harvesting (B) for citrus fruits (Mahajan *et al.*, 2007).

5

Postharvest technologies to maintain the quality of tropical and subtropical fruits

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Abstract: Tropical and subtropical fruits offer many diverse aromas, textures, tastes and shapes and include many different bioactive compounds. These attributes have increased consumer demand for produce of this type. The commercial success of tropical and subtropical fruits worldwide has favored the development of postharvest technologies and handling techniques for these fruits, especially in developed countries. Some adequate postharvest technologies have now been adopted in developing countries, where most tropical and subtropical fruits are produced and where postharvest technologies were virtually non-existent for many years in the past. For most of these products there have been advances in techniques for harvesting, packing, selection and grading, quality evaluation, transportation (by land, rail, sea and air) and control of storage conditions (temperature, humidity and atmospheric composition) and control of postharvest physiological disorder, insects and microbiological decay. There have also been improvements in methods of packaging, storage and processing. However, in some cases the best methods are not used and need to be made available to stakeholders, especially in developing countries. This chapter describes postharvest technologies and handling practices to maintain the quality of tropical and subtropical fruits.

Key words: tropical and subtropical fruits, packing, packaging, storage, transport, ripening, processing.

5.1 Introduction

A wide variety of fruit is grown in the tropics and subtropics, under a diverse range of climatic conditions and soil types. Some crops, such as bananas, have been widely used within the tropics and also exported to temperate countries for many years, whereas many others are currently still grown almost exclusively for

local or regional use, and are barely known or completely unknown in international markets. However, consumer demands and improvements in postharvest technologies and treatments are increasing the number of species available in international markets. Fresh tropical and subtropical fruits are living organs that continue to respire, ripen, senesce and deteriorate as soon as they are harvested. Most of the fruits are very perishable due to their characteristic shapes, structure, high water content, appearance, physiological characteristics and growing conditions (Zhang *et al.*, 1998; Yahia, 2006). Due to these characteristics postharvest losses of these fruits have been estimated to be very high, especially in developing countries, and their postharvest life can be very short (for some only a few days) (Worrell *et al.*, 1998; Brosnan and Sun, 2001; Yahia, 2006; Yahia, 2005).

The respiration rates of tropical and subtropical fruits vary depending on the type and variety of product, the level of maturity, the extent of injuries, and the product temperature. The respiration rate is inversely related to shelf life and therefore the higher the respiration rate, the shorter the postharvest life. Harvesting of tropical and subtropical fruits is commonly done at ambient temperatures ranging from 25°C to more than 35°C (sometime even much higher), resulting in high metabolic activity and a potentially short storage life (Noomhorm *et al.*, 1991), unless adequate postharvest treatments and techniques are used appropriately and in a timely fashion. In many tropical countries and in large-scale operations, it is difficult to take advantage of the low temperatures during the early hours of the day. Therefore, the continuous exposure of the fruit after harvest to high temperatures accelerates metabolic activity, encourages the growth of decay-producing microorganisms, and increases the production of the natural ripening agent, ethylene, therefore increasing the rate of deterioration. For avocado as an example, at higher temperatures the processes of ripening and development of anthracnose proceed at faster rates (Spalding and Reeder, 1974). This is true for almost all tropical and subtropical fruits, especially the very highly perishable ones and during hot weather (Yahia, 2006). Rapid, uniform cooling as soon as possible after harvest is critical for removal of field heat and for lowering the rate of metabolic activity, thus reducing the rate of deterioration, and helping to establish a longer postharvest life (Shewfelt, 1986; Dennis, 1984; Anon., 1984; Farnham *et al.*, 1967; Fraser, 1992; Gibbon, 1972; Gormley, 1975; Peirce, 1987). Other complementary postharvest technologies such as controlled and modified atmospheres, heat treatments and irradiation, among others, can also assist in reducing the deterioration in quality of tropical and subtropical fruits.

5.2 Maturity and harvesting indices

The maturity stage at harvest of tropical and subtropical fruits influences the ripening rate and storage life. Harvesting at the ideal moment is crucial for the quality development after harvest and for attaining the longest postharvest life. Fruits harvested before the appropriate stage of maturation and ripening may not

Table 5.1 Maturity indices of banana, mango, papaya and pineapple fruits

Fruit	Maturity indices
Banana	Fullness of finger (disappearance of angularity), days after flowering or fruit set
Mango	Fruit shape, internal color, formation of shoulder, external color change in some cultivars
Papaya	Surface color (6–33% yellow), soluble solids content (11.5% or higher)
Pineapple	Shell color: 'color break' to 25% yellow stage

attain optimum quality after harvest, may become more sensitive to certain disorders (such as chilling injury), or even may never ripen. Fruits harvested after the optimum stage may not have a sufficiently long postharvest life. For example, harvesting of bananas and mangoes at the mature-green stage hastens their ripening by lowering their response threshold to endogenous ethylene concentration. Bananas, therefore, can be harvested at the mature green stage (see Plate V in the colour plate section, between pages 238 and 239) and ripened later. Papaya and pineapple, however, should be harvested when partially ripe to attain good eating quality when ripe. Maturity indices commonly used for bananas, mangoes, papaya and pineapples are shown in Table 5.1. Many characteristics of fruits have been tested as maturity and harvesting indices, including morphological, anatomical, physiological and biochemical features and components (Table 5.2). Adequate maturity and harvesting indices are those features or components that change during the development of the fruit, correlating adequately and consistently with the quality of the fruit, without being affected greatly by factors such as the environment.

5.3 Harvesting

It is recommended that most tropical and subtropical fruits are harvested early or late in the day (i.e. during the cooler hours) to reduce field heat. Some turgid fruits (such as citrus) should only be harvested late in the day to avoid injury to the skin. Careful handling from harvest onwards throughout the whole postharvest supply chain is of paramount importance. Fruit also needs to be picked at precisely the right stage of development. Almost all tropical and subtropical fruits for fresh consumption are harvested manually (by hand). Mechanical harvesting (harvesting by machine) is not very common, however, some machines can be used to facilitate manual harvesting. Some examples include belt conveyors used for melons, platforms or moveable worker positioners that have been used for dates, citrus and bananas, and shakers for harvesting some types of nuts. Manual harvesting has several advantages over mechanical harvesting. It can result in much less mechanical injury to the fruits and it is selective, which is important because the stage of maturity of most fruits in the same location can vary widely

Table 5.2 Some examples of features used as maturity and harvesting indices in some tropical and subtropical fruits and methods for their measurements

Index	Fruit	Method of measurement
Size	Many fruits	Various measuring devices, weight
Shape	Angularity of banana fingers, full cheeks of mango	Dimensions, ratio charts
External fruit	Several fruit	Visual, color charts
Internal color	Flesh color of some fruits such as mango	Visual, color charts
Elapsed days from full bloom		Computation
Mean heat units during development		Computation from weather data
Development of abscission zone	Some melons, feijoa	Visual or force of separation
Netting	Some melons	
Specific gravity	Watermelons	Density gradient solutions, flotation techniques
Texture/firmness		Firmness testers, deformation
Starch content		KI test, other chemical tests
Sugar content	Grapes	Hand refractometer, chemical tests
Acid content, sugar:acid ratio	Citrus, grapes, pomegranates, melons, kiwifruit	Titration (acid content)
Juice content	Citrus fruits	Extraction of juice
Oil content	Avocados	Chemical test
Dry matter content	Avocados	Oven
Astringency (tannin content)	Dates	Ferric chloride test (tannin content)

and therefore they need to be picked at intervals during the season. Product harvested by machine is of very variable quality and at different maturity stages. Fig. 5.1 shows some harvesting aids used in harvesting avocado in Mexico.

Mechanical harvesting is faster than manual harvesting, with fewer of the management problems associated with workers. However, mechanical harvesting can cause damage to the fruit and even to the tree (for example, in the case of excessive shaking) and trees also need to be managed so that they are compatible with machine harvesting. In addition it is very costly, and may result in social problems by displacing employment. Some nuts are harvested mechanically, as nuts are better able to withstand this practice. This is due to their low water content and the fact that they are commonly protected by shells. Nut harvesters usually attach a shaking mechanism to the tree and remove most of the nuts in a few minutes. The nuts are either caught on a fabric-covered frame or are picked up

Fig. 5.1 Harvesting aid used for avocado in Mexico.

from the ground by sucking machines or by hand. High labor cost or unavailability of labor in some regions may promote more research and more interest in mechanical harvesting.

5.4 Conditioning

Some fruits, such as citrus, need to be conditioned after harvest and before cooling, packing and marketing. Conditioning of citrus to induce resistance to chilling in sensitive cultivars, for example, is done by exposing the fruit to moderate temperatures of 15–18°C for several days, to humid air at 30–37°C for one to three days, or to intermittent warming by raising the temperature of the fruit to 20°C once a week for 16–20 hours for sensitive fruits such as grapefruits or for five hours for mandarins (see the chapter on citrus in Volume 2 of this work).

5.5 Quality

The term 'quality' is associated with the degree of excellence of a product. Overall quality, value and acceptance of tropical and subtropical fruits depend on the

values of a combination of complex attributes such as color, appearance, flavor, texture, nutritional characteristics and safety (Maarten, 2003; Skrzyski and Konopacki, 2003). Color is probably the most important appearance attribute, brought about by several pigments, including carotenoids, betalains, flavonoids (including anthocyanins) and chlorophyll. Fruit color influences consumer acceptance, affects the perception of sweetness and flavor and can even evoke emotional feelings in humans (Ornelas-Paz *et al.*, 2008). Fruit color is commonly used as a ripening index and is useful in determining the harvest date, as well as predicting the length of time for which a product can be stored and marketed. Besides their involvement in fruit color, the pigments mentioned above exert protective actions against several diseases in humans (Yahia, 2010; Yahia and Ornelas-Paz, 2010). Thus, pigments of tropical and subtropical fruits are important for product appearance, as indicators of ripeness of these fruits and for human nutrition and health.

Texture is defined as the group of mechanical properties of a food that determine the sensations that humans feel when they eat food. This attribute greatly influences the acceptability of the product to the consumer (Tijskens, 2003). Several texture attributes have been reported for fruits, including crispness (which is perceived during the first bite, when the fruit is broken), mealiness (which is perceived by the formation of lumps during chewing of the fruit), juiciness (the property that creates the sensation of a progressive increase in the free fluids in the oral cavity during chewing) and firmness (which is the resistance to mastication) (Zerbini *et al.*, 1999). The texture of fruits is affected by cell anatomy, water content and the composition of cell walls. These variables are highly affected by ripening. Changes in turgor pressure and composition of the middle lamella and primary cell walls are the events that negatively affect the binding between cells during the ripening process. The resulting separation of cells is responsible for the loss of texture (Bartley and Knee, 1982). Firmness is the most important attribute of texture for fruit quality, tending to decrease during storage and marketing. It also determines the mechanical properties of fruits (Gunness *et al.*, 2009), in other words their ability to withstand postharvest handling and resistance to mechanical damage and the attack of insects and spoilage microorganisms. Consumers commonly prefer firm fruits (Bartolomé *et al.*, 1995), although variations may exist among consumers in different regions and cultures.

Sugars have high importance among the chemical components of tropical and subtropical fruits. They are implicated in fruit flavor and determine the caloric value of fruits (Montero *et al.*, 1996). Unripe fruits are rich in starch, which is progressively hydrolyzed during ripening; causing increases in the content of low molecular weight sugars such as glucose, fructose and sucrose. Climatic conditions, particularly temperature, can influence the accumulation of starch and its subsequent hydrolysis. For example, high summer temperatures favor the accumulation of starch and can delay the onset of hydrolysis (Kingston, 1992).

Tropical and subtropical fruits are also rich in organic acids, mainly citric, malic, ascorbic and tartaric acids. Ascorbic acid is particularly important for human health, exerting many protective effects (Davey *et al.*, 2000; Harrison and

May, 2009). The content of these acids is dynamic, being affected by several factors, mainly the ripening process and storage conditions. Generally, the content of organic acids decreases during ripening while the pH increases. Organic acids are involved in flavor, texture, pH and color of fruits, altering their sensorial quality (Montero *et al.*, 1996). The ratio of sugar to acids is commonly used as a ripening index for some tropical and subtropical fruits.

Volatile compounds are also important for the quality of tropical and subtropical fruits. These types of fruit release a wide range of volatile compounds, some of which are considered extremely important for fruit quality. The flavor of fruits depends on a combination of aroma and taste sensations. The small number of basic taste sensations (sweet, sour, salty, bitter, and umami) contrast with the thousands of different aroma sensations that can be detected by the human olfactory epithelium (Kays, 1991).

Safety is another important component of fruit quality. Value and acceptance of fruits depend on their levels of pesticide residues and load of pathogenic microorganisms. Organic cultivation confers value and quality to fruits because of the potential lack of residues of agrochemicals. The compliance of tropical and subtropical fruits with the statutory, compulsory or mandatory requirements of importing countries is an unavoidable and essential prerequisite. An increasing number of importing countries are demanding adoption by exporting countries of agreed inspection and examination procedures, and prescribed food safety systems, together with certification by governments of exporting countries that products are in compliance with mandatory import requirements. The procedures are becoming more and more restrictive, with the implication that the supply chain needs to be responsive and flexible to handle the restrictions imposed.

Postharvest techniques and treatments impact tropical and subtropical fruit appearance, texture, flavor and nutritional value, as well as safety and shelf life. It is common nowadays for horticultural crops, including tropical and subtropical fruits, to be transported long distances after harvest, not only within countries but across international borders. Unless great attention is paid to quality and the effects of handling and storage, long-distance transport may result in heavy losses. A demand for high quality, clean and safe farm produce is prompting the parties involved to become more concerned about improving postharvest quality and reducing losses. An expansion in the international fruit trade and growing consumer demand for high quality tropical and subtropical fruits have also created considerable interest and investment in the development of new or improved postharvest technologies.

Satisfactory application of postharvest technologies firstly requires the existence of adequate infrastructure and for producers and traders to have sufficient knowledge of the recommended procedures. Existing understanding of the effects of various postharvest technologies can be increased by research and development in prioritized areas. Generally, postharvest systems are made up of many interacting operations that involve handling of the produce from harvesting, packing, packaging, transportation, storage, and handling at destination. Operations that involve temperature and relative humidity control, together with

decay and insect control, are incorporated at various stages of the supply chain. Successful postharvest handling depends on the initial quality of the crop at harvest, including the degree of maturity. Careful handling to minimize mechanical damage, proper management of environmental conditions and good sanitation are also important. In addition, it must be recognized that different tropical and subtropical fruits sometimes require specific different postharvest handling and management technologies and treatments.

In order to increase tropical and subtropical fruit trade, effective sanitary requirements need to be established, efficiency increased and production be made more sustainable. It should be kept in mind that countries exporting tropical and subtropical fruits are at a different level of development and have different cultural and technical thresholds. Another vital issue that still needs to be resolved is the lack of awareness of consumers in developed countries of the different tropical and subtropical fruits and ways in which they can be stored, prepared and consumed at home. Therefore promotional efforts are also needed.

5.5.1 Methods for quality measurement

The physical and chemical characteristics of tropical and subtropical fruits vary constantly during development and ripening. Dramatic changes in structural carbohydrates, water, soluble sugar, starch, organic acids, proteins, amino acids, phenolic compounds, lipids, vitamins, pigments and aromatic compounds, among other constituents, occur during maturation and ripening of these fruits. These changes impact the quality attributes of fruits. Methods for quality evaluation can be classified as destructive or nondestructive, the former being most commonly used.

Destructive measurement of quality

Firmness is commonly measured by several types of equipment containing a head with a tip of known contact area. The tip penetrates the fruit to a certain depth and at a certain speed, and the firmness of the fruit is measured as the resistance to penetration of the tip. The force required to deform fruit can also be used as a measure of firmness. The two instruments used most frequently are the Magness-Taylor and Effegi testers. Determining firmness is usually a very simple test, involving the penetration of horticultural products under controlled conditions in order to identify characteristics such as the breaking point and consistency of the pulp. These objective measurements, of fruit firmness/softness, can be related to sensory perception. They can thus indicate stage of fruit maturation and ripening, and also indicate certain quality characteristics.

Starch from tropical and subtropical fruits is hydrolyzed to low-molecular weight sugars as ripening advances. Therefore starch content is used as a ripening and quality indicator. Starch content can be measured colorimetricaly. Often, fruit is cut and sprayed with an iodine solution. Starch reacts with iodine, causing the sprayed fruit surface to turn dark-purple in color. The disadvantage of this measurement is its subjectivity and the fact that the reactions between iodine and amylose and between iodine and amylopectin, the two components of starch, are

different. Individual determination of amylose and amylopectin in fruits gives more accurate information about the maturation and ripening stage (Fan *et al.*, 1995). Enzymatic determination of starch in fruits is also used, but this determination is expensive and time-consuming (Travers *et al.*, 2002).

The sugar content increases during fruit ripening and therefore is very often used for quality and ripening determination. Individual sugars can be measured by different analytical methods, such as the use of high pressure liquid chromatography (HPLC); however, this method is time-consuming and requires sophisticated equipment. The sugar content can be quickly determined by refractometers as the content of total soluble solids or °Brix. Refractometers measure the refraction (deflection) of light as a function of density of a solution. Increasing density of a substance (for example, when sugars are dissolved in water) increases the refractive index proportionately. The disadvantage of this measurement is that the values obtained represent not just sugars but all soluble solids, including salts, organic acids, proteins, etc. However, the main components of the juice of tropical and subtropical fruits are sugars.

The content of organic acids in tropical and subtropical fruits commonly decreases during ripening, and also indicates certain quality characteristics. Measurement of titratable acidity allows the rapid determination of the content of total organic acids in fruits. The method consists of titrating a known volume of fruit juice with aqueous sodium hydroxide (generally 0.1 N) until a pH of 8.2 is obtained using phenolphthalein as an indicator and a pH meter. Calculation of titratable acidity involves the volume and concentration of the used sodium hydroxide solution, the volume of fruit juice and a factor related to the main acid in the sample. Titrable acidity is expressed in terms of the main acid contained in the fruit sample.

The content of dry matter is defined as the mass of the sample that remains after the water is completely removed by any method (Lee, 1981). This method is commonly used to determine the maturity of some fruits such as avocado. This determination is relatively easy and can be carried out by drying a representative portion of the product in an oven at temperatures around 70°C or by drying in a microwave oven (Swarts, 1978). The specific gravity of fruits changes during ripening and therefore can be used as a ripening index for some fruits such as avocados, mangoes and guavas. Fruits that float are considered unripe.

Nondestructive measurements of quality

Measurements of tristimulus color taken by colorimeters or spectrophotometers are frequently used to assess the quality and maturity of tropical and subtropical fruits. Changes in chlorophyll fluorescence during fruit ripening can also be used to determine the harvest time and quality of some fruits. This is measured using a fluorometer, which sends out an excitation light and measures the emitted light; chlorophyll fluorescence is the difference between the maximum and minimum fluorescence. This technique is mainly used for apples although it could also be used for green tropical and subtropical fruits, especially for those that are commonly harvested at the mature green stage of ripening (DeEll *et al.*, 1995).

Near infrared (NIR) spectroscopy is an analytical technique that allows measurement of the organic components of foods. Monochromatic radiation is applied to the fruit, which is placed in a dark chamber. This chamber contains a detector, which collects the radiation reflected by the fruit. The wavelengths used in these measurements generally range from 600 and 2400 nm and the spectra obtained are analyzed by specialized software. This technology has been successfully used for the determination of firmness, acidity, color, sugars (total soluble solids) and dry matter content in several tropical and subtropical fruits. Schmilovitch *et al.* (2000) developed several models to predict the firmness, acidity and total soluble solids in 'Tommy Atkins' mangoes using infrared spectroscopy. A portable version of an NIR spectrophotometer has also been used to determine the dry matter content in mangoes (Sirinnapa *et al.*, 2003). Greensill and Newman (1999) classified pawpaw fruit in four stages of ripening using NIR measurements (720–815 nm). Other applications of this technology include the determination of internal darkening and the content of pigments in tropical and subtropical fruits (Slaughter, 1995; Hiromu, 1998).

The composition of fruits can be determined by measuring their delayed light emission. This technique involves measuring the electromagnetic energy emitted by a fruit after it has been subjected to electromagnetic energy in the optical range. The intensity of the emitted energy is generally in the range of nanowatts and persists for two to five seconds after interruption of the excitation beam of light. The energy emitted by the fruit is affected by the wavelength (usually in the visible range) and intensity of the excitation energy, the exposure of the fruit to the dark, thickness of the sample, excitation area, temperature and chlorophyll content (Gunasekaran, 1990). This technology can be used to measure the internal pigmentation of fruits, especially for those in which the chlorophyll and surface color are highly related to their degree of maturity and quality. Forbus *et al.* (1991a) also found that the delayed light emission of several cultivars of persimmon decreased as ripening advanced and that such decrease correlated with the changes in several quality attributes (firmness, chlorophyll, tristimulus color, and the content of β-carotene and total soluble solids). Similar results were reported for muskmelon (Forbus *et al.,* 1991b).

Internal characteristics of fruits and vegetables can be evaluated by magnetic resonance imaging (MRI). When high radio frequency waves are applied to a fruit in combination with a constant external magnetic field, some nuclei in the sample (^{1}H, ^{31}P and ^{13}C) accept energy. The nucleus will release energy when the application of radio frequency waves ends, generating a signal. The magnitude and shape of this signal can give information on the composition of the fruit. This technology has been applied to measure quality and ripening in some tropical and subtropical fruits, including cherimoya, mango, grapes and citrus (Coombe and Jones, 1983; Joyce *et al.*, 1993; Galed *et al.*, 2004; Goñi *et al.*, 2007).

The composition of tropical and subtropical fruits can also be assessed using ultrasound techniques. The attenuation of the ultrasonic waves applied depends on fruit composition. The separation, depth and angle of the transducers on the fruit as well as the applied frequency are crucial in this evaluation. Models to

predict the quality or ripening of fruits can be obtained from the recorded frequency spectra. Mizrach *et al.* (1997, 1999) developed models to predict the storage time, firmness, acidity and sugar content of mangoes based on their ultrasonic properties. Mizrach and Flitsanov (1999) established a method to evaluate the maturity and firmness of avocados using this technology. They found that the values of ultrasonic attenuation increased as the ripening stage of avocados advanced and that such values of attenuation were closely related to fruit firmness and oil content. Mizuno *et al.* (1991) found significant correlations between the ultrasonic properties of melons and their firmness. They concluded that the acoustic response of the melons can be used for assessment of their maturity.

Sonometry is helpful to determinate the quality and ripening stage of some tropical and subtropical fruits. This method consists in applying an acoustic or mechanical impulse to the fruit and measuring the resonant frequency, which will depend on fruit composition. The use of this technology in tropical and subtropical fruits is limited. Gatchalian *et al.* (1994) successfully determined the stage of ripening of coconuts by sonometry. The electrical properties of fruits and vegetables as a function of their composition can also be useful to determine their quality and ripening stage. However, this technique is not widely used for quality/ripening determination of fruits (Tollner *et al.*, 1992).

5.6 The cold chain

Temperature management (i.e. temperature control, refrigeration) is the most important method to maintain the quality of fresh tropical and subtropical fruits, reduce qualitative and quantitative losses, and extend their postharvest life. Temperatures lower or higher than those that are ideal for a certain fruit can result in deterioration and losses. Although cooling is very important for preservation of tropical and subtropical fruits, it is very important to avoid chilling injury, as most of these commodities are sensitive to this disorder. 'The cold chain' (Yahia, 2010) refers collectively to all management stages involving a reduction in temperature or temperature maintenance that perishables must pass through to ensure they reach the end-consumer in a safe, wholesome and high-quality state. Another definition of the cold chain is the progressive removal of heat from the produce, starting as soon as possible after harvest (Yahia, 2010). Cold chain management should start immediately after harvest and then continue throughout the handling chain. The cold chain consists of several components (Yahia, 2010); fast cooling (also called precooling), refrigeration/cold storage, refrigerated transport, refrigerated display during marketing and refrigerated holding (at home, in restaurants, etc.). Cold storage equipment is commonly different from the equipment required for precooling and refrigerated transport, as it is commonly not very effective for fast cooling. It is vital to avoid or reduce exposing fruit after harvest to the sun and to high temperatures for a prolonged period. Produce should be cooled down as quickly as possible to its lowest optimum storage and/or shipping temperature. Shading fruit in the field after harvest, during transport to

the packing facility, and during waiting at the packing facility, can be very helpful in this respect. In general, feasible methods to maintain an optimal cold chain are vital for adequate handling of tropical and subtropical fruits (Yahia, 2010) and there should not be significant delays in cooling produce, nor interruptions in the temperature management program.

Conduction and convection are the two main heat-transfer modes in fresh produce cooling to remove field heat immediately after harvest and during short- or long-term storage. In conduction, the field or generated heat is transferred within the produce to its coldest surface. In convection, the field or generated heat is lost from the surface of the produce through a cooling fluid which is moving air or water. It must be ensured that the cooling medium used is hygienic, safe and does not contaminate the produce. In the case of air-based cooling systems, which are very common for the cold storage of tropical and subtropical fruits (e.g., room cooling, forced-air cooling), it is essential to maintain a hygienic environment within the facility. Cooling methods using water (hydrocooling) carry the greatest risks of contaminating tropical and subtropical fruits, therefore the water quality should be checked regularly.

5.6.1 Fast cooling (precooling)

Fast cooling (also called precooling) is the removal of field heat from freshly harvested produce so as to reduce its temperature as close as possible to that of refrigerated storage or shipping. This slows down its metabolism and reduces deterioration prior to transport or storage (Brosnan and Sun, 2001; Majeed, 2005; Lawrence, 2007). Among the tropical and subtropical fruits that need to be precooled immediately after harvest are grapes, guavas, mangoes, papayas, avocados and pineapples. Many tropical and subtropical fruits are susceptible to chilling injury (CI), and therefore they need to be cooled according to their individual temperature requirements. Bananas require special ripening treatment and therefore are not commonly precooled.

A delay in cooling produce that will most probably reduce product quality will do so for four main reasons: (1) it allows respiration-associated normal metabolism to continue at high rates consuming sugars, acids, vitamins and other constituents; (2) it fosters weight loss; (3) it increases the level of decay; and (4) it increases susceptibly to ethylene damage. A 1-hour delay in cooling some fresh fruits after harvest can result in up to 10% quantitative and qualitative losses during marketing (Kader and Rolle, 2004). A rule of thumb is that a 1-hour delay in cooling reduces a product's shelf life by one day (Hugh and Fraser, 1998). It was stated (Sullivan *et al.*, 1996) that when it comes to produce quality, every minute counts and that precooling is among the most cost-effective and efficient quality preservation methods available to commercial crop producers. The effect of delayed cooling on the quality of tropical and subtropical fruit has been well demonstrated in the literature for many fruits. Crisosto *et al.* (2001) reported that table grapes suffer water loss and stem browning following cooling delays. One of the postharvest factors that influence the shelf life of citrus fruits is the delay between harvest and

cooling (Kader, 2002). Precooling of mango fruit immediately after harvest delays ripening without any deterioration in fruit quality (Puttaraju and Reddy, 1997; Yahia *et al.*, 2006). For figs, precooling immediately after harvest decreased the weight loss during storage at 0°C and doubled the storage life to four weeks (Celikel and Karacali, 1997). Table 5.3 shows the maximum cooling delay that is tolerable after harvest for some tropical and subtropical fruits. Most tropical and subtropical fruits need to be pre-cooled as soon as possible, no later than one day after harvest.

Cooling in general introduces a single controlled handling step at the postharvest stage for tropical and subtropical fruits and may be accomplished using several different techniques. Different precooling techniques include: room cooling, forced-air cooling, hydrocooling, contact icing, and vacuum cooling. All these methods involve the transfer of heat from the commodity to a cooling medium, such as water, air or ice. The choice of cooling methods for tropical and subtropical fruits depends on the following factors.

Type of fruit: tropical and subtropical fruits vary in their cooling requirements. For example, grapes and fresh dates require rapid cooling after harvest to

Table 5.3 Allowable maximum cooling delays for some tropical and subtropical fruits. The delay time is only an estimate. Temperature at harvest may modify the actual allowable cooling delay (adapted from Thompson *et al.*, 2001)

Fruit	Allowable delay (hours)	Disadvantage of cooling delay	Advantage of cooling delay	Comments
Avocado	12	Premature ripening with high fruit maturity	None	Less delay is allowable at high temp. and with high fruit maturity
Cantaloupe	8	Water loss	None	
Grape	4.8 h at < 29°C	Shrivelling, stem browning and increased decay	None	Treat with SO_2 within 12 h
Grapefruit	24	Increased water loss, rind defects and decay	None	Treat for decay within 24 h
Honeydew melon	16	Pulp softening and premature ripening	None	
Kiwifruit	6	Water loss and softening	Wound curing at 18°C for 48 h reduce Botrytis	
Lemon	24	Increased decay	None	Treat for decay within 24 h
Mandarin	8	Increased rind defects and decay	None	Treat for decay within 24 h
Orange	16	Increased rind defects and decay	None	Treat for decay within 24 h
Pomegranate	16	Water loss	None	

near-freezing temperatures (e.g., 0°C), whereas bananas, mangoes and honeydew melons are sensitive to CI and do not require such a rapid rate of cooling and would be damaged by such low temperatures. Similarly, because of disease and cross contamination problems that can be caused by wetting of certain fruits, hydrocooling or icing must be avoided for some commodities.

Packaging needs: the package type and design has a big impact and subsequent effect on the method and rate of cooling of tropical and subtropical fruits. As an example for pineapple, a forced air cooling system is more efficient compared to other precooling methods, but this requires a specially designed unit and compatible packaging (Majeed, 2005). Also, the need to transport tropical and subtropical fruits by sea imposes certain types of packaging (FAO, 2005). The use of CSIRO packaging that maintains high humidity while minimizing the risk of free water on the fruit has been suggested for use with litchi (Olesen *et al.*, 2003).

Capacity design: if the turnover of produce to be cooled per day or per hour is large, it may be necessary to use a faster commercial method than would be used for smaller volumes to absorb the expected bigger heat load. For dates as an example, evaporative cooling is designed to handle small volumes but it is very limited in its ability to lower the temperature of the dates beyond the wet bulb temperature (Alhamdan and Al-Helal, 2008).

Capital investment and running cost: capital investment and running costs vary significantly among different pre-cooling methods. As an added value service, the expense of the selected technique must be covered through selling prices or other economic benefits (Elansari, 2009). Three cooling methods are commonly applied on tropical and subtropical fruits – forced air cooling, room cooling and hydrocooling – in addition to which evaporative cooling is a cheaper alternative. The most common method being utilized for precooling of fresh tropical and subtropical fruits is forced air-cooling, which is used for a wide range of commodities (Guillou, 1960; Parsons *et al.*, 1972; Thompson *et al.*, 1998).

Room cooling

Room cooling is widely used and is desirable for fruits that are marketed quickly after harvest, have a long shelf life, are stored without packing and require only mild precooling after harvest. Packaged fruits to be cooled by this method must be tolerant of the slow removal of heat because heat transfer occurs slowly by conduction through the package walls. For pineapple, citrus and breadfruit, room cooling is considered adequate (Majeed, 2005). Room cooling consists of placing the fruit containers in a refrigerated room. Cold air from an evaporator moves horizontally across the top of the room, then downwards, passing slowly through the containers, therefore cooling the fruit, and then back to the evaporator. The air speed necessary for room cooling is generally greater than that required for storage. Unpacked fruits cool more quickly than packaged fruits, especially if a good air flow is used. The air flow required for room cooling is at least 60–120 m min^{-1}. The advantage of this precooling method is that the fruit can be precooled and stored in the same room. The main disadvantage of room cooling is the slow cooling rate particularly if stacking and spacing are not adequate to allow free and

uniform air flow or if the refrigeration capacity is inadequate. Other disadvantages of this method are that more space inside the room is required and it can cause an increased loss of water in the fruit. Room cooling can also be used for refrigerated storage (see section on 'Mechanically refrigerated storage' in 5.6.2, below). However, it must be remembered that precooling and storage are two separate processes that have vastly different requirements. In other words, the specific requirements for achieving fast, uniform cooling must be considered independently of the cold storage requirements.

Forced-air cooling

Forced-air (pressure) cooling (Figure 5.2) was developed as a rapid substitute or improvement for room cooling and can cool fruit in 20% of the time required by room cooling (i.e., 4–6 hours) (Taverner, 2007). Pressure cooling or forced air cooling is being successfully utilized for many tropical and subtropical fruits, such as avocados in several countries (Ginsberg, 1985), speeding up the rate of cooling and ensuring an even temperature distribution throughout shipping and storage. For mangoes it is also utilized efficiently, lowering their temperature rapidly from 25–35 to 11°C in less than 120 minutes (Shoker *et al.*, 1994). Forced air cooling for pineapple can also be effective, but requires the use of specially designed compatible packaging. The recommended temperature to which to precool pineapples is a minimum of 8°C (Majeed, 2005). Forced-air cooled litchi cultivars 'Kwai May Pink' and 'Wai Chee' retained their color better and decayed less compared to water-cooled fruit, but lost more water (Olesen *et al.*, 2003).

Fig. 5.2 Forced-air pre-cooling units in a refrigerated room.

'Hong Huay' (syn. 'Tai So') packed loose in side-vented cartons were cooled from 26°C to 6°C in 70 minutes using 3–5°C air moving at 2 m s^{-1} (Ketsa and Leelawatana, 1992), and 'Mauritius' (syn. 'Tai So') packed in commercial shipping cartons were cooled from 25–27°C to 3°C in one hour using 3°C air and a 2.5 cm static pressure difference. For litchi fruit packed into plastic bags, Bagshaw *et al.* (1994) stated that forced-air cooling takes at least 12 hours.

A portable forced-air cooler that can be constructed and installed inside an existing cold room using a canvas or polyethylene sheet (Kitinoja and Kader, 2003) is shown in Figure 5.3. The sheet is rolled over the top and down the back of the boxes to the floor, sealing off the unit and forcing air to be pulled through the vents. As a basic requirement, the vent area should be at least five per cent of the surface area of the cartons stacked against the cooler. Certain calculations should be carried out in order to make sure that the available cooling capacity is sufficient to pull down the product heat load needed for precooling. Talbot and Flitecher (1993) utilized a trial mounted cooling unit equipped with two 10.5 kW packaged air conditioner units, a high pressure blower and a self constructed cooling chamber as a mobile precooling facility to cool a pallet of containerized product. Boyette and Rohrbach (1990) promoted a similar idea that applies 2 to 3 tons of refrigeration air conditioning with an integrated fan unit to supply the cooled air through a length of insulated flexible duct which holds the product being precooled. Elansari (2008) developed a portable precooling unit adapted to treating dates in southern Egypt. The unit uses hermetic scroll compressors which

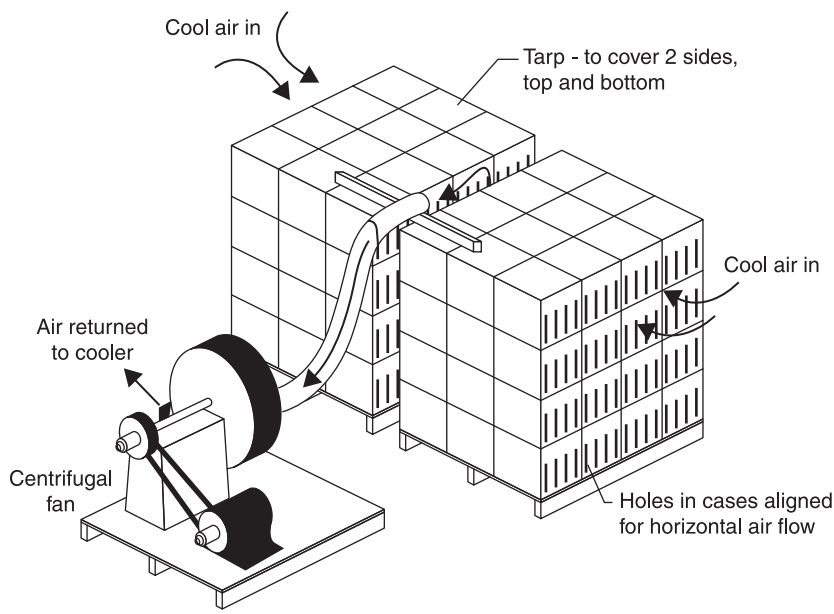

Fig. 5.3 Supplementary system for forced horizontal air flow that can be installed inside an existing cold store.

are cheap, compact and reliable and are tolerant to some liquid return and contamination. This is very important for the precooling process since its mode of heat transfer is considered a transient rather than a steady state process. The scroll compressor is quieter and vibrates less than reciprocating types which is an important feature for a portable precooling system. A similar unit was used successfully in the tropical regions of Australia and New Zealand.

Hydrocooling

Clean and sanitized water is utilized as the cooling medium in hydrocooling (or shower or immersion systems) for fruits that tolerate water contact, either unpackaged or packaged, in water-resistant containers in order to avoid water absorption that leads to their collapse. Hydrocooling was reported to be used for papayas and pineapple, but is not appropriate for some fruits where it leads to skin browning such as in breadfruit (Noomhorm *et al.*, 1991; Majeed, 2005). An economical batch-type hydrocooling system using crushed ice mixed with water was developed and tested (Elansari, 2008). The hydrocooler effectively removed field heat from 'Barhee' dates. The simplest version of a hydrocooler is a tank of cold water in which produce is immersed. A batch-type hydrocooler can be constructed to hold an entire pallet-load of produce. It was found that hydrocooling is a very effective method to pre-cool dates in order to extend their postharvest life and maintain their quality during distribution. 'Barhee' dates at the Khalal stage should be pre-cooled to 0°C immediately after harvest to achieve the longest possible storage period (Al-Redhaiman, 2004). Continuous shower hydrocooling at 4–5°C for mango fruit for 30 minutes was reported to be effective in reducing the fruit temperature by 16°C and significantly retarded ripening, thereby extending the storage life by three to four days, and retaining fruit quality (Puttaraju and Reddy, 1997). Noomhorm *et al.* (1991) designed a precooling apparatus to evaluate different precooling techniques applied to tropical fruits such as mango and papaya. They reported that mango and papaya could best be precooled by hydrocooling and forced-air cooling. Litchi fruit can be cooled more quickly by hydrocooling than by air cooling but the benefits are ambiguous (Olesen, 2003). Ketsa and Leelawatana (1992) found that hydrocooled fruit was less susceptible to browning than control fruit or forced-air cooled fruit. Pornchaloempong *et al.* (1997) confirmed a similar effect as well as no significant difference in the prevalence of decay among the different treatments. Coates (1994) found significantly higher decay in hydrocooled than in control fruit. Olesen *et al.* (2003) stated that the core temperature of water-cooled fruit took ten minutes to cool from 28°C to 5°C in water at 2°C. The core temperature of the forced-air cooled fruit took three hours to cool from 22°C to 5°C in still air at 2°C.

Alternative, inexpensive, technology

Because of the limited temperature control facilities currently available in the tropical and subtropical fruits supply chain in developing countries (Yahia, 2010; 2005; Yahia *et al.*, 2004), alternative inexpensive technologies should be explored. Evaporative cooling has been applied to tropical fruits in different regions. Roy

and Pal (1989) developed a zero-energy chamber using locally available material in India. The chamber was designed for on-farm use, operated by evaporative cooling, and was constructed from double brick with sand-filled cavity walls. The shelf life of tropical fruits kept in the chamber increased by two to 14 days (15–27% increase) as compared to storage at room temperature. Also in India, a portable modified evaporative cooler called a two-stage evaporative cooler (TSEC) has been developed to improve the efficiency of evaporative cooling for high humidity and low-temperature air conditioning of tropical fruits (Jain, 2007). A similar concept was reported by Kitinoja and Kader (2003) where a low cost zero energy cool chamber was developed at IARI, New Delhi, using locally available raw materials such as bricks, sand, bamboo, dry grass and jute cloth. The performance of these cool chambers at different locations was found to be satisfactory for short-term storage of mangoes. The major advantage of the use of these cool chamber storage facilities was the maintenance of fruit firmness by lowering the physiological loss in weight and other metabolic processes. Eventually the shelf life of mature green mangoes was increased by three to four days using cool chamber storage as compared to storage under ambient conditions. Alhamdan and Al-Helal (2008) used an evaporative cooler unit that was operated automatically by controlling ventilation and rates of water addition to the cooling pads based on inside temperature and relative humidity in order to cool down dates in Saudi Arabia for long-term storage, and date quality was observed for a period of six months. The results obtained indicated the possibility of using evaporative cooling of dates in remote producing areas. A porous evaporative cooler with prospects for use for short-term preservation of tropical and subtropical fruits soon after harvest was designed for the tropical environment of Nigeria and made out of locally available materials (Anyanwu, 2004). With a total storage space of 0.014 m^3, it consisted of a cubical shaped porous clay container located inside another clay container. Results revealed that the cooler temperature depression from ambient air temperature varied from 0.1–12°C. The results also illustrated superior performance of the cooler over open air preservation of vegetables soon after harvest during the diurnal operations. In Ethiopia an evaporative cooler was evaluated over a storage period of 28 days. It maintained the temperature between 14.3 and 19.3°C and the relative humidity between 70.2% and 82.4% during the storage period (Tefera *et al.*, 2006). The shelf life of mangoes kept in the evaporative cooling unit increased from three to 28 days, compared to storage at ambient conditions.

5.6.2 Storage

Ventilated storage

As already mentioned, it is essential to remove the 'field heat' from tropical and subtropical fruits before shipment, storage or marketing. Naturally ventilated structures are still used in some countries for storage of a variety of crops. In ventilated storage, controlled ambient air is applied for the cooling of the produce and maintenance of lower temperatures. Any type of building can be used

provided that it allows the free circulation of air through the structure and its contents. The capital investment and operating costs needed for such facilities are much lower than for mechanical refrigerated stores and the method is suitable for some fruits marketed locally or stored for a very short period. This method requires continuous inspection and sorting to minimize losses. If feasible, insulated walls are to be used to provide better insulation, otherwise the structure needs to be shaded by trees or alternatively a proper covering material is to be used to provide insulation from the heat of the sun. In order to get the most out of the non-refrigerated storage (ventilated storage), the store should be clean and free of infection. Also, harvesting should be carried out at the optimum maturity stage and there should be no delay between harvest and storage.

Figure 5.4 presents a model for a ventilated store for fruits. This unit was officially approved as the standard model for farm-level storehouses by the Ministry of Construction in Korea in 1983 (Lee, 1981). The narrow air inlets are at the base of the building in order for the air to gain velocity and it rises up via density differences. The floor is perforated in order to allow free movement of air upward. Another fan is located on the top to exhaust the hot air carrying heat and moisture outside. The entire building is set below ground level, taking advantage of the cooling properties of soil. The main concern with this method is the relative humidity of the ventilating air which is a function of many environmental factors described in the psychometric chart. Such environmental factors are very difficult to control, and therefore large losses occur when the relative humidity values are lower than the equilibrium value. On the contrary, when the relative humidity is larger than the equilibrium value, an increase in the ventilating airflow rate reduces

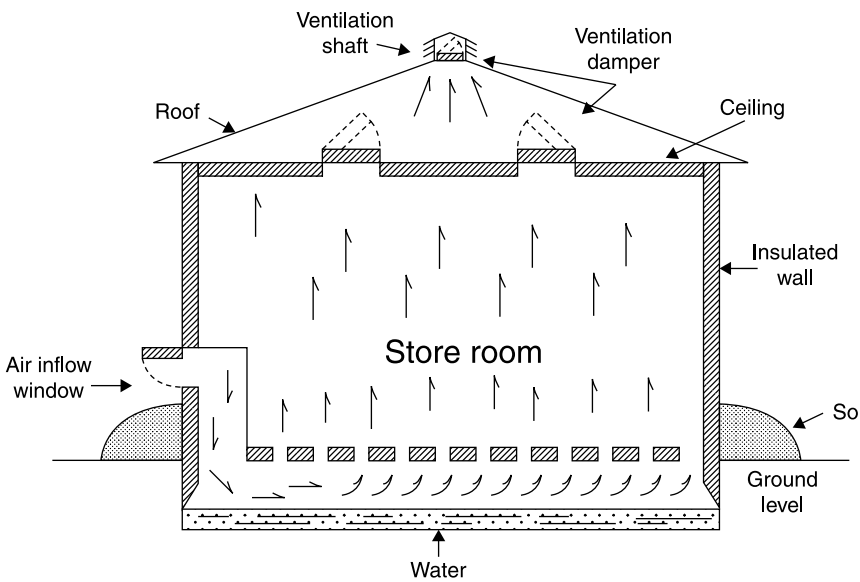

Fig. 5.4 A model for a ventilated store.

product losses during storage (Tashtoush, 2000). In general, fruits are very susceptible to moisture loss in storage. In an unfavorable environment they can suffer damaging water loss in a few hours. Under natural ventilation, dates' texture became hard and they deteriorated in quality compared to those stored in mechanically refrigerated storage (Alhamdan and Al-Helal, 2008).

Mechanically refrigerated storage

Mechanically refrigerated storage is a thermally insulated space, equipped with refrigeration units that allow cold air to circulate and can be used as both a storage method and a precooling method. However, it is important to note that precooling and refrigerated storage are two different stages in the postharvest cold chain that have very different requirements. Although it is simple, it is considered to be the slowest cooling method, as the bulk or containerized commodity has to be placed in a refrigerated room for several hours or days (Talbot and Chau, 2002). In mechanically refrigerated storage, cold air from evaporator coils gradually cools the product by circulating around it. There are many ways to circulate the cold air inside a room, depending on the type of the evaporative coil. For tropical and subtropical fruits, the method most commonly applied to cool a warehouse space is to install ceiling air coolers. This is an economical design that has a short delivery period and is very popular (Jackmann, 2007), however its application is limited to smaller warehouses. In this design (which is the same as that described in 'Room cooling' in section 5.6.1 above), cooler air is blown horizontally just underneath the ceiling, sweeping over and down through the stacks of the fruit placed on the floor, and then moves horizontally into the return vent to be recycled. Product loads must be stacked in ways that enable adequate cold air circulation, in order to remove the heat from the produce.

Cold stores should be designed to maintain a high level of relative humidity around the product to reduce water loss. Cold stores for mechanically refrigerated storage usually include commercial condensing units that work with direct expansion (DX), although this is not the best choice for storage of perishables (Elansari, 2009). A problem associated with this kind of cold store is the choice of evaporator coils, which usually have a small surface area and large delta T that increase the water loss from the produce. Delta T is the temperature difference between the refrigerating surface (coil evaporators) and the storage air. The water-holding capacity of air decreases as the temperature falls. The temperature of the refrigeration coil can reach the dew point, the temperature at which air can carry no more moisture and water vapor condenses out onto the coil. A difference in vapor pressure caused by varying temperatures in the cold store can also cause water vapor movement from or to fruits in contact with the air. Selecting a coil with a sufficiently large cooling surface so that the temperature difference between the refrigerating surface and the storage air is as small as possible is helpful to reduce water loss. The coil is the coldest element in the room, hence by keeping the temperature difference between coil and storage air small, the dew point and dry bulb temperature (air temperature) will be about the same and the air can carry a lot of water. Good thermal insulation, a complete vapor barrier on the warm side

of the insulation, and properly located controls are important factors that help in maintaining a good level of RH inside the cold store. Other design considerations include durable floors, adequate and well-positioned doors for loading and unloading, and a room of sufficiently large size for expected future needs.

Small room coolers for small-scale operations can be purchased in prefabricated form, or used refrigerated transport vehicles (e.g. trailers or reefer containers) can be purchased. However, refrigerated transport trailers are not designed with sufficient refrigeration capacity to rapidly precool produce. If rapid cooling is needed, extra refrigeration capacity must be considered. Moreover, transportation vehicles are too narrow for the frequent product loading and unloading needed in a precooling facility. An independent specially constructed room is much more convenient for precooling operations.

Temperature control and fluctuation remain the major problems in the handling of fresh horticultural crops in developing countries (Yahia, 2008). Temperature during storage must be stable as changes may affect respiration and hence marketable quality of tropical and subtropical fruits. Precise temperature and relative humidity monitoring are essential to guarantee the optimum conditions during cooling and storage. Precision temperature monitoring instruments, including time-temperature monitors, are increasingly being employed in cooling and storage facilities. Storage rooms should not be loaded beyond their capacity for proper cooling to be achieved. Fruit temperature rather than air temperature should be measured in these facilities. Table 5.4 indicates the temperature and relative humidities recommended for several tropical and subtropical fruits. A high relative humidity of 85 to 90% is recommended for most tropical and subtropical fruits to delay wilting from moisture loss.

Chilling injury (CI)

Many sub-tropical and tropical species are sensitive to CI at temperatures below about 15°C (Brown, 1985), although some may be stored safely as low as 0–5°C if cooled soon after harvest (Tan, 2006). CI manifests as a variety of symptoms including surface and internal discoloration, pitting, water soaking, failure to ripen, uneven ripening, development of off flavors and heightened susceptibility to pathogen attack (Kader and Rolle, 2004). Table 5.5 describes the symptoms of CI in some tropical and subtropical fruits.

For example, mango fruit is susceptible to chilling injury (Abou Aziz *et al.*, 1976; Yahia *et al.*, 2006), and therefore when stored below a certain critical temperature it would suffer stress resultant symptoms manifested as brown patches on the skin, and as the severity of the injury progresses discoloration of the pulp and stone is expressed (Yahia *et al.*, 2006; Ravindra and Goswami, 2007). The critical temperature for mangoes is influenced by factors such as the variety and stage of fruit maturity, and is reported to be in the range of 8–12°C (Murray and Georget, 1994; Bender *et al.*, 2000; Kader, 2002; Yahia *et al.*, 2006). Avocado, like many other sub-tropical fruits, is subject to CI when held at temperatures approaching 0°C. Wardlow (1939) determined that 5.5°C was a good compromise temperature for 'Hass' avocados as it provided three weeks' storage with an acceptable quality.

Table 5.4 Recommended conditions of some tropical and subtropical fruits (adapted from Kader, 2002)

Fruit	Scientific name	Holding temp.°C	RH %	Highest freezing temp.°C	Ethylene production*	Sensitivity to ethylene*	Postharvest life
Acerola	*Malpighia glabra*	0	85–95	−1.4			6–8 weeks
Avocado: Fuchs, Pollock	*Persea Americana*	13	85–90	−0.9	H	H	2 weeks
Banana	*Musa paradisiaca var. sapientum*	13–15	90–95	−0.8	M	H	1–4 weeks
Black sapote	*Diospyrus ebenaster*	13–15	85–90	−2.3			2–3 weeks
Breadfruit	*Artocarpus altilis*	13–15	85–90				2–4 weeks
Cactus pear fruit	*Opuntia spps*	5	85–90	−1.8	VL	M	2–4 weeks
Carambola (starfruit)	*Averrhoa carambola*	9–10	85–90	−1.2			3–4 weeks
Cherimoya (custard apple)	*Annona cherimola*	13	90–95	−2.2	H	H	2–4 weeks
Coconut	*Cocos nucifera*	0–2	85–90	−0.9			4–6 weeks
Date	*Phoenix dactylifera*	−18–0	75	−15.7	VL	L	6–12 months
Durian	*Durio zibethinus*	4–6	85–90				6–8 weeks
Feijoa, pineapple guava	*Feijoa sellowiana*	5–10	90		M	L	2–3 weeks
Fig, fresh	*Ficus carica*	0–5–0	85–90	−2.4	M	L	7–10 days
Grape	*Vitis vinifera*	0–5–0	90–95	−2.7	VL	L	1–6 months
Guava	*Psidium guajava*	5–10	90		L	M	2–3 weeks
Jaboticaba	*Myrciaria cauliflora, Eugenia cauliflora*	13–15	90–95				2–3 days
Jackfruit	*Artocarpus heterophyllus*	13	85–90		M	M	2–6 weeks
Kiwifruit	*Actinidia chinensis*	0	90–95	−0.9	L	H	3–5 months
Langsat Lanzone	*Aglaia sp.; Lansium sp.*	11–14	85–90				2 weeks
Litchi	*Litchi chinensis*	1–2	90–95		M	M	3–5 weeks
Longan	*Dimocarpus longan, Euphria longan*	4–7	90–95	−2.4			2–4 weeks
Mamey sapote	*Calocarpum mammosum*	13–15	90–95		H	H	2–3 weeks
Mango	*Mangifera indica*	13	85–90	− 1.4	M	M	2–3 weeks

Continued

Table 5.4 Continued

Fruit	Scientific name	Holding temp.°C	RH %	Highest freezing temp.°C	Ethylene production*	Sensitivity to ethylene*	Postharvest life
Mangosteen	*Garcinia mangostana*	13	85–90		M	A	2–4 weeks
Olives, fresh	*Olea europea*	5–10	85–90	−1.4	L	M	4–6 weeks
Papaya	*Carica papaya*	7–13	85–90				1–3 weeks
Passion fruit	*Passiflora spp.*	10	85–90		VH	M	3–4 weeks
Persimmons, Kaki	*Diospyros kaki*	0	90–95	−2.2	L	H	1–3 months
Pineapple	*Ananas comosus*	7–13	85–90	− 1.1	L	L	2–4 weeks
Plantain	*Musa paradisiaca var. Paradisiaca*	13–15	90–95	−0.8	L	H	1–5 weeks
Pomegranate	*Punica granatum*	5–7.2	90–95	−3	VL	L	2–3 months
Rambutan	*Nephelium lappaceum*	12	90–95		H	H	1–3 weeks
Sapodilla chicosapote	*Achras sapota*	15–20	85–90		H	H	2 weeks
Soursop	*Annona muricata*	13	85–90				1–2 weeks
Spondias, Mombin, Wi apple, Jobo Hogplum	*Spondias spp.*	13	85–90				1–2 weeks
Sugar apple, Custard apple	*Annona squamosa; Annona spp.*	7	85–90		H	H	4 weeks
Tomatillo, Husk tomato	*Physalis ixocarpa*	7–13	85–90		VL	M	3 weeks
White sapote	*Casimiroa edulis*	20	85–90	− 2.0			2–3 weeks

*L: low, VL: very low, M: medium, H: high, VH: very high

Table 5.5 Symptoms of chilling injury in some tropical and subtropical fruits

Fruit	Approximate temperature at which chilling starts (°C)	Symptoms
Avocado		
West Indian	10.0–11.1	Pitting, browning of pulp near the seed on in tissue midway between the seed and the skin, failure to soften when transferred to a higher temperature, off-flavor, vascular strands develop a brownish appearance
Other cultivars	4.4–6.1	
Banana	12.8	Subepidermal brown streaking, clear latex, loss of flavor, delayed ripening, hardening of central placenta, building up of tannins, skin discoloration, slow starch to sugar conversion, decrease in ascorbic acid level, watery dark green patches on the skin, bitterness of fingers
Grapefruit	Variable	Pitting of flavedo, oil glands rarely elevated above the rest of depressed areas, reddish brown pits (red blotch), browning of membrane or carpellary walls between the segments
Litchi	Variable	Dull skin
Mango	4.4	Lack of sweetness, dull skins, improper ripening, brown patches
Papaya	6.1	Impaired ripening, pitting of skin, water-soaking of flesh, failure to hydrolize sucrose to reducing sugar
Pineapple	6.1	Impaired ripening, brown or dull shell color, water-soaked flesh, wilting of crown or is easily pulled off, green spotting, failure to develop good flavor in the flesh

Because of the susceptibility of many tropical fruits and some subtropical fruit to CI, refrigeration cannot be used to full capacity. In other words, temperature must be held close to a safe limit, to avoid deterioration in fruit quality (Spalding and Reeder, 1974). Table 5.4 indicates the lowest safe temperature for some tropical and subtropical fruits.

5.6.3 Transport

Tropical and subtropical fruits are transported to diverse destinations by land, air and water. Those that need to be packed in a packing facility are transported there from the field (see Plate VI in the color section between pages 238 and 239). Mechanical damage, especially due to bruising, during transport from the field to the packing facility is usually a major concern. Deterioration at this stage can also be caused by exposure to the sun either while waiting to be transported or during transport if the product is not adequately covered. The warming up of the load or

parts of the load of the fruit, especially due to restricted air flow through the load, can increase fruit temperature very significantly and can also increase the accumulation of gases such as ethylene and CO_2, leading to significant deterioration. Excessive air flow through the load during transport can increase moisture loss and deterioration. It is difficult to control the temperature of air shipments, but produce shipped by air should be precooled and adequately protected from sun, and adequately packaged and covered.

Land transport

Currently, truck transportation (Fig. 5.5) is the most important mode of transport for tropical and subtropical fruits. Bulk transportation of citrus, bananas, watermelons, melons and pineapples is commonly done by trucks, especially in developing countries. Generally truck transportation is flexible, suitable for transportation over short distance and offers the possibility of door-to-door fruit distribution. Unrefrigerated trucks can be used to transport fruits to nearby locations, but the transportation time must not exceed ten hours at ambient temperatures and the trucks must be well ventilated. Trucks transporting tropical and subtropical fruits to distant locations must be refrigerated. Rail transportation of some tropical and subtropical fruits is still practiced, although to a very limited extent. Refrigeration facilities are available for rail transportation. The advantage of this transportation mode is its low cost and the high volume of fruits that can be transported. The velocity of rail transportation is acceptable, especially for long trips. Its main disadvantage, however, is the excessive time taken for loading and unloading of rail cars.

Fig. 5.5 Refrigerated truck.

Marine transport

Marine shipping is relatively inexpensive, especially compared to air transport, and is therefore becoming important for transportation of tropical and subtropical fruits. Internal transportation of tropical and subtropical fruits by boat is common in countries such as Japan, Thailand and the Philippines. Bananas, mangoes, citrus, pineapples, papayas and avocados are examples of many tropical and subtropical fruits commonly transported by sea. Modified and controlled atmospheres and refrigeration facilities are available for marine transportation. Marine transport of tropical and subtropical fruits can be carried out under conventional ventilation when the trip is shorter than 12 hours.

Air transport

Air transport of tropical and subtropical fruits allows sale of fresh, very perishable fruits in distant markets, but this mode of transportation can only be used for fruits of high commercial value. Its disadvantages are the high cost per kg of fruit, the interruption of scheduled flights due to bad weather, difficulties in managing the fruit containers in airport terminals, long distances between airport terminals and the destination/origin of the fruit, uncertainty about the available space on regular scheduled flights, and potential fruit damage due to inadequate handling. This transportation mode offers the possibility of commercialization of tree-ripened fruit, such as mango, avocado and papaya, in distant markets.

Relative humidity and its control

Fresh tropical and subtropical fruits contain large amounts of water (85–90% or more). Water loss and dehydration starts immediately after harvest, causing shriveling and softening, and may promote decay and some physiological disorders. These effects decrease the quality of fruits and reduce their economic value. Most fruits are considered unmarketable when they lose 5–10% of their total moisture content. Moisture loss in tropical and subtropical fruits can be reduced by maintaining the fruit in an atmosphere with a high relative humidity (RH), reducing the storage temperature, providing the exact amount of air flow required to remove heat, and coating or packaging the fruit. Most vehicles used to transport fruits are not equipped with devices to monitor and regulate RH. Refrigerated vehicles commonly use direct expansion cooling units, which reduce the moisture content in the atmosphere during the defrost cycle. Temperature variations during transport also remove moisture from the atmosphere. It is recommended that transport containers and storage rooms of tropical and subtropical fruits be equipped with humidifiers to maintain high RH (85–90%) in order to avoid fruit dehydration.

Grouping of compatible fruits during storage and shipping (mixed loads)

Shippers commonly transport several types of tropical and subtropical fruit in the same vehicle and store them in the same room. Each of these types of fruit may require storage at different temperatures, relative humidities and atmospheres, among other requirements. In particular, mixing different tropical and subtropical fruit together during storage or shipping may result in the mixing

Table 5.6 Some tropical and subtropical fruits that can be safely mixed during storage or shipping

Conditions	Fruits
0–2°C, 90–95% relative humidity. Several are ethylene producers	Cashew apple, coconuts, dates, figs, grapes (without sulfur dioxide), longan, loquat, litchi, persimmons, pomegranates
0–2°C, 90–100% relative humidity. Many products of this group are sensitive to ethylene	Grapes (without sulfur dioxide), kiwifruit, pomegranates
5°C, 90–95% relative humidity	Cactus (prickly) pears, caimito, cantaloupes, clementines, lemons, litchis, mandarins, oranges, pepino, tamarillo, tangelos, tangerines, ugli fruit
7–10°C, 85–90% relative humidity, sensitive to chilling injury, many are sensitive to ethylene	Cactus pears, caimito, durian, feijoa, granadilla, grapefruit, guava, lemon, lime, mandarin, okra, olive, orange, passionfruit, pepino, pineapple, pomelo, sugar apple, tamarind, tangelo, tangerine, tomatillo
13–15°C, 85–90% relative humidity, chilling sensitive, many produce high concentration of ethylene	Atemoya, avocados (certain cultivars), babaco, bananas, bitter melon, black sapote, boniato, breadfruit, carambola, cherimoya, coconuts, feijoa, granadilla, grapefruit, guava, jaboticaba, jackfruit, langsat, lemons, limes, mamey, mangoes, mangosteen, melons (except cantaloupes), papayas, passionfruit, pineapple, plantain, pumpkin, rambutan, sapodilla, sapote, soursop, sugar apple, tomatillos
18–21°C, 85–90% relative humidity	Watermelon, white sapote

of chilling-sensitive with non-chilling sensitive commodities or ethylene-producing with ethylene-sensitive commodities, resulting in injuries to some of them. Odors produced by certain types of fruit and vegetables may also accumulate and be absorbed by other fruits. For example, citrus fruits easily absorb the aroma of onions and sprouts. Avocados, bananas and peaches produce high levels of ethylene, which can promote ripening and may cause softening and even decay in some other fruits. Table 5.6 indicates the types of fruits that can be safely mixed during storage or shipping of tropical and subtropical fruits.

5.6.4 Supplements to temperature management

There is no substitute for refrigeration and maintenance of the cold chain (Yahia, 2010) to preserve fresh tropical and subtropical fruit quality, but there are several supplementary techniques and treatments that can also be used. These include sorting, use of chemicals (such as pesticides, growth regulators, and waxes), film wrapping, heat (hot water or hot air for insect or decay control) treatments, irradiation, modified and controlled atmospheres (Yahia *et al.*, 2004).

5.7 Centralized packing operations

Centralized packing in a packinghouse (Fig. 5.6) is more comfortable for workers than packaging in the field and therefore packing efficiency is usually higher. Under this system, it is also possible to pack different grades of fruit and apply different treatments (such as washing, waxing, heat, chemicals). A few tropical fruits are packed in the field (Fig. 5.7).

Fig. 5.6 Banana packinghouse (courtesy of Prof. Adel A. Kader).

Fig. 5.7 Banana field handling operation in Malaysia.

5.7.1 Cleaning, washing and sanitation

Several tropical and subtropical fruits are washed before packing (see Plate VII in the color section between pages 238 and 239). Washing eliminates soil and other materials, reduces fruit temperature, and can be the vehicle for the application of other treatments such as chemicals used for sanitation. Oranges are sometimes washed with a high pressure spray to remove scale insects and surface mold. Chlorine, as liquid sodium hypochlorite or chlorine gas, at a concentration of 50 to 200 ppm, in water at a pH of 6.5 to 7.5 is a commonly used sanitation treatment for many tropical and subtropical fruits. The washing water should be changed frequently depending on fruit volume and rate of contamination, and chlorine activity should be checked frequently. Water ozonization is an alternative to the use of chlorine.

5.7.2 Sizing and grading

Tropical and subtropical fruit need to be graded by size to ensure that fruit of uniform size are packed together. Sizing of mangos treated with hot water as an insect quarantine treatment is also required so that the fruit is treated with an adequate hot water regime. Sizing is done with different machines either by weight or by dimensions. Traditional dimension sizers measure the fruit at two, three and four contact points. Fruit sorting by rollers consists of passing the fruit on a bank of rollers which have different sized spaces between them. The fruit that fit between the rollers fall from the bank and are delivered into specific containers according to their size. Electronic sizers, which capture several video images of the fruit and calculate volume based on these images, are increasingly in use. Some of these sizers are able to weigh each fruit 250 times in 0.1 s at their normal speed. The fruit are rotated as they pass under a video camera, which records multiple images. The pictures are then analyzed by specialized software to determine fruit diameter, shape, density and color. These sorters are also able to determine shades of yellow in green lemons and differentiate yellow oranges from red oranges. They also allow fruit grading based on blemish incidence (number, area, intensity and position). According to these evaluations each fruit is delivered to the appropriate outlet for the individual size/grade. Some fruit, such as grapes, melon and citrus can be sorted based on °Brix using near infra-red (NIR) equipment which is commercially available.

5.7.3 Waxing

Tropical and subtropical fruits contain a superficial cuticle, which develops during fruit growth. Suberin, cutin and waxes are its main components. The cuticle protects the fruit against dehydration, regulates gas exchange, and may also even protect against decay, and contribute to fruit appearance. However, it is removed or can be altered during fruit washing and some other handling operations, increasing the susceptibility of fruit to damage. Protective coatings have been applied to foods since the twelfth century, when in China paraffin was used to

cover fruit as a method of preservation. Nowadays the application of waxes to fruit is practiced commercially to overcome the negative effects of washing and some handling operations. Adequate waxing can, for example, reduce water loss, restrict gas exchange and improve fruit appearance.

Examples of fruit that are sometimes waxed are oranges, limes and lemons, mangoes, avocados, passion fruit, litchi and pineapples. The type of wax should be chosen, and waxing should be carried out, according to relevant legislation and guidance from authorities. Commercial waxes generally contain carnauba wax, beeswax, candelilla wax, mineral oil, vegetable oil, shellac wax, polyethylene-based waxes, paraffin and emulsifiers. Carnauba wax is low in brightness and cost. Shellac wax gives the best shine although it becomes whitish when fruit are removed from cold storage. Shellac wax is usually expensive. Polyethylene-based waxes provide effective control against weight loss and also provide acceptable shine. Waxes are applied using specialized equipment as aqueous emulsions or foams at 38°C or more. Waxing is commonly followed by brushing to distribute the wax and generate shine. Brushed fruits are then treated with hot air to dry and fix the wax.

Commercial coatings may promote decay as the waxed lenticels turn into moist chambers in which spores can become trapped. However, some waxes contain chemicals such as fungicides and disinfectants and other compounds, including sprout inhibitors, antioxidants and aroma precursors, can also often be included. Sealing the avocado pedicel with waxes containing benomyl and thiabendazole reduced decay caused by *Colletotrichum* and *Dothiorella* (Darvas *et al.*, 1990). Waxing also effectively controlled the decay of mango fruits by *Alternaria alternata* (Prusky *et al.*, 1999). Hallman *et al.* (1994) tested the effectiveness of different covers (Primafresh 31, Sta-Fresh 360HS, Sta-Fresh 600 and Nature Seal) to control Caribbean fruit flies in grapefruits, carambola and mangoes. The effectiveness ranged from 33% for Primafresh 31 to 100% for Sta-Fresh 600. Some components of Nature Seal (methylcellulose and shellac wax) favored the mortality of the larvae of flies. Bayindirli *et al.* (1995) demonstrated that two commercial coatings (Jonfresh® and Semperfresh®) can reduce the ripening, respiration and transpiration rates of 'Satsuma' mandarins. Covered fruits also retained good levels of ascorbic acid, soluble solids, acidity and weight. Semperfresh coatings also reduced the postharvest weight loss in bananas (Al-Zaemey *et al.*, 1989). Paull and Chen (1989) demonstrated that waxing reduces the ripening rate in papaya as well as the weight loss in the range between 14 and 40%. In contrast, coating heat-treated guavas with carbauba wax can induce irregular ripening, high acidity and low content of total soluble solids.

5.7.4 Drying

Washing water must be removed from fruits in order to reduce fruit decay, as water stimulates the growth of bacteria and fungi. In geographical areas with high temperatures and low relative humidity, moisture evaporates quickly from the fruit. In contrast, in humid places fruit need to be dried. Several items of equipment

are commonly used for this purpose. Most commonly, fruit are passed through a hot air tunnel, a treatment which quickly eliminates superficial water from the fruit. Some fruits such as oranges and grapefruit require drying after waxing. A wax emulsion on oranges can be dried with exposure to air at 49°C for 2.5 minutes. Metallic rollers covered with absorbent materials are also used to dry fruits after washing. While fruits pass over a bank of these rollers the washing water is removed. Water in the rollers is eliminated by a device that presses the absorbent material. A bank of brushes similar to those used for washing can also be used to dry fruit. Polyethylene or nylon bristles are suitable and it is common to use banks of 20 or more brushes in order to ensure proper drying.

5.7.5 Disease control

Tropical and subtropical fruits are prone to several diseases and therefore disease control treatments are important to reduce decay and deterioration (see Chapter 6 in this volume). Diseases are the major cause of postharvest losses in tropical and subtropical fruits. The most important decay-causing organisms are *Colletotrichum gloeosprioides* (causing anthracnosis), *Diplodia natalensis* (causing stem-end rot), *Ceratocystis paradoxa* (causing black rot in banana and pineapple), and *Penicillium* and *Fusarium* (causing brown rot on pineapple). Anthracnose is the major postharvest problem in several tropical fruits, and latent infection commonly occurs in green fruit before harvest.

Preharvest treatments and methods of disease control are important. These include the use of healthy seedlings, as well as proper field sanitation practices. Pesticides are still used to control decay before and after harvest, but physical treatments such as postharvest heat treatments are effective and safer for consumers. Hot water treatments (48–55°C for 3–15 minutes) can be used for some fruits such as mango and papaya. Fungicides are applied through washing water and/or by incorporation in wax coatings. They should also be used in accordance with relevant legislation.

5.7.6 Insect control

Many insects infect tropical and subtropical fruits (see Chapter 7 in this volume). These include the Mediterranean fruit fly (*Ceratitis capitata*) in several regions of the world, several *Anastrepha* species in South, Central and parts of North America, and the West Indies, and the genus *Dacus* in Africa and Asia, among several others. Many importing countries implement strict plant quarantine measures to prevent the introduction of pests. Traditionally, chemical fumigants have been widely used for fruit disinfestation. Methyl bromide (MBr) is often used on several commodities, including grapes, nectarines, peaches, citrus and plums because of its wide-ranging action against a large number of pests (Aegerter and Folwell, 2000). Furthermore, large, bulky shipments can be rapidly and easily treated. Conditions of fumigation (concentration of MBr, time and temperature) depend on the fruit and pest types. Generally, this disinfestation process is

performed at 4.4°C or above. However, MBr can damage fruits, especially if the product fumigated has been recently waxed or has condensed water on the surface. Low temperatures increase the incidence of MBr damage. Another potential disadvantage of MBr is that it has now been identified as a chemical that damages the ozone layer. Some uses of MBr in various countries have already been restricted and its use for certain applications is scheduled to be eliminated in the next few years (the timescale varies in different countries), according to the Montreal Protocol and the Clean Air Act.

Efforts are being made to develop alternatives. Phosphine has been tested, but the exposure time for insect elimination is higher: 48–72 hours for phosphines compared to two hours for MBr. This prolonged time of fumigation is not desirable for many tropical and subtropical fruits because they generally have a reduced postharvest life. Elimination of larvae of Queensland fruit fly (*Bactrocera tryoni*) has been successfully achieved in citrus by the use of phosphine (16h/20°C/0.98 g m^{-3}) (Williams *et al.*, 2000). Nakakita *et al.* (1974) demonstrated that phosphine paralyses the insect and decreases the respiratory rate. Other fumigants that have also been tried include carbonyl sulphide and methyl iodide. There is currently some interest in some plant volatiles that could serve as a fumigant against certain insects that feed on fruit surfaces.

Heat treatments are an effective, non-chemical method, widely used to treat mangoes, papaya and some other tropical fruits. Heat treatments maintain produce at a certain (high) temperature for a fixed period of time, through the application of hot water or hot air. This treatment destroys different stages of various fruit fly species which might be present inside the fruit. For example, a hot water treatment at 46.1°C for 65 to 110 minutes (depending on fruit weight) is commonly used for mango in several countries. For papaya a two-stage heating process has been used to eradicate the Mediterranean fruit fly consisting of treatment at 42°C for 30 minutes, and then at 49°C for 20 minutes. Brown *et al.* (1991) inhibited the development of *Anastrepha suspensa* in grapefruits using hot vapor (43.5°C/24 hours). *Ceratitis capitata, Dacus cucurbitae* and *Dacus dorsalis* were effectively controlled in papayas by hot air treatment (fruit core temperature of 45.2–47.2°C/2 hours) (Armstrong *et al.*, 1989). Immersion of guavas in hot water (46.1°C/35 minutes) successfully eliminated *Anastrepha suspensa* (Probit 9) (Gould, 1994). Hot air treatment (fruit core temperature of 47.2° C/2 hours) eliminated the Mediterranean fly fruit in nectarines (Obenland *et al.*, 1999). Hot air treatment (43.5°C/240 minutes) reduced the incidence of *Diplodia spp, Phomopsis spp* and *Anastrepha suspensa* (Loew) in grapefruits (Miller and McDonald, 1991; 1992). A combination of hot air and modified atmosphere (1 kPa O_2 or less) for two hours is helpful in controlling *Anastrepha ludens* in oranges and mango (Shellie *et al.*, 1997; Yahia and Ortega, 2000; Yahia, 1998; 2007b).

The heating rate, insect density and life stage of the insect alter the effectiveness of heat treatments. Hansen and Sharp (1997) demonstrated that the effectiveness of heat treatment (40°C/67 minutes) was sequentially reduced as insect density (*A. suspense*) increased. Similar results were obtained for larvae of flies. Low heating rate can induce thermotolerance in insects. Thomas and Shellie (2000)

demonstrated that larvae of *Anastrepha ludens* (Loew) biosynthesized heat shock proteins under heating at 44°C, increasing the thermotolerance of the insects. The main disadvantage of heat treatments in insect control is potential fruit damage. Lay-Yee *et al.* (1998) demonstrated that hot air treatment (fruit core temperature of 48.5/49.5°C for 60 minutes or more) caused scald in papayas. Immersion of guavas in hot water (46.1°C/35 minutes) increased chilling injury susceptibility, fruit decay and postharvest weight loss (McGuire, 1997).

The use of cold to control insects in tropical and subtropical fruits is rare because many of these fruits are susceptible to chilling. Several cold treatments (10 days/O°C, 11 days/O.6°C, 12 days/1.1°C, 14 days/1.7°C, 16 days/2.2°C) have been recommended to control insects. This technology has shown good results in citrus, grapes, kiwifruit, persimmon and pomegranate. Heat pretreatments can induce cold tolerance in some fruits. Heat-treated pomelos tolerate the quarantine treatment based on cold. Cold is used to control the *Caribbean* fruit fly in carambola previous to transport from Florida to California. *Rhagoletis pomonella* (Walsh), *Conotrachelus nenuphar* (Herbst) and *Ceratitis capitata* (Wiedemann) are successfully controlled by subjecting the fruit to low temperatures (0–2°C) for 60, 33 and 12–20 days, respectively.

Besides their benefits for fruit preservation, modified and controlled atmospheres (MA/CA) can be effective in controlling insects in tropical and subtropical fruits (Yahia, 1998; 2009). Insect control by MA/CA is possible for nuts and dried fruits because they are tolerant of considerably low O_2 and high CO_2 atmospheres. Generally, insects are controlled using atmospheres with 0.5 kPa O_2 or less and/or 50 kPa CO_2 or more, for periods ranging from a few hours to about four to five days, depending on temperature and insect stage (Yahia, 1998; 2009; Yahia and Ortega, 2000). Johnson *et al.* (1998) controlled orangeworm in nuts using an atmosphere containing 0.5 kPa O_2 at 10°C for six days. Several insects (*Platynota stultana* Walsingham, *Tetranychus pacificus* McGregor and *Frankliniella occidentalis* Pergande) can be successfully controlled by using a CO_2-rich atmosphere (45 kPa CO_2 and 11.5 kPa O_2) at 2°C for 13 days (Mitcham *et al.*, 1997). The potential disadvantage of this method is fruit fermentation.

Insect control in tropical and subtropical fruits can also be achieved by using irradiation at doses <1 kGy. Irradiation can cause death or sterility of insects, and is a potential substitute for the chemical control. The typical irradiation dose is close to 0.3 kGy, which may induce minimal changes in the fruit. X-ray irradiation (0.195 and 0.395 kGy) had no detrimental effects on quality of 'Clementine' mandarin and allowed the control of the Mediterranean fruit fly (*C. capitata*) (Alonso *et al.*, 2007). Sound-emitting lamps and attractants are also tools for insect control, but their use in tropical and subtropical fruits is limited.

5.7.7 Packaging

Packaging is an important operation in the tropical and subtropical fruit supply chain. Some fruits (such as grapes) are commonly packed in the field, while several others (such as avocado, mango, papaya, pineapple) are commonly packed

in central packing stations (Fig. 5.6). Field packing can be very simple requiring only packers and boxes (Fig. 5.7), or more complex with specially designed sheds and machines (for trimming, grading, etc.). Field packing has several advantages. It reduces the time between harvest and cooling, reduces the possibility of manipulation of the product, and initial investments in this system are lower compared to packing in a centralized packinghouse. However, quality control and sorting into different grades during field packing are not easy, and in addition it is difficult to carry out certain treatments (such as washing, application of fungicides, waxing, heating, etc.).

Field containers for most tropical and subtropical fruits in different regions are made of plastic and are of different sizes, shapes and designs, with a capacity of 10 to 20 kg. Plastic boxes have several advantages compared to the wooden boxes commonly used in the past and still in use in some regions, as they are lighter, cause less product abrasion, need significantly less maintenance, are easily cleaned, washed, and disinfected, and have better ventilation, but they usually cost more than wooden boxes.

Once in the packaging house, several packaging materials are used to package tropical and subtropical fruits. Boxes made from particleboard or wood and multi-layer paper bags are commonly used for fruits that are precooled before being packed or require minimal cooling. Boxes made of wood or particleboard are very rigid and are able to withstand stacking up high, transportation and precooling. They are not deformed under wet conditions and can be spaced out in the transport vehicle by means of strips of wood. Melons, avocados and prickly pears are commonly packed in this kind of box.

Many fruits that are hydrocooled before packaging or packed in small quantities are brought to market in corrugated cardboard boxes (Figs 5.8 and 5.9). Mangoes, bananas, watermelons and papayas are examples of fruits packed into boxes of this kind. The disadvantage of this type of boxes is their tendency to absorb moisture and lose strength as their moisture content increases. This prevents their use for products that are hydrocooled after packing. Holders made of cardboard or wood are required to stack cardboard boxes on pallets. These holders are placed in each corner of the pallet, increasing the strength of the stack. Cardboard boxes treated with wax, oils or resins have increased resistance to moisture, allowing the fruit packed in them to be hydrocooled and ice cooled. Plastic boxes are usually sufficiently strong to be stacked but are easily broken when they fall or are handled roughly. One of the advantages of using plastic containers is that they can be manufactured according to the needs of a particular user, and they are useful if the product is hydrocooled after packaging.

If consumer packaging is to be used (Figs. 5.10 and 5.11), the product may be placed in this packaging in the packinghouse, near the harvest site, or once the product reaches the final market. Consumer packages are made from several types of materials including cellulose acetate, cardboard, rubber hydrochloride, polyester, oriented polystyrene, vinilidene chloride, irradiated polyethylene, nylon, vinyl and polypropylene, among others. These materials are used to make boxes, cups, bags, trays, coatings and meshes of different sizes. These packages

Fig. 5.8 A package for avocado.

Fig. 5.9 An orange package from Turkey.

Fig. 5.10 A consumer package for papaya.

Fig. 5.11 Packaged fresh cut tropical fruits in Malaysia.

should provide adequate protection against injury and water loss and should be sufficiently permeable to allow adequate exchange of gases and water vapor. Grapes, guavas, oranges, limes and avocados are commonly packed into these kinds of containers. Consumer packages are generally transported from the packinghouse to the retail store in large master containers, the purpose of which is to simplify the handling of the consumer packages. These master containers are commonly made of cardboard.

5.7.8 Modified (MA) and controlled atmosphere (CA)

An MA is an atmosphere with a different compositon from normal air (20–21 kPa O2, 0.03 kPa CO2, 78–79 kPa N2, and traces of other gases). A CA is an atmosphere with a composition that is both different from normal air and strictly controlled (Yahia, 2009; 1998). MAs and CAs with lower levels of oxygen and/or higher levels of CO_2 than normal air can reduce rates of respiration and ethylene production and reduce the action of ethylene, therefore delaying ripening and prolonging postharvest shelf life. Other advantages include decay control, alleviation of CI and reduction in losses of nutritional and health components (Yahia, 2009).

Modified atmosphere packaging (MAP)

MAP involves the packaging of fruits in permeable materials and an MA developing either passively (through fruit respiration and gas permeating through the package) or semi-actively (by adding one or more gases to or eliminating them from the package). Low density polyethylene (LDPE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), polyvinylidene chloride (PVDC) and polyethylene terephthalate (PET) are the most common flexible materials (polymers) used in MAP (Yahia, 2007a). Coextruded polyolefin is adequate for MAP of guavas (Jacomino *et al.*, 2001). Polyethylene and polystyrene are desirable to preserve figs in MAP (D'Aquino *et al.*, 1998). Preservation of avocados and loquat by MAP has been successfully achieved using polyethylene and PVC as packaging material (Meir *et al.*, 1997; Piga *et al.*, 1996), but the use of RD 106 film was not useful for mango preservation by MAP (McCollum *et al.*, 1992). Typically, the levels of O_2 and CO_2 in MAP are low and high, respectively. The packaging material must have specific permeability properties to ensure that O_2 and CO_2 concentrations reach levels that are optimal for reducing fruit metabolism without affecting its cellular functions (Yahia, 2007a). Low permeability films generally cause off-flavors and the accumulation of fermentative metabolites in the fruit, while high permeability films are not useful because they do not allow the development of adequate atmospheres. Micro-perforations are helpful to modify the permeability properties of low permeability films, but other sophisticated means are available to adequately modify the surrounding atmosphere for a specific fruit. Beside fruit respiration, elimination of O_2 can be achieved by several antioxidants (such as butylated hydroxyanisole, butylated hydroxytoluene α-tocopherol, β-carotene, *tert*-butylhydroquinone and propyl

gallate) which are generally incorporated into the films. Oxygen levels can also be diminished by introducing small sacs containing iron inside the package. CO_2 levels can be reduced or increased inside the package by using several compounds such as iron oxide, calcium hydroxide, ferrous carbonate, metal halide, calcium oxide, activated charcoal, ascorbate and sodium bicarbonate. Ethylene is commonly eliminated by using $KMnO_4$, which is incorporated into porous solids such as activated alumina, vermiculite and silica gel. Ethylene removal has also been achieved by using activated charcoal (alone or soaked with bromine), bentonite, tetrazine and various crystalline aluminosilicates such as zeolites. The Orega system removes ethylene using an inorganic and porous material composed mainly of zeolite, pumice, activated charcoal, cristobalite and clinoptolite. ProFresh, EverFresh, Hatofresh Systems, SendoMate, BO film and Frisspack are some of the ethylene-removing systems that have been available commercially for MAP. These systems have shown utility to preserve bananas, kiwifruit and grapes.

MAP has shown beneficial effects for the preservation of tropical and subtropical fruits. Chamara *et al.* (2000) preserved the chemical and physical attributes of bananas (firmness, total soluble solids, weight, acidity and pH) by MAP. Ladania and Dhillon (1989) increased the postharvest life of 'Perlette' grapes introducing an SO_2 generator inside ventilated corrugated packages. MAP decreased the respiration and softening rates but increased sucrose content in persimmon (Gwang *et al.*, 2000). MAP has also been extensively investigated for packaging minimally processed tropical and subtropical fruits (Yahia, 2007a). The extension of the shelf life of tropical and subtropical fruits by MAP is attributed in part to the control of microbiological damage. Incidence of *Aspergillus niger* and *Zygosaccharomyces rouxii* during the storage of raisins was reduced as a result of the combined effects of MAP (40–80 kPa CO_2) and potassium sorbate and sodium benzoate (El-Halouat *et al.*, 1998). The combination of MAP and several fungicides has shown beneficial effects in controlling decay in citrus (D'Aquino *et al.*, 1998). Opperman *et al.* (1999) added an SO_2 generator inside MA packages, reducing the incidence of *Botrytis cinerea* in grapes. However, inadequate MAP may increase the microbiological decay of fruits. For example, the use of RD 106 film was not useful for mango preservation at 12°C because the MAP increased microbiological decay activity (McCollum *et al.*, 1992). Inadequate MAP of fruits can cause the accumulation of fermentative metabolites, and therefore can cause fruit damage, but biosensors based on immobilized enzymes are now available to monitor the concentration of fermentative metabolites inside MA packages (Smyth *et al.*, 1999).

MA and CA storage

Among the tropical and subtropical fruits only kiwifruit is stored in a CA for prolonged durations (Yahia, 2009). McDonald and Harman (1982) stored kiwifruit for a long time under several CA systems at 0°C. Atmospheres containing more than 4 kPa CO_2 with 15–20 kPa O_2 caused retardation in the softening of kiwifruit. This effect increased as the CO_2 content of the atmosphere increased from 4 to 10 kPa. Low O_2 (2–3 kPa) with 3–5 kPa CO_2 delayed the rate of kiwifruit softening

and increased storage life up to three to four months beyond normal air-storage life. In a further study, the same authors (Harman and Mcdonald, 1989) found that storage of kiwifruit in 4, 6 or 8 kPa CO_2 + 15–20 kPa O_2, 5 kPa CO_2 + 2 kPa O_2 or 3 kPa CO_2 + 3 kPa O_2 for 16 weeks did not markedly alter the appearance or chemical composition of the fruit relative to those stored in air at 0°C. Atmospheres of 14 kPa CO_2 for 16 weeks caused low titratable acidity, abnormal texture, abnormal appearance and off-flavors in the fruit. Fruit stored in 5 kPa CO_2 + 2 kPa O_2 or 3 kPa CO_2 + 3 kPa O_2 for 24 weeks retained good texture, appearance and flavor. Although storage under CA is preferable for kiwifruit, MA technology has also shown good potential for long term storage of this fruit. An MA (0–21 kPa O_2, 0–5 kPa CO_2) successfully controls the softening rate in kiwifruit (Hertog *et al.*, 2004). Ben-Arie and Sonego (1985) demonstrated that MAP of kiwifruits in polyethylene bags results in an improved keeping quality, and longer storage life of 'Bruno' kiwifruits. They concluded that the postharvest life of kiwifruits could be extended to six months by storing the fruit at −1°C in sealed 0.04–0.05-mm-thick polyethylene bags containing a C_2H_4 absorber (Ethysorb). Several studies have demonstrated that CA and MA storage is possible for avocados for nine weeks (3 kPa O_2 and 8 kPa CO_2 at 5°C) (Yahia, 2009). Kim *et al.* (2007) successfully stored mangoes under a CA for two weeks. Similar results have been reported for guavas, litchis, peaches, bananas, grapes, muskmelons and citrus (Yahia, 1998; 2009).

Transport in MA and CA

MA and CA technologies are used when transporting several tropical and subtropical fruits, especially during marine transport (Yahia, 1998; 2009; Yahia and Singh, 2009; Singh *et al.*, 2009) (Fig. 5.12). For example, MA transport has been used for more than 30 years in banana transport from Central America to the rest of the world (Yahia, 1998; 2009). In the past few years the use of (and interest in using) MAs and CAs for transport has increased. In recent years there have been many advances. Since transport periods for tropical and subtropical fruits can be long (up to several weeks), MA and CA transport can be very helpful to maintain quality. In fact, for tropical fruits MA and CA transport is much more promising than MA or CA storage. The successful use of MAs and CAs in this context can also encourage transport by sea, rather than air, since MA/CA marine transport is cheaper than air transport. Atmospheres for transport can be developed passively, semi-actively or actively. The most common systems for transport in the past 30 years have been developed on a semi-active basis. These systems, which have been (and are still) used for the transport of bananas, are usually less efficient, but less expensive than active systems. The use of CAs for transport first became feasible in the late 1980s when technologies to build gas-tight containers and adequate gas control systems became available and it became possible to establish controlled gas mixes. The use of air separation technologies in the late 1980s, especially the introduction of membrane technology in 1987, made CA transport practical and feasible for several tropical fruits such as banana and mango (Yahia, 1998; 2009).

Fig. 5.12 Loading a modified atmosphere container.

5.8 Ripening

5.8.1 Hastening of ripening

Some tropical and subtropical fruits are artificially ripened after arrival at their destination. Ethylene gas is commonly used for this process, and temperature is an important factor for the process of ripening. Ideal conditions for ripening are temperatures of 20 to 24°C at high relative humidity (RH = 90 to 95%). Temperatures above 27°C accelerate softening and may cause tissue discoloration, excessive decay and off-flavors. Temperatures above 35°C inhibit ethylene production and action, and consequently inhibit the process of ripening. Ripening is also impaired when fruits are exposed to chilling temperatures. The ripening rate can be manipulated by temperature management between 14 and 24°C. Ethylene concentrations commonly used for fruit ripening range between 10 to 100 ppm. Removal of ethylene, using different agents such as potassium permanganate, is used to delay ripening in various fruits.

5.8.2 Control of ripening and senescence

Refrigeration is the method most commonly used to delay the ripening rate of tropical and subtropical fruits, although some of these fruits are susceptible to chilling injury. Fresh tropical and subtropical fruits, especially those shipped to distant markets, need to be handled under refrigerated conditions. However, the cold chain may be nonexistent for some tropical and subtropical fruits, especially in developing countries (Yahia, 2005; 2010). Several technologies have been developed to delay ripening in conjunction with refrigeration, including CA, MA and heat treatments. The ripening capacity of kiwifruits was sequentially reduced by low oxygen (2 kPa O_2 + 5 kPa CO_2) and ultra low oxygen (0.7 kPa O_2 + 0.7 kPa CO_2 and 1 kPa O_2 + 1 kPa CO_2) storage at 0°C (Antunes and Sfakiotakis, 2002) and was considerably lower than that of kiwifruit stored in air. Inhibition of ripening by CA storage was attributed to a reduced ethylene-producing activity, which was due to low 1-aminocyclopropane-1-carboxylate (ACC) oxidase activity rather than reduced ACC production or ACC synthase activity. Enrichment of the storage atmosphere with carbon dioxide (10 kPa) delayed the ripening in custard apple during storage, while ethylene did not influence the ripening process (Broughton and Guat, 1979). CA storage delayed and suppressed respiratory and ethylene peaks during ripening in guavas, especially at low O_2 ($\leq$5 kPa) atmospheres. High CO_2 was not beneficial, causing a reduction in ascorbic acid levels. CA storage was effective in delaying ripening, reducing weight loss and maintaining firmness of fruit (Singh and Pal, 2008). Baldwin *et al.* (1999) demonstrated that polysaccharide-based coatings can diminish the ripening rate of whole mangoes, and this effect was attributed to the low permeability of the coating.

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been shown to delay the postharvest ripening of several tropical and subtropical fruits. The delay in ripening is extended if 1-MCP is combined with other treatments, such as waxing. 1-MCP treatment delayed the onset of climacteric ethylene production and respiration in persimmon fruit, and also significantly retarded the activities of pectin methyl esterase (PME) and polygalacturenase (PG) during ripening (Luo, 2007). This gas reduced the respiration rate and completely suppressed PG activity (Jeong *et al.*, 2003). 1-MCP has been regarded as an effective ripening inhibitor in nectarines and mangoes (Liguori *et al.*, 2004; Singh and Dwivedi, 2008). Nitrous oxide (N_2O) at levels of 40–80% reduces the ripening rate of bananas. This gas reduced the ethylene synthesis and respiration rate and showed potential to control ripening of bananas during handling, transportation and storage (Palomer *et al.*, 2005). Alar and cobaltous chloride have been shown to delay the ripening rate of litchi (Nagar, 1994). These compounds reduced the activity of PME, peroxidase, cellulase and the rate of respiration. Zheng *et al.* (2007) demonstrated that dipping of intact mangoes in oxalic acid solution (5 mM 10 min^{-1} at 25°C) can delay the ripening rate and decay incidence. Irradiation, especially in the form of gamma rays, has been authorized in approximately the past two decades at levels of up to 1 KGy (Kilo Gray), and is commercially used in some countries. Gamma irradiation has been shown to slow down the ripening process. Some tropical fruits such as

papayas, mangoes and litchis are somewhat resistant to irradiation. A market test of irradiated Hawaiian papayas in 1987 showed that consumers preferred these fruits over hot water treated papayas (Moy, 1993).

5.9 Processing

Tropical and subtropical fruits are processed into many different products using many different techniques (see Chapter 11 in this volume). For example pineapple is commonly processed into products such as canned slices or rings, pulps or juices. These fruits are processed using traditional methods such as canning, concentration, fermentation and dehydration, as well as newer methods such as freeze-drying. The preliminary operations required are diverse and include washing, sorting, peeling, cutting, grinding, and blanching, among others. The raw materials need to be processed as soon as possible after harvest to avoid spoilage. Washing aims to eliminate any dirt stuck to the fruit before it enters the processing line, thus avoiding possible contamination of the raw material. It must be performed with clean water, possibly with added chlorine either as sodium hypochlorite or chlorine gas. Sorting and selection is needed after washing to separate fruits which have defects or to grade them on the basis of ripeness, color, shape and size, depending on the material and type of process to be performed. The fruits are usually peeled: the skin of the fruit is removed using physical devices such as knives or similar instruments, or heat or chemical methods. Peeling can improve the sensory quality of processed products, since the skin may contain pigments that are affected by thermal processing. Cutting to certain shapes and sizes is important to ensure even heat penetration during thermal processing and uniform drying. Products made from pieces of a similar size also have a better package appearance, since the packed material is more even in terms of its shape and weight. In the specific case of drying, cutting enhances the surface/volume ratio, which increases the efficiency of the process. Blanching is widely employed to soften the tissue to facilitate the filling of the containers and to inactivate enzymes which cause an unpleasant smell and flavor, as well as defects in the natural color of the product. Blanching needs to be followed by an efficient cooling process. The use of steam, rather than hot water, is recommended in the blanching process, to avoid the loss of soluble solids and water-soluble vitamins.

High temperature is the preservation method used to produce pasteurized and canned products such as juices and pulps. Such thermal processes involve sterilization or pasteurization in jars, bottles or other containers serving the same function. Other containers include tin cans. The bulk sterilization of products and their packaging in aseptic containers is another procedure based on the utilization of ultra high temperatures. Sterilization can be applied to any product that has been peeled, cut or has undergone some other preparation procedure, provided that it has been packaged in an appropriate container and sealed hermetically to prevent the penetration of micro-organisms and oxygen. Sterilization prevents the survival of pathogenic or disease-causing organisms.

Pasteurization is crucial to such products as pulps or juices. It consists of a thermal treatment less drastic than sterilization, but sufficient to inactivate micro-organisms present in the foods, and enzymes that may cause the food deterioration. Like sterilization, pasteurization is performed according to an appropriate combination between time and temperature.

Drying is one of the oldest food preservation techniques, which can be carried out by exposing the product directly to sunlight, or by the use of artificial (hot air) drying or chemicals. In dried fruits water activity drops down to levels at which neither micro-organisms nor deteriorating chemical reactions can develop. Generally fruits with less than 18% moisture are not favorable for the development of fungi, bacteria or important chemical or biochemical reactions.

Sugar is generally added in the processing of jams, jellies and sweets. The fruit is boiled, after which the sugar is added in variable amounts, depending upon the kind of fruit and the product being prepared. The mixture must then continue to boil until it reaches a level of soluble solids suitable for its preservation.

Consumption of fresh-cut or minimally processed tropical and subtropical fruits (Fig. 5.11) has increased in recent years (see Chapter 10 in this volume). Mangoes, melons, citrus, watermelons, pineapples, cactus pear fruits and papayas are minimally processed and then packed either with pieces of the same fruit, or as a fruit mixture. The typical steps in minimal processing of fruits include sorting, washing, peeling and cutting (including elimination of fruit core and stem-end), draining, centrifugation and packaging. The objective of fruit sorting is to discard fruits at an inadequate stage of ripening or size. Rotten fruits also must be eliminated. Two washing steps are generally performed: one immediately after fruit sorting and the other after fruit cutting and peeling. Water sprayers, rotary drums, rotary brushes or washing machines are generally used to wash the fruit. The sensory and microbiological quality of the washing water should be taken into consideration and the water temperature must be lower than 5°C. The water requirements to wash fruit before and after cutting are 5–10 L kg^{-1} and 3 L kg^{-1}, respectively. Preservatives and browning inhibitors can be added to washing water. Fruit must be dried after washing, generally by centrifugation. Then, fruit must also be peeled and cut up. Peeling of fruit is performed mechanically (mainly by abrasion forces), chemically (using enzymes, acids and alkalis) and physically (using hot water, high-pressure steam and freezing). Manual peeling has been regarded as the best method for fruit peeling. The cutting process consists of subjecting the fruit to the action of vertical and horizontal cutting blades. Cutting machines for fruit and vegetables can be classified as choppers, slicers, cutters of cubic forms and shredders. The cutting process must be carried out under refrigerated conditions because cutting accelerates the respiration rate. Cut tissues eliminate barriers to the diffusion of gases, but tolerate higher concentrations of CO_2 and lower concentrations of O_2 compared to intact products. Cut fruit is then washed, mixed (mainly by convective or diffusive mixing) and packed.

MAP is commonly used to preserve minimally processed fruits. The typically high levels of CO_2 inside packages of minimally processed fruits reduce the respiration rate and the activity of some ripening and browning-related enzymes

such as polyphenol oxidase (PPO) and PME. CO_2 also reduces the microbial load. Modification of the atmosphere with CO (carbon monoxide) reduces the levels of *E. Coli* and *C. botulinium.* CO also diminishes the activity of PPO. Sulfur dioxide also can be used in the MAP of some minimally processed fruits. This gas exerts anti-browning and antimicrobial effects. Infrared irradiation can also be helpful in avoiding browning and the loss of water and aroma compounds in minimally processed fruits. The use of ionizing irradiation is approved for this kind of product, which may reduce respiration rate, inhibit some enzymatic reactions and the growth of microorganisms.

Minimally processed fruits are highly susceptible to decay. Preservation of this kind of food can be achieved by using organic acids and related compounds. Citric acid is widely used to preserve cut fruits. This acid is employed to avoid fruit browning by PPO. Several antioxidants are also used to prevent browning of minimally processed fruits (Gonzalez *et al.*, 2010). Control of fungi and pathogen microorganisms can be achieved by acetic, propionic and sorbic acids. Other acids (malic, succinic and tartaric acids) have shown potential for preservation of minimally processed fruits, but their use has not been extensively studied. There are other kinds of antimicrobial agents that can be used to preserve minimally processed fruits, such as fatty acids (palmitic and linoleic acids), fatty acid esters, polyhydric alcohols, and some others. The use of a combination of methods to preserve minimally processed fruits has shown promising results. Treatments such as bleaching, osmotic dehydration, the addition of chemical preservatives, pH modification and the use of appropriate packaging can be combined. An example is the dipping of mango chunks in syrup at 85°C for five minutes before a second dipping in another syrup containing sodium metabisulfite (4 mg) and sodium benzoate (413 mg) per kg of fruit for eight hours before finally packing the fruit in plastic bags. The mango chunks were preserved for 30 days when stored at 27°C or 90 days at 2°C (Yahia *et al.*, 2006).

Quality control of processed products is very important. It must be a planned activity, with written specifications and standards for raw materials and other ingredients, the inspection of critical process control points, and finally examination of the entire system, including an inspection of the finished product.

5.10 Conclusions

Optimum temperature, humidity, and atmosphere are critical for the storage and shipping of tropical and subtropical fruits. The precise conditions required depend on the nature of the individual product, the length of time the product is to be held and whether the product is packaged or unpackaged. Compared with several temperate fruits, tropical and subtropical fruits are more difficult to store and ship because of their perishable nature, and the fact that many of them are sensitive to chilling injury. Provided that fruits are harvested at the ideal stages of maturity and handled carefully, refrigeration offers the greatest potential for increasing postharvest life. It should be emphasized that the benefits of cooling will be

jeopardized if the commodity is not kept properly refrigerated after precooling. Precooling and storage are two separate processes that have vastly different requirements. In other words, the specific requirements for achieving fast, uniform cooling must be considered independently of the cold storage requirements. Several other complementary treatments and techniques are used for tropical and subtropical fruits, such as MA/CA (especially for marine transport), heat treatments (especially for control of quarantine pests and some decay organisms, such as in the case of mango fruit) and irradiation (especially used for quarantine pest control in fruits such as mango and guava), among others. The increased demand for tropical and subtropical fruits in international markets requires the use of the available techniques and technologies. Major research is still necessary, though, to develop additional adequate techniques, especially for the control of pests and decay.

5.11 References

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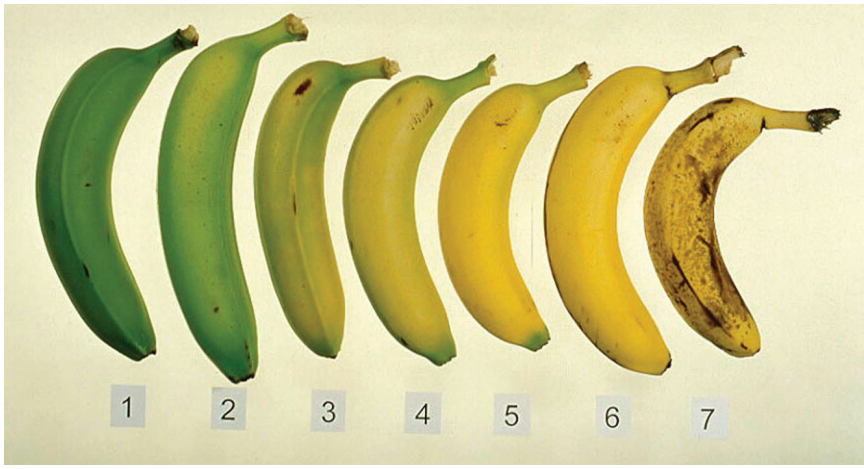
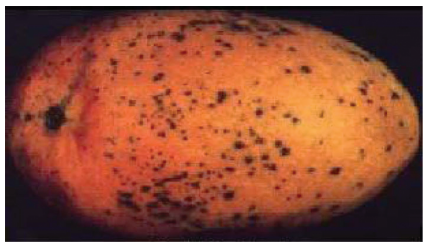


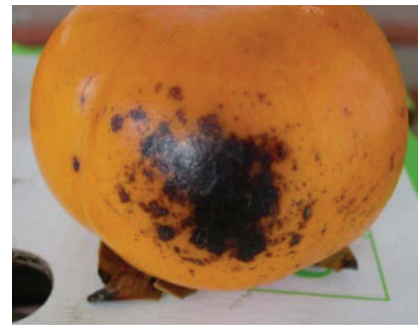
Plate V (Chapter 5) Banana ripening chart (courtesy of Prof. Adel A. Kader).

Plate VI (Chapter 5) Harvested banana being transported to packinghouse in Saudi Arabia.

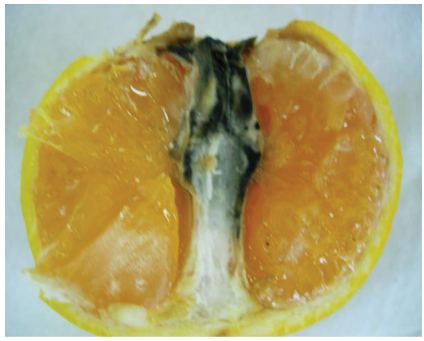
Plate VII (Chapter 5) Banana washing at packinghouse in Costa Rica (courtesy of Prof. Adel A. Kader).



(a)



(b)



(c)

(d)

Plate VIII (Chapter 6) (a) Black spot disease on mango caused by *Alternaria alternata*; (b) black spot disease on persimmon caused by *Alternaria alternata*; (c) black rot on mandarin caused by *Alternaria citri*; (d) stem-end rot on grapefruit caused by *Botrydiplodia theobromae*.

6

Postharvest pathology of tropical and subtropical fruit and strategies for decay control

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Abstract: This chapter discusses the major causes of postharvest losses in tropical and subtropical fruits due to fungal pathogens. First, the etiology, biology and environmental and horticultural factors that are involved in infection and development of decay are reviewed. Here, understanding the relationship between production conditions and the postharvest handling chain is crucial for improving fruit quality. Details on the host–pathogen interactions of major pathogens of tropical and subtropical fruits and their modes of infection are provided. Special emphasis is given to *Colletotrichum* as it has a very wide host range and causes extensive postharvest losses in many fruits. Information about natural (constitutive and induced) fruit defense mechanisms in major tropical and subtropical fruit is also discussed. The chapter addresses current control strategies based mainly on the use of synthetic fungicides but also includes information on safer alternatives.

Key words: quiescent infection, anthracnose, biological control, integrated control, fruit defense mechanisms, fungal attack mechanisms, induced defense mechanisms, nectrotrophic pathogens, hemibiotriphic pathogens.

6.1 Introduction

Decay of tropical and subtropical fruits is a major cause of postharvest losses and is due mainly to fungal infections (Armstrong, 1994). While reports on the level of these losses are conflicting, they are estimated at 50% or more in developing countries due to the lack of adequate handling and refrigeration facilities; losses may be lower in developed countries. Because of the lack of precise statistics,

however, it is hard to determine the extent of real losses that may vary depending on the commodity and the producing country. Postharvest decay can be traced to infections that occur either between flowering and fruit maturity or during harvesting and subsequent handling, storage, marketing, and even after consumer purchase. Efforts have been made to minimize these losses through developing a better understanding of the biology and etiology of postharvest diseases as well as by developing adequate postharvest handling technologies and control strategies.

A number of fungi from several genera cause postharvest decay on agricultural produce. Among these are representatives of *Botrytis*, *Penicillium*, *Mucor*, *Alternaria*, *Colletotrichum*, *Lasiodiplodia*, *Rhizopus*, *Fusarium* and *Aspergillus* (Snowdon, 1992). Due to the wide variety of tropical and subtropical commodities grown throughout the world, this review will focus mainly on fruit crops that have widespread consumption and play an important role in trade as exports. Concomitantly, only research on the main pathogens will be discussed in detail. In many cases, with regionally consumed fruits, pathogens responsible for postharvest losses have not been well characterized.

This chapter will provide a general overview of postharvest diseases of tropical and subtropical fruits. It will touch on the etiology and biology of major pathogens, provide details about factors affecting infection and disease development, and discuss host–pathogen interactions. Current, primary control strategies and alternative approaches to disease management will also be discussed. Generally speaking, several factors contribute greatly to high levels of postharvest losses in tropical and subtropical fruits that are difficult to address in a simple manner such as with the use of an effective fungicide or biocontrol agent. Tropical environments are often hot and humid, conditions that are optimum for the survival and development of fungi and fungal propagules such as spores. Additionally, unless there is a large export market and the commodity is produced and managed in a conventional manner such as a large orchard with known cultivars, there is a lack of the infrastructure that can help to keep postharvest losses low. This infrastructure can include such things as adequate harvesting equipment and sanitized packing boxes, timely transport to a packing facility, rapid access to refrigeration either during transport to the packing facility or at the packing facility itself, access to controlled atmosphere (CA) storage facilities or modified atmosphere (MA) packaging, etc.

6.2 Preharvest and postharvest factors affecting disease development

Susceptibility of fruit to pathogen attack after harvest is very much influenced by fruit quality before and at harvest. Therefore, the relationship between production conditions and postharvest decay development needs to be well understood in order to have effective control strategies. Many of the production and postharvest factors affecting quality, as well as disease development after harvest, however, are still poorly understood in tropical and subtropical fruits.

6.2.1 Preharvest factors

Cultivar and rootstock genotype

Cultivar and rootstock have an important role in determining different physiological and quality characteristics of fruit that, in turn, also influence susceptibility to the development of postharvest disease. New cultivars and rootstocks with improved quality are developed through breeding programs that unfortunately only rarely take into consideration susceptibility to postharvest pathogens in the early stages of the breeding process (Arpaia, 1994).

Rootstock studies conducted on 'Hass' avocado showed that rootstock had a significant impact on postharvest anthracnose susceptibility. The severity and incidence of anthracnose was significantly lower on 'Hass' grafted to 'Velvick' Guatemalan seedling rootstock compared to scions grafted on 'Duke 6' Mexican seedling rootstock. Anthracnose susceptibility in the two different rootstock-scion combinations was related to significant differences in the concentration of antifungal dienes in leaves, as well as the levels of mineral nutrients in both leaves and fruits (Willingham *et al.*, 2001). In another study, cultivar was also reported to affect susceptibility of avocado and banana to *Colletotrichum gleoesporiodes* (Dinh *et al.*, 2003) and *C. musae*, respectively (Shillingford and Sinclair, 1977). In citrus, Ritenour *et al.* (2004) showed that rootstock significantly affected the development of postharvest decay on Navel orange, 'Ray Ruby' grapefruit, 'Oroblanco' and 'Valencia' orange.

Weather

Weather has a marked effect on the development of plant diseases, from the amount of inoculum that overwinters to the amount of pesticide residue that remains on the crop at harvest (Conway, 1984). Environmental conditions during growth, such as low temperature, wind, rain or hail can have a direct effect on a crop and not only determine the yield but also fruit quality after harvest. Wind and rain may be responsible for the dispersal of spores of many pathogens that survive in soil and dead plant parts in an orchard. Abundant inoculum and favorable conditions for infection during the growing season often result in heavy infection when the produce is harvested. For example, conidia of the fungus that causes brown rot on citrus fruit are dispersed from the soil surface and from rotten fruits after heavy rains especially near harvest time, causing infections that later develop during storage or shipment (Graham *et al.*, 1998). Infection of many tropical and subtropical fruits by anthracnose in the orchard is greatly dependent on temperature, inoculum dispersal and relative humidity (McMillan, 1986). Moisture conditions in the field at harvest are very important in rot development. Hence, fruit should not be harvested after prolonged rains. Moreover, relatively high temperatures in the field during harvest can render tropical fruit more susceptible to chilling injury and contribute to the development of disease. The combination of high temperature and relative humidity favors the growth of postharvest pathogens which in turn can significantly increase disease development when the fruit reaches a retail market (Fitzell and Muirhead, 1983).

Cultural practices

Cultural practices such as tree pruning, handling of debris, and removal of decayed fruits may have a marked influence on the survival and quantity of pathogen inoculum in an orchard (Everett *et al.*, 1999). Therefore, sanitation programs to reduce the inoculum in the orchard are an extremely important aspect of disease management. Picking bags contaminated with conidia can become a major source of inoculum, and research has shown that nylon mesh bags may reduce *Botrytis* build-up compared to enclosed bags. Korsten (2006) indicated that delaying harvesting of litchi fruit to as close to actual packing time as possible and developing harvesting techniques that caused less fruit injury could greatly increase fruit quality and extend shelf life. In grapes, early season horticultural practices have a substantial effect on bunch and berry structure that can affect fruit quality and influence its storage potential. 'Berry thinning' performed manually or by chemical induction of flower abscission with gibberellin prevents excessive fruit set (Coombe, 1973). Failure in thinning may result in grape bunches that have a 'corn'-like appearance with tightly compressed berries, creating a favorable microclimate for infection and development of decay either before or after harvest (Marois *et al.*, 1986; Vail and Marois, 1991).

An open canopy in kiwifruit allows for plenty of air movement in the orchard which is an unfavorable condition for *Botrytis* conidial germination and establishment. Good orchard hygiene such as the removal of sources of *Botrytis* conidia including dead leaves, petals etc. can reduce the risk of *Botrytis* infection of the fruit and subsequent postharvest decay (Michailides and Elmer, 2000).

In relation to postharvest decay, Snowdon (1992) has noted the importance of mechanical injuries such as scrapes, bruises or abrasions inflicted during harvesting and packing that result in increased susceptibility to postharvest decay.

Physiological and nutritional condition

The physiological condition of harvested fruit, as well as fruit maturity and ripening process, markedly affect its susceptibility to pathogen attack and disease development during storage and transit. It is a common principle that quality at harvest cannot be improved but merely maintained for a limited period of time. Harvesting fruits at the optimal maturity stage can therefore result in optimal quality and maximum shelf life. In most cases, advanced fruit ripening and the onset of senescence in various fruit renders them more susceptible to infection by pathogens (Kader, 2004). Either an excess or a shortage of water during the growing season can result in harvested fruit being more susceptible to postharvest diseases (Snowdon, 1992). Grobler *et al.* (2002) found that endophytic *Botryosphaeria* spp. was more prominent in trees exposed to continual water shortage and salt stress.

On the other hand, fruit can be made less prone to decay by management of crop nutrition. In this regard, calcium has been more closely associated with disease resistance than any other mineral cation associated with the cell wall (Sams, 1994). In general, produce containing adequate levels of calcium does not develop physiological disorders and can be stored longer with lower decay

incidence (Hernández-Munoz *et al.*, 2006). Conversely, high nitrogen content in fruit predisposes them to decay (Conway, 1984). Hofman *et al.* (2002) in a study on 'Hass' avocado reported that the lower the content of minerals, such as calcium and magnesium in the fruit, the higher the incidence of anthracnose. The mineral content of immature and mature mango fruit (cv. Nam Dork Mai) grown under different soil conditions also had a direct relationship to postharvest fruit quality and rot development (Whangcha *et al.*, 2001). Fruit grown in soil with higher Ca/N and (Ca + Mg)/K ratios remained firm, resistant to decay and had longer storage life.

Fungicide sprays

Fungicides are used extensively to control postharvest disease in fruits and vegetables. The timing of application and type of fungicide used depend primarily on the target pathogen, the timing of the infection, and the commodity. For postharvest pathogens that infect produce before harvest and generally remain quiescent until after harvest, field application of fungicides is often necessary. This may involve the repeated application of protectant fungicides during the growing season, and/or the strategic application of systemic fungicides. For example, in the control of mango anthracnose (*Colletotrichum gloeosporioides*) in Australia, trees are sprayed regularly with a protectant fungicide such as mancozeb during flowering and fruit development. If rain occurs during flowering, however, a systemic fungicide is also applied to inactivate infections already established and to guard against new infections. Protective sprays applied on a 7 to 14 day schedule have been extensively used to control anthracnose on mangoes (Prusky, 1996; Saaiman, 1995), papayas (Alvares and Nishijima, 1987) bananas (Stover and Simmonds, 1987) and avocados (Muirhead *et al.*, 1982).

6.2.2 Postharvest factors

Inoculum load

Organic matter (soil, plant parts) and remnants of rotten fruit in a packinghouse processing area can serve as substrates for decay-causing pathogens. Dump tanks, if not frequently cleaned and disinfected, become sources of contamination due to the accumulation of high quantities of spores. For these reasons, it is important to have and routinely apply a rigorous sanitation program in packinghouse sheds, cold storage rooms, and on the packing line itself (Cappellini *et al.*, 1984). Inoculum sources may also come from trucks and other transport vehicles used to move the fruit from the orchard to the packinghouse. Additional sources of inoculum are container or cool trucks, port terminals, distribution centers, repack facilities, markets and other retail locations (Kader and Rolle, 2004).

Several different sanitizers are usually used to disinfect surfaces, packing lines and dip tanks. These include chlorine (sodium and calcium hypochlorite), chlorine dioxide, peracetic acid and different quaternary ammonium-based products. Caution, however, should be exercised when using some of these sanitizers as

they may be corrosive to metal or may be phytotoxic and cause injury to the fruit if used improperly in dip tanks (Mari *et al.*, 1999; Eckert and Sommer, 1967).

Temperature and relative humidity

Penetration of the host by a pathogen does not automatically result in the development of decay, unless favorable conditions exist. These conditions include appropriate temperature and relative humidity. Spore germination and mycelial growth largely depend on temperature, and the optimal and minimal temperature may vary according to fungal species. In fact, most of the decay that occurs in the marketplace, particularly that of tropical fruits, results from overexposure to damaging temperatures. Temperatures either below or above the optimal range (e.g. freezing, chilling, high temperature) for a specific commodity can cause accelerated decay development. Temperature management is the most effective tool to minimize decay development and extend shelf life. It begins with rapid removal of field heat followed by refrigerated transport and storage. In this regard, maintaining the cold chain during all the postharvest processing, from packing to the marketplace, is crucial to preserving quality (Kader and Rolle, 2004).

High relative humidity (RH), required to protect fresh produce from dehydration and weight loss, also carries the potential to stimulate pathogen development during storage. Prospects for decay development are enhanced by water films or water condensation on a fruit surface. These conditions may arise when fruit is packaged with sealed plastic films having no permeability to water vapor or result from temperature fluctuations during shipment which can lead to condensation of water. Many fruits are more susceptible to pathogen attack when their tissue is in a turgid state as a result of being kept under high RH. Under these conditions fungal spores use the moisture to germinate and penetrate the tissue (Barkai-Golan, 2001).

6.3 Modes of infection by postharvest pathogens

6.3.1 Surface damage

Most postharvest pathogens of tropical and subtropical fruits are incapable of infecting the fruit directly through the intact fruit surface. These pathogens are largely dependent on the presence of surface injuries for penetration, and hence are called ‘wound pathogens’. The nature of surface wounds may vary from cracks in the cuticle and underlying tissues created during fruit growth, maturation and ripening, to mechanical damage inflicted during harvesting and subsequent handling of the fruit. For example, pathogens such as *Penicillium digitatum* and *P. italicum*, the cause of green and blue mold of citrus, respectively, are totally dependent on surface wounds (Kavanagh and Wood, 1976; Macarisin *et al.*, 2007). For this reason, the method of harvesting (hand vs. mechanical) can significantly impact postharvest decay development. To minimize mechanical damage of fruit skin, proper management decisions that include the selection of an optimum time to harvest in relation to produce maturity, and the appropriate climatic conditions for harvest, must be considered.

Black rot, caused by *Thielaviopsis paradoxa*, is the most common and best-known postharvest disease of pineapple fruit. The pathogen gains entrance through natural growth cracks and wounds on the fruit surface generated during picking or packing. After penetration through the cut stem-end the pathogen grows rapidly upwards and into the flesh (Snowdon, 1992).

Pathogens can also invade fruit tissue though physiological damage of the tissue caused by cold, heat, and CO_2. In most cases the physiological damage is manifested by localized browning and death of fruit peel tissue forming vulnerable sites for entry of wound pathogens.

6.3.2 Infection through natural openings

Without the presence of surface injuries, some pathogens can penetrate fruit through natural openings such as stomata and lenticels. For example, germinating spores of *C. gloeosporioides* can penetrate young papaya fruits in the orchard through stomata at various developmental stages (Prusky, 1996). Penetration through lenticels was described also for *Alternaria alternate* spores in mango and persimmon fruit (see Plate VIII in the color section between p. 238 and p. 239) (Prusky *et al.*, 2006).

6.3.3 Insect mediated infection

The activity of various types of invertebrates can play a critical role in contaminating produce with inoculum. The most substantial evidence in this regard has been shown in relation to *B. cinerea* infection of kiwifruit through flowers facilitated by thrips (*Thrips obscuratus*) and honey bees (*Apis* sp.) (Michailides and Elmer, 2000). High incidence of *Botrytis* infections were also reported on kiwifruit when garden snail (*Helix aspersa*) damage was present; slime secreted by the snail stimulated the germination of the conidia. Honey bees and other types of insects visiting flowers potentially have the capability to disseminate *Botrytis* (Michailides and Elmer, 2000).

6.3.4 Infection through flower parts

Pathogen infection of tropical fruits through flower parts has rarely been studied. The sporadic nature of floral infections by pathogens combined with extended latent periods prior to decay development has made this a difficult topic to study. Michailides and Morgan (1996) showed that colonization of persistent sepals and fruit receptacles in California kiwifruit by *B. cinerea* starts as early as 30 days after fruit set, and this colonization correlates significantly with postharvest decay after three to five months of cold storage. The authors found that harvest infections along with infections through wounds could explain only 10% of the postharvest decay. In contrast, colonization of sepals of kiwifruit in New Zealand plays a minor role (if any) in postharvest gray mould decay. Instead the fresh stem scar becomes infected during harvest (Elmer *et al.*, 1995). Pineapple is another

example where floral infection followed by an extended latent period can occur. The flower of pineapple is the portal for several major pathogens, and the period of latency ranges from four to six months (Rohrbach and Apt, 1986; Rohrbach and Pfeiffer, 1976).

6.4 Attack mechanisms

Fungal pathogens can be divided into three classes: necrotrophic, biotrophic, or hemibiotrophic fungi. Necrotrophs are opportunistic pathogens that invade wounds to access dead tissue. They grow intercellularly producing lytic enzymes and toxins to decompose surrounding tissue and utilize the dead tissue as a source of nutrients (Wolpert *et al.*, 2002). In contrast, biotrophs, which are not known to attack tropical and subtropical fruits, do not kill their host immediately. They are in fact dependent on living tissue to complete their development. Generally these fungi do not produce large quantities of extracellular enzymes or toxins during their parasitism on the host. Hemibiotrophic fungi are regarded as a subgroup of nectrotrophic fungi that require living host tissue during part of their life cycle and then later switch to a necrotrophic stage.

Colletotrichum is a hemibiotroph on many tropical and subtropical fruits. *Colletotrichum* spores adhere to and germinate on the plant surface, where they produce appresoria that give rise to germ tubes. The tip of the germ tube, developing from the appressorium, penetrates through the cuticle with an infection peg. After penetration and colonizing one or more host cells, the biotrophic hyphae (Kramer-Haimovitch *et al.*, 2006) subsequently gives rise to secondary necrotrophic hyphae (Latunde-Dada *et al.*, 1996; Mendgen and Hahn, 2001). Both *Botrytis* and *Monilinia* can either invade host tissue through wounds or breach the fruit cuticle by extending an infection peg from an appressorium. These initial infections can then remain quiescent or latent for long periods (Fourie and Holz, 1995; Pezet *et al.*, 2003). Depending on the physiological status of the fruit, these hyphae may continue the infective process or remain quiescent.

As reviewed by Prusky (1996), the quiescence of the latent infections established by biotrophic pathogens can be attributed to several factors: (a) lack of nutrients in the fruit needed for pathogen development after infection; (b) the presence of preformed or inducible antifungal compounds in resistant unripe fruits; and (c) an unsuitable environment for the activation of fungal pathogenicity factors.

Activation of quiescent biotrophic infections seems to involve a set of coordinated events including physiological and biochemical changes that occur in the host during ripening and senescence that lead to decreased host resistance and increased susceptibility. Concomitant to the developmental loss of resistance that accompanies fruit ripening, there also appears to be an activation of processes by the pathogen that compromise host defenses, directly or indirectly, by the detoxification of antifungal agents produced by the host (Prusky, 1996; Prusky and Lichter, 2007). The physiological changes that accompany fruit ripening and host senescence – e.g.,

changes in host pH, sugar content, cell-wall components, and the oxidation of wounded tissue – trigger responses by the infecting fungus. The acidification of the tissue by organic acids (oxalic and gluconic) or its alkalinization by ammonia, and the possible interference with the production of reactive oxygen species (ROS) by the host in response to fungal infection, as well as fungal ROS production, may contribute to rapid necrosis of the tissue. Further amplification of the decay can result from activation of fungal genes coding for cell wall degrading enzymes. Regardless of the mechanisms that are involved in the onset and termination of quiescence, however, quiescent infections appear to be a case of coevolution, as they can be seen as advantageous to both pathogens and hosts in a natural ecosystem. In relation to a pathogen, quiescent infection of climacteric fruits by *C. gloeosporioides* represents an example of pathogen adaptation to host physiology. Flaishman and Kolattukudy (1994) indicate that the involvement of ethylene in the termination of quiescence strongly suggests that *Colletotrichum* spp. must have coevolved to develop a mechanism to use the host's ripening hormone as a signal to reactivate the infection process. This mechanism prevents contact of the pathogen with host tissues that have high levels of antifungal compounds.

In a recent study, Droby *et al.* (2008) demonstrated that citrus fruit volatiles released after wounding of the fruit rind play an important role in host recognition by *P. digitatum* and *P. italicum* (Droby *et al.*, 2008). When exposed to volatiles from citrus fruit peel tissue, the percentage of germinated spores of *P. digitatum* and *P. italicum* markedly increased compared to the unexposed controls. In contrast, *Botrytis cinerea* and *P. expansum*, non-pathogens of citrus fruit, were either not affected or inhibited by the citrus peel volatiles. The citrus example is illustrative of a situation where a pathogen has become uniquely adapted to the physiology of its host.

In another report by Macarisin *et al.* (2007), it was found that *P. digitatum* in its early stages of infection suppresses a defense-related hydrogen peroxide (H_2O_2) burst in host tissue, thereby compromising fruit defenses. In contrast, *Penicillium expansum*, a non-pathogen of citrus, triggers production of a significant amount of H_2O_2 in citrus fruit exocarp. The results of this study demonstrated that H_2O_2 is a critical element in citrus fruit defense. Whether it acts directly on the fungus or as a signaling molecule needs to be elucidated. An example of a pathogen that attacks a wide range of tropical and subtropical fruits is *Alternaria. A. alternata*, known to cause black spot disease on a variety of fruits after harvest and causes significant economic loss (Prusky *et al.*, 1997). On mangoes for example, the pathogen penetrates fruit tissue through lenticels during fruit growth. In persimmon fruit it penetrates either through surface wounds or directly through the cuticle (Prusky *et al.*, 1997). In both cases the pathogen remains quiescent until fruit ripen, after which symptoms become visible. It has been suggested that the transition from the quiescent to the active necrotrophic stages is modulated by changes in ambient pH (Eshel *et al.*, 2000; Prusky and Lechter, 2008).

Black rot, caused by *Alternaria citri*, can affect all species of citrus, where it develops through the central columella of the fruit (Brown and McCornack, 1972; Brown and Eckert, 2000). Penetration of the pathogen takes place either at the stylar

or stem end of the fruit around the calyx (button) of immature fruit. The pathogen remains quiescent until after harvest, after which the decay process is initiated and is evidenced during storage as an internal black rot spreading from the fruit core (see Plate VIII c between pages 238 and 239). External symptoms are not often apparent. The ability of the pathogen to produce an endopolygalacturonase appears essential for *Alternaria* isolates to cause black rot (Isshiki *et al.*, 2001). None of the black rot strains isolated from citrus to date have been found to produce host specific toxins (HST). In general, affected citrus fruit are more brightly colored than normal fruit due to the ethylene generated in response to infection (Timmer *et al.*, 2003).

Another mechanism of pathogen attack is via the fruit's stem end. Stem-end rots are usually caused by a complex of pathogens that varies with the fruit type. These rots usually originate from quiescent infections at the stem end of the fruit. In citrus for example, stem-end rots caused by *Botrydiplodia theobromae* (Syn, *Diplodia natalensis*) (see Plate VIII d) and *Phomopsis citri* develop from quiescent infections located in the stem end button (calyx and disc) (Brown and Wilson, 1968; Brown, 1986). Infections occur in the orchard under wet conditions that allow the release of fungal spores from pycnidia. These spores infect flowers in the initial stages of fruit development and establish primary infections at the stem end of the developing fruit. Infections remain quiescent until fruit is harvested and then become active. Visible decay becomes evident, however, only after senescence of the fruit's button.

Stem-end rot pathogens of mango (*Mangifera indica*) include *Dothiorella dominicana*, *Dothiorella mangiferae*, *Lasiodiplodia theobromae* (Syn. *Diplodia natalensis*), *Phomopsis mangiferae*, *Cytosphaera mangiferae*, *Pestalotiopsis* sp. and *Dothiorella* 'long', as well as other fungi (including *Alternaria alternata*). These fungal species were found to occur endophytically in the stem tissue of mango trees prior to inflorescence emergence (Johnson *et al.*, 2008).

6.5 Fruit defense mechanisms

The development of postharvest disease is a process that involves contamination of a fruit surface with a pathogen's propagules, followed by attachment, germination, penetration and establishment of the pathogen. This is followed by pathogen development in the tissue and the production of decay symptoms. To be capable of producing a disease, the pathogen needs to overcome constitutive and induced host-defense mechanisms, and exert pathogenicity factors (cutinases, pectolytic enzymes, cellulytic enzymes, toxins, etc.) that facilitate its development through the use of the released nutrients. Plants have evolved a number of strategies to resist fungal infection. These strategies include the following.

6.5.1 Preformed antifungal compounds

The ability to provide direct evidence for the role of preformed compounds in resistance of tropical and subtropical fruit to postharvest diseases is problematic

due to the difficulty in determining the concentration of inhibitory compounds that may actually come into contact with the invading pathogen. In several systems studied, changes in concentrations of inhibitory compounds did not coincide with changes in susceptibility (Schulz, 1978, Sitterly and Shay, 1960). Dopamine, for example, was isolated from the peel of unripe banana in concentrations that inhibited *C. musae in vitro* and was therefore presumed to be a possible preformed antifungal compound involved in fruit resistance. However, changes in the concentration of dopamine were not correlated with changes in decay development (Muirhead and Deverall, 1984). In mangoes, a mixture of antifungal compounds consisting of 5-12-cis-heptadecenyl resorcinol and 5-pentadecenyl resorcinol was found at a fungitoxic concentration in the peel of unripe mango fruit that were resistant to *A. alternata* and markedly decreased in ripe and susceptible fruit (Droby *et al.*, 1986; Droby *et al.*, 1987).

The resistance of unripe avocado fruit to the activation of latent infections of *C. gloeosporioides* is correlated with the presence of high concentrations of preformed antifungal compounds. The major antifungal compound has been shown to be 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (Prusky *et al.*, 1982). The amount of this compound decreases tenfold, to sub-fungitoxic concentrations, during fruit ripening. The decrease correlates with the activation of quiescent infections. Similarly, it has been suggested that in citrus the resistance of young mature green lemons is related to the presence of a preformed monoterpene aldehyde, citral, that decreases in older yellow fruit, enabling decay to develop rapidly (Rodov *et al.*, 1995).

6.5.2 Induced antifungal compounds

Inducible defense responses include the early formation of physical barriers to pathogen development and/or the activation of biochemical defenses. Physical barriers may include strengthening of the plant cell wall via phenolic deposition and lignification to prevent the pathogen progressing beyond the first few penetrated cells. Biochemical defenses include the activation of pathogenesis related (PR) proteins, some of which are enzymes (e.g. chitinase and β-1-3-glucanase) known to hydrolyze components of fungal cell walls, and the *de novo* synthesis by the plant of antimicrobial secondary metabolites, known as phytoalexins.

In green banana, resistance to *C. musae* has been associated with a growing necrotic reaction within the peel. Five antifungal compounds not present in healthy tissue have been isolated from these necrotic tissues (Brown and Swinburne, 1980). In some cases, the induction of antifungal compounds during fungal penetration can lead to the confinement of the pathogen by an antimicrobial barrier. Stange *et al.* (1993) reported that 'wound gum' accumulated in injured citrus exocarp and predominantly consisted of the antifungal compound, 3-[4-hydroxy-3-(methyl-2-butenyl) phenyl]-2-(E)-propenal. This compound increased in concentration as a function of time, resulting in the complete quiescence of the invading pathogen.

Levels of antimicrobial secondary metabolites may also be induced by various physical treatments. For example, grapefruits exposed to UV irradiation, and subsequently inoculated with *P. digitatum*, exhibited increased resistance to the development of green mold decay. The increased resistance following UV irradiation was associated with the induction and accumulation of chitinase proteins in flavedo tissues. UV irradiation induced the accumulation of a 25 kD chitinase protein but had almost no effect on the levels of β-1,3-endoglucanase proteins. The induction of the 25 kD chitinase protein by UV irradiation was detected after 48 hours, and increased further after 72 and 96 hours (Chalutz *et al.*, 1992; Droby *et al.*, 1993). Resistance of citrus fruit to development of green mold has also been attributed to increased levels of phytoalexins produced in response to fungal infection (Kim *et al.*, 1991; Rodov *et al.*, 1992).

Heat may also inhibit pathogen spread by inducing defense mechanisms in the outer cell layers of the epicarp (Dettori *et al.*, 1996; Ben-Yehoshua *et al.*, 1997). Hot water rinsing and brushing (HWRB) induced the accumulation of 21, 22 and 25 kDa proteins that cross-reacted with citrus and tobacco chitinase antibodies and 38, 42 and 43 kDa proteins that cross-reacted with citrus and tobacco β-1,3-glucanase antibodies. The increase in the accumulation of glucanase and chitinase proteins may be part of a complex of fruit disease resistance mechanisms induced by the HWRB treatment (Pavoncello *et al.*, 2001).

Exposure of harvested mangoes to CO_2 at a flow rate of 100 mL of CO_2 min^{-1} for 24 hours significantly reduced anthracnose development. However, the optimum effective dosage of CO_2 varied according to cultivar, 20% CO_2 for 'Keitt', 60% CO_2 for 'Tommy Atkins'. A concomitant increase of 5-(7,12-heptadecadienyl) resorcinol was also detected in 'Keitt' fruits in response to the 20% CO_2 treatment (Kobiler *et al.*, 1998). Dipping fruit in hot water (55°C) for five minutes also resulted in an increase in 5-(7,12-heptadecadienyl) resorcinol.

6.6 Major pathogens of subtropical and tropical fruits

Although postharvest diseases are an inherent problem in fruit production worldwide, they may represent a greater problem under tropical conditions where high temperatures and humidity are common throughout the growing season and during the period of harvest and postharvest handling. These factors favor the development of postharvest diseases and are difficult to control without expensive handling and storage facilities. Aside from direct economic considerations, diseased produce also poses a potential health risk (Phillips, 1984; Bills *et al.*, 1992). A number of fungal genera such as *Penicillium, Alternaria* and *Fusarium* are known to produce mycotoxins under certain conditions (Barkai-Golan and Paster, 2008).

The identification of the causal pathogen and its biology can provide the basis of control strategies during storage and handling of fruits. Of the pathogens listed in Table 6.1, fungi of the genera *Colletotrichum*, *Botrytis*, *Lasiodeplodia*, *Diaporthe*, *Alternaria* and *Aspergillus* are considered to be the major pathogens responsible for

Table 6.1 Principal pathogens affecting subtropical and tropical fruits during postharvest life

Fruit	Pathogen	Disease
Avocado (*Persea americana*)	*Colletotrichum gloeosportoides*; *C. acutatum*	Anthracnose
	Various fungi including: *Dothiorella* spp. *Lasiodiplodia theobromae* *Stilbella cinnabarina* *Phomopsis perseae*	Stem-end rot
	Ewinia carotovora	Bacterial soft rot
Banana (*Musa sapientum*)	*Colletotrichum musae*	Anthracnose
	Various fungi including: *Fusarium roseum* *Verticillium* spp., *Acremonium* sp. and *Colletotrichum musae* *Thielaviopsis paradoxa, Deightoniella torulosa*	Crown rot
	Various fungi including: *Colletotrichum musae* *Lasiodiplodia theobromae*	Stem-end rot
	Nigrospora sphaerica *Thielaviopsis paradoxa* *Verticillium theobromae* and/or *Trachysphaera fructigena*	Cigar-end rot
Cactus (Prickly) Pear (*Opuntia ficus-indica*)	*Penicillium* sp.	Penecillium rot
	Alternaria spp.	Alternaria black rot
	Dothiorella ribis	Dothiorella fruit rot
Cherimoya (*Annona cherimola*)	*Colletotrichum gloeosporioides*	Anthracnose
	Phomopsis anonacearum	Black Canker
	Botryodiplodia theobromae	Botryodiplodia rot
Citrus (*Citrus medica*)	*Penicilltum digitatum*	Green mold
	Penicillium italicum	Blue mold
	Alternaria citrti	Black center rot
	Phomopsis citri, Lasiodeplodia theobromae	Stem-end rot
	Phytophthora citrophithora and/or P. parasitica	Brown rot
	Colletotrichum gloeosportoides	Anthracnose
Durian (*Durio zibethinus*)	*Colletotrichum gloeosporioides*	Anthracnose
	Cylindrocladium scoparium	
	Geotrichum citri-aurentii	Sour rot
	Lasiodiplodia theobromae	Stem-end rot
	Phytophthora palmivora	Phytophthora rot
	Aspergillus niger	Apergillus black rot
	Penicillium sp.	Penecillium rot
Feijoa (*Feijoa sellowiana*)	*Botrytis cinerea*	Grey mold

Fig (*Ficus carica*)	*Alternaria alternata*	Alternaria rot
	Aspergillus niger	Black mold rot
	Fusarium moniliforms	Endosepsis (pink rot)
	Various yeasts and bacteria including Saccharomyces sp. and Candida sp.	Souring
Guava (*Psidium guajava*)	*Colletotrichum gloeosporioides*	Anthracnose
	Aspergillus niger	Aspergillus rot
	Mucor hiemalis	Mucor rot
	Phomopsis destructum	Phomopsis rot
	Rhizopus stolonifer	Rhizopus rot
Kiwifruit (*Actinidia deliciosa*)	*Botrytis cinerea*	Grey mold
Litchi (*Litchi chinensis*)	*Alternaria* sp.	Black rot
	Aspergillus sp.	Aspergillus rot
	Penicillium sp.	Penicillium rot
	Botryodiplodia sp.	Stem-end rot
	Colletotrichum sp.	Anthracnose
Loquat (*Eriobotrya japonica*)	*Colletotrichum gloeosporioides,*	Anthracnose
	Botrytis cinerea	Grey mold
	Pestalotiopsis funereal	Pestalotiopsis rot
	Phytopthora cactoarum	Phytophthora rot
Mango (*Mangifera indica*)	*Colletotrichum gloeosporioides*	Anthracnose
	C. acutatum	
	Dothiarella spp.	Stem-end rot
	Lasiodiplodia theobromae	
	Phomopsus mangiferae	
	Pestalotiopsis mangiferae	
	Rhizopus stolonifer	Rhizopus rot
	Aspergillus niger	Black mold
	Alternaria alternata	Alternaria rot
	Botrytis cinerea	Grey mold
	Penicillium expansum	Blue mold
	Mucor circinelloides	Mucor rot
Mangosteen (*Garcinia mangostana*)	*Botryodiplodia theobromae* and *Diplodia sp.* and *Phomopsis* sp.	Stem-end rot
	Pestalotia flagisettula	Pestalotia rot
	Rhizopus sp.	Rhizopus rot
Papaya (*Carica papaya*)	*Colletotrichum* spp.	Anthracnose
	Phoma caricae-papayae	Black rot
	Phomopsis caricae-papayae	Phomopsis rot
	Rhizopus stolonifer	Rhizopus rot
	Phytophthora palmivora	Phytophthora fruit rot
Passion Fruit (*Passiflora edulis*)	*Alternaria passiflorae*	Brown spot
	Phytophthora nicotianae var. *parasitica*	Phytophthora rot
	Septoria passiflorae	Septoria spot

(*Continued*)

Table 6.1 Continued

Fruit	Pathogen	Disease
Persimmon (*Diospyros kaki*)	*Alternaria alternata*	Black rot
Pineapple (*Ananas comosus*)	*Thielaviopsis paradoxa*	Water blister
	Penicillium funiculosum	Fruitlet core rot
	Fusarium moniloforme var. *subglutinans*	
	Saccharomyces spp.	Yeasty rot
	Erwinia ananas	Bacterial brown rot
Pepino (*Solanum muricatum*)	*Alternaria solani*	Alternaria rot
Pomegranate (*Punica granatum*)	*Aspergillus* spp. *and Alternaria* sp.	Heart rot
	Botrytis cinerea	Grey mold
	Penicillium sp.	Penicillium rot
	Aspergillus sp.	Aspergillus rot
Starfruit (*Averrhoa carambola*)	*Altenraria alternata*	Alternaria rot
	Cladosporium cladosporioides	Anthracnose
	Botryodiplodia theobromae	Stem-end rot

postharvest diseases of most subtropical and tropical fruit, although an exception is noted with pineapple, citrus and durian (*Durio zibethinus*) (Coates *et al.*, 1995; Madden, 1992). The most observed diseases on these latter mentioned fruits are *Thielaviopsis paradoxa* causing water blister, *Penicillium* spp causing green and blue mold, and *Phytophthora palmivora* causing phytophthora fruit rot, respectively. Nevertheless, sporadic, serious postharvest disease losses are also caused by *Rhizopus* spp causing transit rot, and *Geotrichum candidum* causing yeasty rot.

Colletotrichum species, and its teleomorph *Glomerella*, is a pathogen with the widest host range among pathogens of tropical and subtropical fruit. The ability to cause latent or quiescent infections has grouped *Colletotrichum* among the most important postharvest pathogens of these crops. It causes significant economic damage with its typical disease symptoms referred to as anthracnose, characterized by sunken necrotic tissue where orange conidial masses are produced (Bailey and Jeger, 1992). Anthracnose diseases appear in both developing and mature plant tissues (Bailey *et al.*, 1992). Two distinct types of diseases occur: those affecting developing fruit in the field and those damaging mature fruit during storage.

The taxonomy of *Colletotrichum* species is changing constantly and remains confusing. The situation is particularly complicated with the species *C. acutatum* and *C. gloeosporioides*, which attack several hosts. Due to their broad (sometimes overlapping) host ranges and phenotypic and genotypic heterogeneity, *C. acutatum* and *C. gloeosporioides* are considered cumulative species composed of diverse subpopulations. Although the traditional methods to classify these fungi into distinct species are based primarily on morphological criteria, deviation from

type-culture characteristics and the presence of intermediate forms have introduced ambiguity into species identification (Freeman *et al.*, 1998).

6.7 Control of postharvest pathogens

6.7.1 Horticultural practices

The first step in ensuring quality of fruit crops is the selection of high-quality, disease-resistant cultivars followed by good handling and marketing practices (Korsten, 2006; Thompson *et al.*, 2002). This requires the implementation of an appropriate production system that utilizes crop management practices that ensure and maintain quality attributes and extend shelf life (Ippolito and Nigro, 2000). Stressful growing conditions, such as poor fertilization, over or under availability of water, and extreme high or low temperatures can have a direct effect on postharvest quality and disease susceptibility (Stretch and Ceponis, 1983; Hawthorne, 1989; Snowdon, 1992). Grobler *et al.* (2002) reported that mango trees exposed to a sustained water shortage and salt stress produced fruit that were more susceptible to *Botryosphaeria* spp. which is responsible for postharvest stem-end rot (Lonsdale, 1993). Because the level of fruit quality present at the time of harvest cannot be improved by postharvest practices, it is recommended to harvest tropical fruits at optimal size and maturity to maximize shelf life potential.

Fruits exposed to excessively high temperatures and mechanical injuries such as scrapes, bruises or abrasions during harvesting, handling, and transportation prior to storage are at a greater risk of postharvest disease. These conditions also increase respiration and ethylene production, thus accelerating senescence and shortening shelf life (Lizada, 1993; Snowdon, 1992; Thompson *et al.*, 2002). As previously stated, high temperatures and relative humidity preceding harvest also favor the growth of pathogens, significantly increasing levels of decay development during postharvest handling, storage, shipment and marketing (Fitzell and Muirhead, 1983).

6.7.2 Physical control methods

Heat

Several reports have been published on the use of heat to control postharvest diseases of subtropical and tropical fruits (Lurie, 1998; Falik, 2004). Heat treatments can be applied to harvested crops by hot water dip (WD), vapor heat treatment (VHT), hot dry air (HDA) or a very short, hot-water rinse (VSWR) and brushing. Heat treatments have a direct effect on postharvest disease development by killing spores or inhibiting germ tube elongation, thus reducing the quantity of effective inoculum and minimizing rots. Heat treatments can also indirectly affect decay development by altering the physiology of the fruit tissue and inducing defense-related compounds (Falik, 2004; Lurie, 1998; Shirra *et al.*, 2000).

Heat treatments have an important commercial application as an alternative to chemical fumigants and other more expensive treatments. Changes in the quality

of mangoes were measured after a vapor heat treatment (VHT at 47°C for 15 minutes) or hot water (HW) treatment at 53°C for five minutes prior to VHT. After treatment, fruit was stored at 10°C for five days followed by 22°C for five days, or stored at 22°C for ten days. Results indictated that the HW+VHT treatment, combined with continuous storage at 10°C, produced the highest quality fruit and was recommended for mango fruit destined for export by air freight (Jacobi and Giles, 1997). A combined hot water spray (HWS) and hot water brushing (HWB) treatment applied to mango was shown to reduce the incidence of *Alternaria alternata* postharvest rot (Prusky *et al.*, 1999). Results of the study demonstrated that the combination of fruit HWB and waxing yielded high quality fruits with less decay development (Prusky *et al.*, 1999).

Nashijima *et al.* (1992), however, reported that forced, hot-air treatment did not significantly reduce the incidence of postharvest diseases of papaya when compared with some fungicides or hot water treatments. Postharvest storage experiments using various organically grown citrus cultivars showed that hot water bruising (HWB) reduced green mold (*Penicillium digitatum*) by 45–55% (Porat *et al.*, 2000). Various combinations of heat treatments were also effective in reducing *Penicillium* molds on 'Oroblanco' citrus (Rodov *et al.*, 2000). Olesen *et al.* (2004) reported that the most effective hot water dip to slow the rate of rot development of litchi by approximately 15% was one minute at 52°C. Hot water treatment combined with gamma irradiation totally eliminated strawberry decay caused by *Alternaria alternata* but induced more rapid fruit softening (Barkai-Golan *et al.*, 1993). Prestorage heat treatment (38°C for three days) was also reported to improve the quality of mango fruit and greatly reduced development of decay caused by *C. gloeosporioides* following cold storage at 4°C for three weeks (Kesta *et al.*, 2000).

UV-C and γ-irradiation

Postharvest diseases of tropical fruits can also be controlled by γ irradiation. Control of the postharvest gray mold disease (*Botrytis cinerea*) of strawberry fruits by gamma irradiation is improved by heat treatment prior to irradiation (Sommer *et al.*, 1968). A synergistic interaction between heat and radiation was observed that resulted in a level of decay prevention that could not be obtained by either treatment alone without excessive fruit injury. The use of gamma irradiation was examined for its ability to reduce decay of pineapple (*Ananas comosus* L., Merr. cv. Queen) caused by the fungal pathogens, *Ceratocystis paradoxa* and *Penicillium purpurogenum*, and also for its ability to prolong storage life (Dayamanti *et al.*, 1992). Results indicated that irradiation at several low doses (≤250 Gy) reduced decay development and increased shelf life. As an alternative to gamma irradiation, UV-C treatment of mango resulted in better overall appearance, lower decay percentage and increased shelf life (González-Aguilar *et al.*, 2007).

6.7.3 Chemical control methods

While several different approaches to postharvest disease control have been explored, chemical control is still the most widely used method. The first

generation of fungicides used to prevent postharvest diseases infection of tropical fruits, mainly caused by the wound-pathogens, *Penicillium, Rhizopus, Aspergillus*, and *Ceratocystis*, were developed during the mid-1960s. The main chemicals used for postharvest disease control are benzimidazoles (thiabendazole, benomyl and carbendazim) and sterol inhibitors (imazalil, prochloraz and propiconazole). These chemicals also provided good control of anthracnose and stem-end rot on citrus, banana, mango, papaya, pineapple and avocado (Eckert, 1990). However, the ability of these fungicides to eradicate infections largely depends upon the time elapsed between infection and application, the infection location, and the extent of pathogen penetration into fruit tissue (Eckert and Ogawa, 1985; Eckert, 1990; Prior *et al.*, 1992). Preharvest treatment of mangoes with these chemical fungicides has also been reported to be effective in controlling postharvest stem-end rot during storage (Johnson *et al.*, 1996; Lonsdale *et al.*, 1991).

Sulphur dioxide (SO_2) has been used to control postharvest diseases of litchi and longan (Han *et al.*, 2001; Tongdee, 1991). Cappellini *et al.* (1986) also reported that sulphur dioxide was effective in controlling grey mold (*Botrytis cinerea*) and other postharvest diseases of grapes.

Potassium sorbate (KS), a common food preservative, can be used to control postharvest decay of citrus fruit. Smilanick *et al.* (2008) investigated the effect of KS combined with heat and other fungicides to control citrus decay and observed that heat increased fungicide residues in oranges. They also noted that sour rot decay, caused by *Geotrichum citri-aurantii*, was reduced by immersion in hot solutions of KS or sodium bicarbonate. *Penicillium digitatum* and *Penicillium italicum* were also controlled on 'Clemenules' and 'Nadorcott' mandarins, 'Fino' lemons, 'Ortanique' mandarins, and 'Valencia' oranges, by immersion in KS. After 60 days storage at 5°C, green and blue molds on 'Valencia' oranges were reduced by 96 and 83% respectively, using KS immersion (Montesinos-Herrero *et al.*, 2009).

Many compounds produced naturally by microorganisms and plants have fungicidal properties. The exploitation of some natural products such as flavor compounds, acetic acid, jasmonates, glucosinolates, propolis, fusapyrone and deoxyfusapyrone, chitosan, and essential oils have been investigated for their ability to control postharvest diseases of fruits and vegetables as well as prolong shelf life (Droby *et al.*, 1999; Tripathi and Dubey, 2004).

Chitosan, for example, is not only an elicitor of host defense responses but also has direct fungicidal action against a range of postharvest pathogens. Chitosan has been shown to control numerous pre- and postharvest diseases on various horticultural commodities. It has been reported that both soil and foliar plant pathogens fungal, bacterial and viral may be controlled by chitosan application (Bautista-Baños *et al.*, 2006). Jiang and Li (2001) reported that chitosan coating enhanced the postharvest life and quality of longan fruit.

6.7.4 Biological control

As a method of managing postharvest diseases, biological control is a relatively new approach that has developed into a science over the past 20 years. The origins

of this approach, its current status, and future directions have been recently reviewed (Droby *et al.*, 2009; Sharma *et al.*, 2009; Janisiewicz and Conway, 2010). The approach is based on the application of high populations of microbial antagonists to fruit surfaces during the postharvest handling of harvested fruits in order to delay and suppress the development of postharvest pathogens. Some management schemes also employ the application of the microbial antagonist one to several times in the field prior to harvest. However, to ensure consistent performance under commercial conditions, microbial antagonists are often used in combination with physical management approaches (e.g. heat treatment), natural antimicrobial compounds (e.g., chitosan), or low doses of fungicides (Droby *et al.*, 2009; Sharma *et al.*, 2009).

Yeasts represent the majority of postharvest biocontrol agents reported in the literature and most of the antagonists that have been developed into products are yeasts (Droby *et al.*, 2009). While several products are in development or awaiting registration in the US or Europe, there are a number of biologically based products that are currently commercially available for controlling postharvest diseases of citrus and other fruits and vegetables: Shemer, based on the yeast, *Metschnikowia fructicola* developed in Israel; BioSave, based on the bacterium, *Pseudomonas syringae* developed in the US; Candifruit, based on the yeast *Candida sake* developed in Spain; Boni-Protect, based on the yeast *Aurerobasidium pullulans* developed in Germany; NEXY, based on the yeast *Candida oleophila* developed in Belgium; and Avogreen based on the bacterium *Bacillus subtillis* developed in South Africa. The review by Sharma *et al.* (2009) provides a comprehensive table of postharvest diseases on various commodities for which biological control has been explored, as well as the antagonists that have been demonstrated to exhibit effective control.

The use of yeast antagonists for the control of postharvest diseases was seen as a protective approach, i.e., in order for the yeast to prevent decay it needed to be present in a wound site prior to the establishment of the pathogen. For this reason, the combining of yeast antagonists with other control measures has been explored (Sharma *et al.*, 2009; Droby *et al.*, 2009; Janisiewicz and Conway, 2010). The use of a combination of methods has greatly improved the reliability and effectiveness of biocontrol agents and also allowed some products to exhibit both eradicant and protectant activity. In this regard, the combination of the yeast, *Candida saitoana*, with either the sugar analog, 2-deoxy-D-glucose, glycolchitosan, or lysozyme, was shown to greatly improve control of a variety of postharvest diseases of citrus and apple (El Ghaouth *et al.*, 2000a; El Ghaouth and Wilson, 2002; El Ghaouth *et al.*, 2000b).

Among tropical and sub-tropical fruits, and most relevant to the current chapter, biological control of postharvest diseases of citrus has been the commodity most extensively studied (Palou *et al.*, 2008). Several antagonists, including *Metschnikowia fructicola*, *Pichia guilliermondii*, *Candida oleophila*, *Candida saitoana* and *M. pulcherrima*, as well as bacterial isolates have been shown to effectively control a variety of postharvest pathogens of citrus (Kinay and and Yildiz, 2008; Sharma *et al.*, 2009). Abraham *et al.* (2010) screened over 60 yeast

isolates and 92 *Bacillus* isolates for their ability to control *P. digitatum* on citrus and identified ten yeast and ten *Bacillus* that reduced lesion size. However, two of the yeast isolates (B13 and Grape) were superior to all the *Bacillus* isolates. Interestingly, Bull *et al.* (1998) demonstrated that the antibiotic, syringomycin E, was produced by the bacterial antagonists (strains of *Pseudomonas syringae*) used in the commercial product BioSave (Jet Harvest Solutions, Longwood, FL, USA) and was responsible for the biocontrol activity.

Early work by Krauss *et al.* (1998) identified 13 potential microbial antagonists out of 30 isolates obtained from various banana tissues. These 13 demonstrated varying ability to control a complex of organisms responsible for crown rot of banana. The antagonists were screened for various biocontrol features such as efficacy, host range, aggressiveness against survival structures such as appressoria, and tolerance to fungicides. While eight of the antagonists exhibited varying combinations of desirable traits, none were sufficient to proceed to more detailed evaluations. The results were promising enough, however, that the authors recommended further studies. De Costa *et al.* (2008) screened a wide array of bacterial and fungal isolates for control of postharvest diseases of banana and indicated that the bacterium *Burkholderia spinosa* was the most effective at controlling anthracnose, crown rot, and blossom end rot of banana. They also indicated that this organism could effectively control anthracnose and stem end rot in avocado, mango and pineapple. Williamson *et al.* (2008) demonstrated that the strain, ESC-11, of the biocontrol agent (*Pseudomonas syringae*) used in combination with low doses of the fungicides (thiabendazole and imazalil) reduced crown rot and anthracnose of banana caused by a complex of fungi, including *Colletotrichum musae, Fusarium* sp., and *Gliocladium* sp.). Effective control was demonstrated in both laboratory and field experiments and further studies of combining low doses of the fungicide with the biocontrol agent were recommended.

Govender *et al.* (2005) and Govender and Korsten (2006) evaluated a strain of the bacterium *Bacillus licheniformis*, alone or in combination with the chemicals prochloraz and stoburilin, for their ability to reduce postharvest diseases (anthracnose and stem-end rot) of mango (*Mangifera indica*) when applied as a dip treatment in a mango packinghouse. Results indicated that the biocontrol agent could be effectively used in an integrated manner with the chemicals and was more effective at control then the standard chemical treatments when mangoes were kept in cold storage to simulate export conditions. Total recovered populations of yeast and bacteria were higher in the fruit treated with the biocontrol agent compared to fruit that had been dipped in hot water and treated with prochloraz.

Biological control of Diploida rot (*Botryodiplodia theobromae*) of guava (*Psidium guajava*) was examined by Hashem and Alamri (2009). They evaluated five yeast strains (*Pichia anomola* Moh 93, *P. anomola* Moh104, *Pichia guilliermondii* Moh10, *Lipmyces tetrasporus* Y-115 and *Metshnikowia lunata* Y1209). Of the yeast tested, the two strains of *P. anomala* were the most effective at controlling postharvest rot of guava, reducing disease by as much as 50%. In

this study, they also demonstrated the ability of *P. anomala* to adhere to hyphae of *B. theobromae* and inhibit both cellulose and pectinase activity by the pathogen. Haggag and Nofal (2006) also demonstrated control of Diplodia rot on different cultivars of the tree fruit species, *Annona*, which produce the fruits, Soursop (*Annona muricota*), Cherimoya (*Annona cherimola*), and Sweetsop (*Annona squamosa*). The most effective control was obtained when a multi-bioagent mixture consisting of a mixture of *Trichoderma* and *Pseudomonad* species was applied. The combination of several species of each genus was more effective than any sole or even single combination applications.

Wijesinghe *et al.* (2010) demonstrated that an isolate of *Thrichoderma asperellum* could control the causal agent of black rot, *Thielaviopsis paradoxa*, of pineapple (*Anansas comosus*). An application of 10^5 conidia per mL of *T. paradoxa* to pineapple fruit was applied followed by application of a formula of *T. asperellum*. Fruit were completely free of disease when stored at 28°C for seven days. Antagonistic activity appeared to be mainly due to coil formation of the *Trichoderma* hyphae around the pathogen hyphae. A strain (ALF 247) of *Trichoderma martiale* was also shown to be effective at controlling black-pod disease (*Phytophora palmivora*) of cacao (*Theobroma cacao*).

Cao *et al.* (2009) combined the antagonistic yeast, *Pichia membranefaciens*, with methyl jasmonate treatment to control postharvest anthracnose rot (*Colletotrichum acutatum*) of loquat (*Eriobotrya japonica*). The application of methyl jasmonate increased the levels of defense-related enzymes (chitinase and β-1,3-glucanase) in the fruit and enhanced populations of the biocontrol agent. The results of the study indicated that control of the anthraconose rot of loquat fruit was better with the combination than with either the yeast or methyl jasmonate alone. The effect of the methyl jasmonate was directly due to the inhibition of *C. acutatum* spore germination and the promotion of yeast growth in the wound and indirectly due to the enhancement of native fruit defense-related enzymes.

Lastly, the use of *Muscador albidus* as a biofumigant may be especially relevant to the control of postharvest diseases of tropical fruit (Schotsmans *et al.*, 2008). This endophytic fungus inhibits or kills a broad range of fungi and bacteria by the production of a mixture of volatile organic compounds. *M. albidus* as a biofumigant has also been demonstrated to have insecticidal properties (Lacey and Neven, 2006). The biofumigant is active at both warm and low temperatures which makes its potential for use in management of postharvest diseases of tropical fruits of special interest.

6.7.5 Integrated control methods

Integrated methods to control postharvest diseases have also been tested, although not on many subtropical and tropical fruits. Some *Bacillus subtilis* strains, isolated from citrus fruit surfaces, were evaluated alone, or in combination with sodium bicarbonate (SB) or hot water treatment for control of citrus green and blue molds caused by *P. digitatum* and *P. italicum*. Applied alone, the isolated strains showed significant ability to control these two diseases, but they were not as effective as

some chemical treatments (fungicide). The treatments comprising a biological control combined with SB, or biological control applied following hot water treatment, were as effective as the fungicide treatment, and gave total control (100%) of both green and blue molds (Obagwu and Korsten, 2003). Preharvest application of *Bacillus subtilis* field sprays integrated with copper oxychloride or benomyl reduced the severity of avocado black spot (BS), caused by *Pseudocercospora purpurea* (Korsten *et al.*, 1997). Combining antagonistic bacteria occurring naturally in the fructosphere with a hot water treatment has been used to control postharvest diseases of banana (de Costa and Erabadupitiya, 2005). Antibiotics produced by various species of *Trichoderrna* have potent antifungal activity against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Corticium rolfsii* and other important plant pathogens. There are many other natural compounds which have been isolated and shown to possess considerable antifungal activity. Although these compounds may be more desirable than synthetic chemicals from a consumer viewpoint, their potential toxicity to humans needs to be evaluated before useable products are developed. On a positive note, many of these compounds are readily biodegradable (Spadaro and Gullino, 2004).

6.8 Conclusions and future challenges

Disease management programs have to be developed specifically for each crop and largely depend on epidemiological and etiological information. Because this data is lacking for many tropical commodities, a great deal of research still needs to be conducted. Lacking this information, the availability and utilization of simple cultural methods previously discussed (adequate sanitization, refrigeration, controlled atmosphere facilities, etc.) could greatly reduce postharvest losses due to postharvest disease. The use of fungicides as an effective tool again requires a basic understanding of the pathogens involved in the disease and a variety of factors that affect pathogenesis. Chemical fungicides, however, have come under scrutiny and their use as a control strategy in the long term is somewhat questionable. This is because of problems associated with (1) failure to effectively control decay in several harvested fruit commodities due to the development of fungicidal resistance, (2) consumers' desire to reduce human and environmental exposure to chemicals, and (3) increased restrictions imposed by governmental regulatory agencies on the use of agro-chemicals and/or allowable residues. These issues have been the driving force for the development of postharvest disease control measures that do not rely on chemical fungicides. Indeed, reduced-chemical postharvest disease control measures have now become an economic imperative, not just an option. Currently, the use of alternative non-chemical control methods as stand-alone treatments, however, does not provide the efficacy and consistency required for commercial postharvest situations. At this time, a more realistic scenario to combat decay of harvested commodities is the use of an integrated control approach combining biological, GRAS chemicals, and physical

control strategies, with or without limited quantities of pre-harvest fungicides, along with proper management and handling practices.

Developing biological control approaches for the management of postharvest diseases of tropical fruits has been especially challenging for several reasons. Outside of a few commodities, many tropical fruits are produced only in relatively small quantities, and are often grown in unmanaged plots. Postharvest management strategies are often lacking or unknown. Additionally, modern refrigeration facilities for storage and shipment are non-existent. These factors may even be true for well-known commodities that are grown in small plots by farmers lacking knowledge or access to modern, technologically advanced management methods. However, the same factors that make the development of biocontrol agents for postharvest use problematic may also be the very reason why biological solutions may be the most appropriate.

The identification, development and registration of biocontrol agents are relatively easier and inexpensive compared to the development and commercialization of a new chemical. Screening for postharvest antagonists is a simple protocol and many effective strains of antagonists have been isolated from locally grown fruits. Production of the antagonists can be done locally and distributed to growers at the appropriate time. Local strains of antagonists may even be better at controlling local strains of specific pathogens, though this has not been demonstrated. Since most postharvest antagonists occur naturally on fruit surfaces and are ingested by consumers, their application poses a very low health risk to both growers and consumers. For these reasons, the development of new antagonists for controlling a wide array of postharvest diseases on numerous different tropical fruit may hold great promise.

6.9 References

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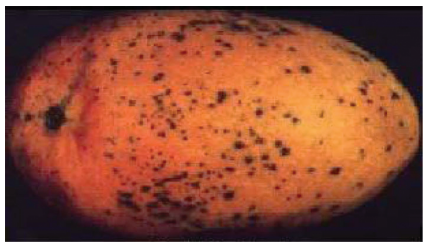
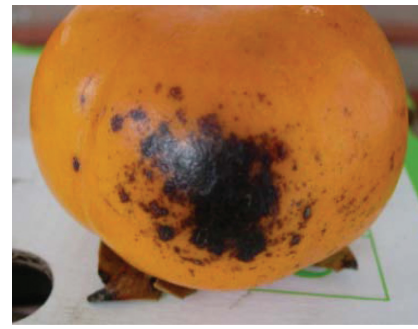
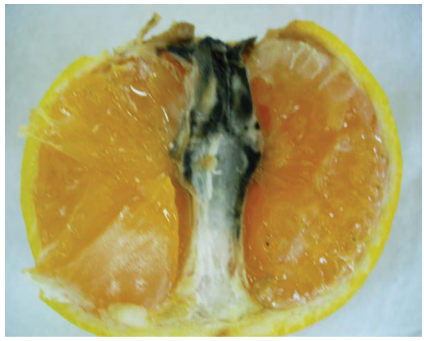
Plate VII (Chapter 5) Banana washing at packinghouse in Costa Rica (courtesy of Prof. Adel A. Kader).



(a)



(b)



(c)

(d)

Plate VIII (Chapter 6) (a) Black spot disease on mango caused by *Alternaria alternata*; (b) black spot disease on persimmon caused by *Alternaria alternata*; (c) black rot on mandarin caused by *Alternaria citri*; (d) stem-end rot on grapefruit caused by *Botrydiplodia theobromae*.

7

Quarantine pests of tropical and subtropical fruits and their control

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Abstract: International trade in tropical and subtropical fruits has become increasingly important, but phytosanitary restrictions continue to limit its growth. Phytosanitary or quarantine treatments are often required to disinfest host commodities of economically important arthropod pests before they are moved through market channels within or between countries to areas where the pest does not occur. This chapter describes some of the important quarantined pests, as well as some of the treatments that can be used for their control and the effects of the commercially developed or potential insect quarantine treatments and systems on the quality of tropical and subtropical fruits, thus allowing the evaluation of the potential of the commercial application of these treatments or systems on the basis of their effect on fruit quality.

Key words: pests, postharvest fruits, quarantine, irradiation, controlled atmospheres, temperature.

7.1 Introduction

Many insect pests, such as tephritid fruit flies, codling moth, oriental fruit moth, mango seed weevil, pineapple mealy bug, oriental citrus mite, pecan weevil, litchi fruit borer, false codling moth, among several others, are of quarantine importance in different regions of the world because they are either absent from an importing region or country, or the importing region or country has a 'zero tolerance' for any live insects whether or not they are economically important. Some important quarantine insect pests are shown in Table 7.1 and Plate IX (in the color section between pages 238 and 239).

Quarantine treatments are applied to fresh tropical and subtropical fruits to prevent the export of quarantined pests to regions free of these pests within the

Table 7.1 Examples of some important quarantine pests

Common name	Scientific name	Common hosts	Distribution
	INTERNAL FRUIT FEEDERS		
	Diptera		
Mediterranean fruit fly	*Ceratitis capitata* (Wiedemann)	Deciduous, subtropical and tropical fruits	Southern Europe, Africa, Central America, South America, Western Australia, Hawaii
Mexican fruit fly	*Anastrepha ludens* (Loew)	Citrus, mango, tropical and subtropical fruits	Mexico, Central America
West Indian fruit fly	*Anastrepha obliqua* (Macquart)	Mango, guava, other tropical and subtropical fruits	Caribbean, South Texas and Florida, Mexico, Central America, Venezuela, Ecuador, Brazil (Rio de Janeiro)
Caribbean fruit fly	*Anastrepha suspensa* (Loew)	Tropical and subtropical fruits	Caribbean, Southern Florida
Sapote fruit fly	*Anastrepha serpentina* (Wiedemann)	Tropical and subtropical fruits	North America (south Texas), Mexico, Central America, South America
Guava fruit fly	*Anastrepha striata* (Schiner)	Guava and other tropical and subtropical fruits	Mexico, Central America, South America, Caribbean (Trinidad)
South American fruit fly	*Anastrepha fraterculus* (Wiedemann)	Citrus, apples, grape, mango, and tropical and subtropical fruits	South America
South American cucurbit fly	*Anastrepha grandis* (Macquart)	Cucurbits, (watermelon, pumpkins, honeydew melon, among others), guava	South America
Melon fruit fly	*Bactrocera cucurbitae* (Coquillett)	Cucurbits, tomato, several other fleshy fruits	Hawaii, Asia, Papua New Guinea, Africa
Carambola fruit fly	*Bactrocera carambolae* (Drew & Hancock)	Carambola (star fruit) and other subtropical and tropical fruits	South America (Surinam, Guyana, French Guiana)
Queensland fruit fly	*Bactrocera tryoni* (Froggatt)	Deciduous, subtropical and tropical fruits	Australia, Pacific Islands
Oriental fruit fly	*Bactrocera dorsalis* (Hendel)	Most fleshy fruits and vegetables	Asia, Hawaii

(Continued overleaf)

Table 7.1 Continued

Common name	Scientific name	Common hosts	Distribution
Invader fruit fly	*Bactrocera invadens* Drew, Tsurua, & White	Mangos, tomatoes, bananas and guavas	Sub-Saharan Africa, southern India
Papaya fruit fly	*Toxotrypana curvicauda* Gerstaecker	Papaya, and various wild hosts	Florida, Mexico, Caribbean, Central America, South America (Colombia and Venezuela)
		Lepidoptera	
False codling moth	*Thaumatotibia leucotreta* (Meyrick)	Avocado, carambola, citrus, mango, pineapple, litchi, and other tropical and subtropical fruit	Sub-Saharan Africa, Israel
Avocado seed moth	*Stenoma catenifer* Walsingham	Avocado, a few wild hosts	Brazil, Venezuela, Ecuador, Peru, Colombia, Bolivia, Panama, Costa Rica, Guatemala, Mexico
		Coleoptera	
Mango seed weevil	*Sternochetus mangiferae* (Fab.)	Mango	Asia, Africa, Australia, Hawaii
		EXTERNAL FRUIT FEEDERS	
		Hemiptera	
Pineapple mealy bug	*Dysmicoccus brevipes* (Cockerell)	Pineapple	Asia, Africa, Australia, South America, Pacific Islands
Pink hibiscus mealy bug	*Maconellicoccus hirsutus* Green	Tropical, subtropical fruit, vegetables and ornamental plants	Asia, Middle East, Africa, Australia, Oceania, reported in Florida, USA in 2002
		Thysanoptera	
Red banded thrips	*Selenothrips rubrocinctus* (Giard)	Many tropical, subtropical fruits, ornamentals and shade trees	Asia, Africa, Australia, Oceania, North America (Florida), Mexico, Central America, Caribbean, South America

same country or in other countries. Several treatments have been developed, but the most commonly applied, especially prior to the 1990s, involved the use of chemical fumigants such as ethylene dibromide (EDB) and methyl bromide (MeBr). On December 14, 1983 EDB was banned by the US Environmental

Protection Agency (EPA) due to its identification as a carcinogen (Anon., 1993). This prohibition caused major restrictions and difficulties in the export and trade of many commodities from several countries. The ban on EDB resulted in MeBr becoming the treatment of choice for achieving quarantine security for fresh horticultural crops in several countries. However, in 1992, MeBr was classified as an ozone depleter (Anon., 1992) and subsequently the US EPA included it as a chemical to be removed from use by 2001 (Clean Air Act, 1991, Title VI section 602). In October 1998, the US Congress, under the 1999 Appropriations Bill, altered the US Clean Air Act (Sec. 764.a) to reflect the restrictions of the Montreal Protocol. This meant that restrictions on the use of MeBr would not apply to postharvest and phytosanitary uses.

Due to the restriction in the use of chemical fumigants, significant research efforts have been directed at the development of alternative physical (non-chemical) quarantine treatments to maintain export markets for fresh horticultural products, including tropical and subtropical fruits. Among the alternative treatments investigated are the use of low and high temperatures, ionizing radiation, microwaves, ultraviolet radiation, infrared radiation, X-rays, modified and controlled atmospheres (low oxygen and/or elevated carbon dioxide atmospheres), or the combination of some of these. The type of treatment or system investigated or selected has been largely determined by commodity tolerance. Several quarantine treatments or systems are now available as alternatives to fumigants. For example, current quarantine treatments for oriental fruit moth include either MeBr fumigation or cold storage for several weeks (Hallman, 2004).

The goal of the quarantine treatments or systems is to ensure the death of real or potential infesting insects to meet quarantine requirements, while minimizing the effects of these treatments on commodity quality (Yahia, 2006). This goal is all the more challenging given that the effectiveness of quarantine treatments and systems is often evaluated based on strict standards such as the 'probit 9' concept, which refers to a mortality rate of 99.9968%, or no more than 32 survivors from a population of 1 000 000. Japan often uses a variation of the probit 9 concept that requires no survivors from a treated population of 30 000 target insects. Given the high quarantine standards that must be met to maintain current export markets, it has become increasingly important to develop innovative, non-contaminating and integrative alternative methods for quarantine treatments. A systems approach can achieve effective insect control, integrating control measures in the field, control of the commodity's maturity at harvest, inspection at the packing station, quarantine treatments, and certification prior to shipment. Figure 7.1 shows the inspection of quarantined fruit at origin (a) and at the import side (b).

7.2 Internal fruit feeders: an introduction

Insect pests that feed internally in fruit are more difficult to detect and to disinfest, and are thus of greater concern in quarantine efforts. This type of feeding is most highly evolved and successful in several groups of the Lepidoptera, Diptera and

(a)

(b)

Fig. 7.1 Inspection of fruit, (a) at origin in a mango packinghouse in Mexico, and (b) at the import side at Pharr, Texas, USA port of entry.

Coleoptera, wherein the adult stage locates the host upon which the larval stage feeds and develops. Larvae enter fruit in one of two ways. In the Lepidoptera, for example, the eggs are placed by adults on or in the vicinity of the fruit, and the first larval stage penetrates the fruit after hatching. In contrast, for internal fruit

feeding Coleoptera and Diptera, the adults choose and penetrate fruits using specialized ovipositors or mouthparts which allow them to place eggs directly in fruit tissues.

7.3 Diptera: Tephritidae

7.3.1 Mediterranean fruit fly (medfly): *Ceratitis capitata* (Wiedemann)

The 'medfly' is generally considered to be the most economically important pest fruit fly. Although its name implies that it is native to the Mediterranean region, and it is an important pest there, researchers place its origin in equatorial Africa (Gilstrap and Hart, 1987). It is now ubiquitous in the tropical regions of the world. It established in Hawaii in 1910 and Central America in the early 1960s. It can infest more than 250 varieties of fruits and vegetables including many ornamental dooryard fruits (Liquido *et al.*, 1990). Hence, as an invasive species it easily establishes populations in urban areas (Lance and Gates, 1994). Unfortunately one of its preferred hosts is coffee (*Coffea arabica* L.). Yet, it is not considered to be a pest of coffee because it only infests the ripened berry, not the internal seed or coffee bean. Because organic coffee fetches a higher price, and because the medfly infestations do not cause economic losses to the coffee growers, the medfly is not controlled in coffee. Thus coffee serves as a primary host plant that sustains a persistently large population of the pest that plagues domestic fruit production in those areas, and such areas are under an essentially permanent quarantine.

Attempts to suppress medfly infestations are typically carried out by public programs including application of the Sterile Insect Technique (SIT) (Vargas *et al.*, 1994). In Guatemala for example, the 'Moscamed' factory at Petapa has a capacity to produce 50 billion sterile medflies per year. Similar factories operate in Mexico, Peru, Argentina and Hawaii. Beginning in 1980, a series of medfly outbreaks occurred in California, USA, with such regularity and expense that a strategy of prophylactic sterile releases has been instituted (Penrose, 1993; Dowell *et al.*, 1998). Such is the seriousness of the threat even to temperate agricultural zones.

7.3.2 Mexican fruit fly (mexfly): *Anastrepha ludens* (Loew)

The mexfly is considered to be the most important insect pest of oranges (*Citrus sinenesis* Osbeck) and mangos (*Mangifera indica* L.) in Mexico (Gutierrez-Samperio *et al.*, 1993). In addition to these major crops, the 'mexfly' is known to infest at least 60 varieties of fruit (Norrbom and Kim, 1988). Hence, exportation of any fruits from infested regions is subject to quarantine restrictions. The fly is found in areas from the southern USA (Texas) to Panama. It is most intensely managed in Mexico and the citrus producing border states of the USA using SIT (Thomas *et al.*, 1999). Quarantine protocols allow the export of host fruits from low prevalence regions where SIT is applied under a set of provisions including detection trapping, inspection of commodities moving in and out of the regulated

zone, and bait-sprays around detection sites (Nilakhe *et al.*, 1991). In Mexico, suppression with SIT is augmented with biological control and bait stations with limited broadcast bait sprays (Ovruski *et al.*, 2000).

Management of the mexfly as an invasive pest has matured beyond reliance on postharvest treatments of potentially infested commodities to provide quarantine security. The 'Maximum Pest Limit' concept considers such factors as fly phenology, infestation rates, survival rate within fruits, and selection at harvest along with the pest management practices at the grove level (Mangan *et al.*, 1997). For example, Aluja *et al.* (2004) demonstrated that Hass avocados, *Persea americana* (Mill.) are not susceptible to mexfly oviposition while still attached to the tree. The female can only penetrate the fruit at the peduncle. By harvesting only unripened fruit picked from the tree and by moving picked fruit to closed sheds away from alternative hosts, postharvest treatments and quarantine restrictions are unnecessary. Solanaceous crops such as jalapeño peppers, bell peppers (*Capsicum anuum* L.), and tomatoes are seldom attacked by mexfly although they are perfectly suitable hosts in the laboratory. In contrast, manzano peppers (*Capsicum pubescens* Ruiz & Pavon), are heavily infested by mexflies, resulting in a ban of their exportation to the USA. The susceptibility of this host is traceable to the behavior of the fly and the production methods of this type of pepper. Manzano peppers, native to the Andes region of South America, are grown in the shade, often intercropped with other fruit trees; habitats favored by mexflies. Tomatoes and jalapeño crops grown in sunny, open fields, or *solares*, are avoided by mexflies (Thomas, 2004).

7.3.3 West Indian fruit fly: *Anastrepha obliqua* (Macquart)

The West Indian fruit fly is a serious pest of mangoes throughout Latin America, though mainly at low elevations. Unfortunately, most commercial mango production is at low elevations. It is also a pest of tropical plums, *Spondias* spp., members of the family Anacardiaceae, as are mangoes. Fruits of this genus, also called 'hog-plums' or 'mombins', are believed to be its primary native plant host. These fruits are grown and consumed locally but there is not much demand for their export. The West Indian fruit fly is also a minor pest of guavas. *Anastrepha obliqua* is another of the 'broad' host utilizing species, with Norrbom and Kim (1988) listing records for 70 species of fruit. As a quarantine pest this species has been problematic, in part because of its checkered taxonomic history. Although named *obliqua* by Macquart in 1843, the name used in the agricultural literature for most of the twentieth century was A. *mombinpraeoptans* Sein, which itself was considered by some as a subspecies of *Anastrepha fraterculus*, the South American fruit fly (Hernandez-Ortiz, 1992). As a consequence many of the host records attributed to *A. obliqua* or to its synonym, *A. mombinpraeoptans*, especially records infesting oranges and apples, were most likely *A. fraterculus* or another closely related species, *Anastrepha suspensa*, the Caribbean fruit fly. And some records when traced back were based on no more than the tree that held the trap where the fly was captured – 'host' records which have been promulgated in

the literature. These dubious host records are problematic for those responsible for writing quarantine regulations that are supposedly based on sound science. The biology of this insect has been studied extensively (Aluja *et al.*, 1987; Aluja and Birke, 1993) and it is clear that it prefers warm climates and infests only tropical fruits. Consequently, it poses little threat to temperate crops and for this reason mangoes and guavas are exported to Canada without restrictions but not to the USA, in part because of the literature records of it infesting citrus. An estimated 200 000 tonnes of Mexican mangoes are exported annually (Heather and Hallman, 2008); most of it receiving an expensive hot water disinfestation treatment. (All mangoes exported to the USA, Japan and some other countries (except mangoes produced in the north of the state of Sinaloa in Mexico) must receive the treatment. Those exported to Europe and Canada are not treated.)

7.3.4 Caribbean fruit fly: *Anastrepha suspensa* (Loew)

The Caribbean fruit fly became established in south Florida in 1965 where its primary host is the berries of plants in the genus *Eugenia* (Myrtaceae). Widely planted as an ornamental hedge, the Surinam Cherry supports a population of this pest fly in urban areas of the state. Native to the islands of the Caribbean, it is generally not considered an economic pest (Weems, 1966). However, this is another species with a wide host range which includes *Citrus* spp. and as a consequence, Florida oranges and grapefruits are quarantined by many foreign countries and by the other citrus producing states of the US (Riherd, 1993).

7.3.5 Sapote fruit fly: *Anastrepha serpentina* (Wiedemann)

The sapote fruit fly is one of the most widely distributed species of neotropical fruit flies, with a geographic range from south Texas to Argentina. In North America, this species is occasionally trapped in commercial citrus production areas. When these areas are under management to achieve pest free status or low pest prevalence for quarantine security, this species must be considered as subject to quarantine. A series of studies, summarized in Norrbom (2004), document that *A. serpentina* can breed in a wide range of commercial fruits, and lists 45 species from 28 genera in 17 families as field hosts. Of the 18 genera and 29 species that are native hosts for *A. serpentina*, five of the genera and 15 species belong to the Sapotaceae. Species in this family are sometimes heavily attacked by *A. serpentina* and the common name in many reports is the 'Sapote fruit fly'. Among commercial fruits this includes the mamey, *Pouteria sapota* (Jacq.) and chicosapote, *Manilkara zapota* (L.). Its native hosts in northern Mexico are members of the genus *Sideroxylon* (Baker *et al.*, 1944). At one time this species was common in the southern USA but, for reasons not well understood, it has all but disappeared. Because of dubious records on *Citrus* it is considered a quarantine pest and triggers export restrictions when captured in surveillance traps. It is easily recognized by its dark markings and is thus sometimes referred to by the common name 'black fruit fly'.

7.3.6 Guava fruit fly: *Anastrepha striata* (Schiner)

The guava fruit fly ranges from northern Mexico to Brazil. In our experience it is difficult to find wild guavas that are not infested by this fly. It is another of the *Anastrepha* with a wide alternate host range. Norrbom and Kim (1988) list 26 fruit species in 12 families as recorded hosts. Commercially grown and exported Mexican guavas are produced in the dry, desert areas in the states of Zacatecas and Aguascalientes, an area which is essentially free of *A. striata* which prefers humid tropical habitats. In consideration of this low prevalence, commercially produced Mexican guavas are certified for export to the USA following irradiation treatment of 400 Gy.

7.3.7 South American fruit fly: *Anastrepha fraterculus* (Wiedemann)

The South American fruit fly is a major pest of commercial fruit production in South America, especially on oranges and apples. In Brazil, Fernandes and Teles (1993) reported that although the citrus groves are also infested with medflies, 99% of the infesting larvae were *A. fraterculus*. Its native hosts in southern Brazil are members of the Myrtaceae, especially guava but also *Eugenia* spp. (Suguyama *et al.*, 1998). Control of this pest, including quarantine measures, is especially challenging because it is established that *Anastrepha fraterculus* is actually a cryptic species complex (Smith-Caldas *et al.*, 2001). Although its geographic distribution is reputedly from Argentina to Mexico, the Central American and Mexican population does not infest commercial fruits and, with slight but consistent morphological differences, should be considered a separate species (Hernandez-Ortiz *et al.*, 2004). Even within South America there is evidence for breeding incompatibility among populations. The species, or one of them, has invaded the Galapagos Islands where it breeds on the introduced guavas (Harper *et al.*, 1989). In mitigation of this potential ecological disaster, efforts are underway for its eradication.

7.3.8 South American cucurbit fly: *Anastrepha grandis* (Macquart)

The larvae of *Anastrepha grandis* feed on cucurbits such as melons, pumpkins and squash (Gomes Silva and Malavasi, 1993) and as a consequence it is considered a quarantine pest by the USDA. It is distributed from southern Brazil and Argentina to Venezuela, mainly along the Andean cordillera (Norrbom, 1991). Ecuador maintains a fly-free zone for the production of honeydew melons (Cabanilla and Escobar, 1993). Under this program, the USDA-APHIS-PPQ allows Ecuadorian melons to enter ports in the northern US. Recently there was an outbreak of this species in Panama which triggered quarantine restrictions. As its Latin name implies, it is one of the largest species in the genus and, based on its larval morphology (Steck and Wharton, 1988) as well as its host plants, it is a unique and distinctive species.

7.3.9 Carambola fruit fly: *Bactrocera carambolae* (Drew & Hancock)

The carambola fruit fly is a member of the 'dorsalis' complex of dacine fruit flies that has become established in the western hemisphere, specifically in the Guianas.

It was first detected in Surinam in 1986 but it is suspected that it was introduced as much as 20 years earlier (Malavasi *et al.*, 1998). It has since spread to neighboring Guyana and French Guiana and thus it also threatens Brazil. It is known to infest at least 15 cultivated fruits aside from its preferred host, carambola or 'star fruit' (*Averrhoa carambola*). Almost all of the known hosts are exotic introduced fruits found in urban areas. However, among the alternative hosts is the genus *Eugenia* (Sauerss-Muller, 1993). The USDA and FAO are coordinating an effort to eradicate this pest.

7.3.10 Queensland fruit fly: *Bactrocera tryoni* (Froggatt)

Eastern Australia maintains a 'Fruit Fly Exclusion Zone', a trade zone demonstrably free of fruit flies, allowing export of fruit without quarantine treatments. The maintenance of the zone is credited with a fourfold increase in exports since its institution in 1988 (Sutherst *et al.*, 2000). The fly-free status is maintained by massive releases of radio-sterilized 'Q-flies'. The Queensland fruit fly is considered to be the most destructive fruit fly in Australia. It infests nearly all commercial fruit crops, exceptions being pineapple and strawberry (White and Elson-Harris, 1992). Drew (1982) lists 62 commercial hosts and 60 wild hosts from 25 plant families in Australia.

7.3.11 Melon fly: *Bactrocera cucurbitae* (Coquillett)

The melon fly is found in many parts of the Old World and has become established in Hawaii. As the name implies, it oviposits mainly in fruits of the Cucurbitaceae (White and Elson-Harris, 1992). It seems that its preferred host is the weedy bitter melon (*Momordica charantia* L.) (Harris and Lee, 1989). However, it is also a quarantine pest in tomato (*Lycopersicon lycopersicum* L.) and papaya (*Carica papaya* L.) (Liquido, 1991). This is one of the species of *Bactrocera* that produces a sex pheromone based on methyl-eugenol (Liu, 1993). A synthetic formulation, 'cuelure', has been successfully applied as a male-annihilation method for suppressing populations and also combined with SIT for local area eradication (Kuba *et al.*, 1993).

7.3.12 Oriental fruit fly: *Bactrocera dorsalis* (Hendel)

The oriental fruit fly is native to Asia and is distributed throughout the Asian Pacific region including Hawaii (Cheng and Lee, 1993). It is an economic pest of 89 species of fruit in 32 plant families (Chu and Chen, 1985). Vargas *et al.* (2003) cite this species as instrumental in stunting the growth of the fruit export business in Hawaii. The mate-seeking behavior of the oriental fruit fly involves a sex pheromone of which the primary ingredient is methyl eugenol. Used as a lure in combination with an insecticide formulation, a control technique referred to as 'male annihilation' has been effective for suppressing oriental fruit fly populations (Cunningham, 1989). Control and quarantine efforts against the oriental fruit fly

are complicated by the fact that it is part of a cryptic species complex, the 'dorsalis' species group.

7.3.13 Invader fruit fly: *Bactrocera invadens* (Drew, Tsurua & White)

In 2002, farmers in Kenya discovered their fruit crops were being ravaged by a hitherto unknown pest fruit fly (Lux *et al.*, 2003). Although similar to the oriental fruit fly, *B. dorsalis*, specialists soon realized that they were dealing with an undescribed species of *Bactrocera* which had probably invaded Africa from Asia. It was subsequently identified as a population of the 'dorsalis' group native to Sri Lanka (Drew *et al.*, 2005). It has now spread into most of the Central African countries and has triggered export restrictions on fruits and vegetables from the region. Among the fruits heavily attacked are mangoes, tomatoes, bananas and guavas (Rwomushana *et al.*, 2008). Because so little is known of its biology a strategy for its control has not yet been formulated.

7.3.14 Papaya fruit fly: *Toxotrypana curvicauda* (Gerstaecker)

The papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, is a key pest of papaya in the New World. This species is not to be confused with the Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock, which is also a serious quarantine pest in Asia. The origin of *T. curvicauda* is apparently the West Indies, and it is currently distributed throughout the Caribbean, Mexico, Central America, Brazil, Colombia, and Florida in the United States. This pest is a specialist on papaya (Knab and Yothers, 1914), but occasionally has been found to develop on other hosts in Florida and Mexico (Castrejon-Ayala, 1991; Landolt, 1994).

Toxotrypana curvicauda is an unusual fruit fly in that it superficially resembles a vespid wasp, due to its elongated body and yellow and black striped markings. The ovipositor of the female is long and curved, and its length exceeds the length of her body. It is used to oviposit groups of ten or more eggs among the seeds of the central cavity of the papaya (Knab and Yothers, 1914). Green immature fruits are favored oviposition sites (Peña *et al.*, 1986). Eggs hatch approximately 12 days after oviposition, and larval development in the fruit lasts about 15 to 16 days (Selman *et al.*, 2009). Upon hatching, the larvae feed on the developing seeds and pulp of the interior, which is evidenced by yellowing of the papaya and often causing the fruit to drop from the tree prematurely. Upon maturation of the last instar, the larva will exit the fruit and pupate in the soil (Knab and Yothers, 1914). Flies emerge in about two to six weeks, depending upon humidity and temperature of the soil (Selman *et al.*, 2009).

Management techniques for *T. curvicauda* include chemical applications before oviposition occurs, bagging of fruit, and collection and destruction of all dropped and prematurely ripe fruit (Selman *et al.*, 2009). Habitat manipulation including orchard design, trap crops and border trapping can also help manage populations (Aluja *et al.*, 1997). Pheromone traps using membrane based synthetic formulations can also be used to determine pest distributions and aid management decisions (Heath *et al.*, 1996).

Quarantine procedures used to disinfest fruit of other tephritid species may be also effective against the papaya fruit fly. Gould (1996) was able to reach Probit 9 mortality levels using hot air treatments of 48°C for 167 minutes. However, further study of adequate quarantine procedures for this species is needed.

7.4 Lepidoptera: Tortricidae and Elachistidae

7.4.1 False codling moth: *Thaumatotibia* (*Cryptophlebia*) *leucotreta* (Meyrick) (Tortricidae)

The false codling moth *Thaumatotibia leucotreta* (Meyrick) is a key pest of citrus, stone fruit, and other crops in many countries in sub-Saharan Africa (Carpenter *et al.*, 2007). Although this pest is currently restricted to the African continent, its adjacent islands and Israel, it is considered to have a high probability of establishment if the pest were introduced into a suitable tropical or subtropical climate due to its wide range of possible host plants (Venette *et al.*, 2003). More than 70 possible hosts are recorded which include: avocado, carambola, citrus, coffee, mango, pineapple and litchi.

Once established, the potential economic impact of the false codling moth is high (Venette *et al.*, 2003), and it is of special importance as a quarantine pest because feeding and damage is internal. The presence of infested fruit is indicated by fruit drop prior to maturation. Also, the site of larval entrance is evidence of infestation, but this may be missed by fruit graders and inspectors, and infested fruit may be packaged for export (Carpenter *et al.*, 2007).

The false codling moth is being mass reared in the Cedar Biocontrol facilty in South Africa (Carpenter *et al.*, 2007). This program is currently focused on the production of biological control agents (Trichogrammatidae) against the pest, but plans are being developed for a large scale program of control of the pest using sterile insect techniques.

7.4.2 Avocado seed moth: *Stenoma cantenifer* (Walsingham) (Elachistidae)

The avocado seed moth (*Stenoma catenifer* Walsingham) is a fruit pest of avocados found only in the New World. The species is restricted to feeding on fruit and seeds of avocado and a few other members of the family Lauraceae. The moth is present in Brazil, Venezuela, Ecuador, Peru, Colombia, Bolivia, Panama, Costa Rica, Guatemala and Mexico. Observations from Guatemala indicate that the pest is restricted to altitudes below 1550 m (Hoddle, 2007). The severity of damage to avocados by this pest can be high and it is difficult to control (Nava *et al.*, 2005). Because *S. catenifer* is not present in the United States and the rest of the world, strict quarantine measures are implemented to prevent its establishment in regions producing avocado.

Adult female moths lay a single egg directly on the fruit, on the pedicel or on the stem near the attachment of the pedicel (Hohmann *et al.*, 2003; Hoddle and Hoddle, 2008). Emerging larvae burrow through the outer skin and down into the

developing seed. Feeding may occur at or on the surface or into the seed. Complete destruction of the seed can occur when small fruit are attacked by several larvae (Hoddle, 2007). Small larvae are cream colored, becoming a darker purple color as they mature, and during the last instars they are dark purple dorsally and turquoise ventrally (Hoddle, 2007). Adult moths are light brown in color with three or more dark spots near the mid-line, and the apical portion of the wing tips is fringed with eight to ten dark spots. At rest, the width at the thorax is approximately half that of the width at the apex of the folded wings.

The presence of *S. catenifer* is relatively easy to detect in infested fruit. The entrance hole is relatively large (ca. 2–3 mm) and is kept open by the continual expulsion of brown excrement (frass) which accumulates around the hole. Also, a white exudate of dried sugars seeps down the fruit from the entrance hole (Hoddle, 2007). Feeding is clearly marked by dark brown tunnels in the developing pulp and seed, some of which usually contain frass. Even cleaned of frass and exudates, the large entrance hole is unlikely to be missed by fruit inspectors.

7.5 Coleoptera: mango seed weevil: *Sternochetus mangiferae* (Fabricius) (Curculionidae)

The mango seed weevil ('mango stone weevil,' or 'mango weevil') is a specialized mango herbivore that was probably originally restricted to the India–Myanmar region (Jagatiani *et al.*, 1988), but is now distributed widely in the tropics. It is currently reported from Eastern Australia, Oceania, Asia, Africa, Hawaii, the Caribbean (Barbados, Dominica, Guadeloupe, Martinique, St Lucia, Trinidad and Tobago, British Virgin Islands, Grenada, Montserrat, St Vincent and the Grenadines), and South America (French Guiana) (EPPO, 2004; NAPPO, 2006). This pest has not been reported from the continental United States, Mexico, or Central America, although it has been intercepted in several US ports from shipments of mangoes from the Caribbean region. The mango seed weevil is considered a high risk quarantine pest in the United States, hence importation of mangoes is prohibited from countries where this weevil is present.

The mango seed weevil is a particularly difficult pest to control and detect because the damaged immature stages are deep within the seed of the fruit with no outward sign of entry. The only sure way to confirm infestation is by cutting open the fruit (Balock and Kozuma, 1964). Thus, infested fruit is often transported unnoticed from region to region.

The biology of *S. mangiferae* is similar to other weevils in that the principal damage is caused by the larvae which feed internally and protected within plant structures. In the case of *S. mangiferae*, the female deposits an egg in half mature (green) to ripe mango fruit and the oviposition puncture is concealed by exudates and sap. One female may lay 15 eggs per day, each singly, with a maximum of 300 over a three month period (Balock and Kozuma, 1964). The time from egg to adult takes five to eight weeks. The larva emerges from the egg in the pulp, then burrows down to the seed where it feeds and develops. Usually, pupation occurs

within the seed, but larvae will sometimes pupate in the pulp (Balock and Kozuma, 1964). Adults pass unfavorable periods concealed under loose bark and hidden in branch terminals on mango trees or in the vicinity of orchards (Balock and Kozuma, 1964). Adult weevils can live for two years, so even with a crop failure in one season some weevils may survive to the next. The presence of weevil larvae in mango fruit is associated with greater fruit drop from the tree (Balock and Kozuma, 1964), especially during early fruit development (Follett, 2002).

7.6 External fruit feeders

Pests that feed externally on fruit (scales, thrips, aphids, mites) are generally not as important as quarantine pests that feed internally on fruit. This is not because they are less damaging or infrequent on fruit crops, but because the majority of these pests are already distributed throughout the world. For example, of 17 species of hard scales (Diaspididae) reported from citrus by Jeppson (1989), all occur on at least three continents, although varying in economic importance. This wide geographical distribution of external fruit feeders, and especially scale insects, has come about because the majority of these pests feed on a wide variety of host plants and are not restricted to fruit feeding. Thus, inadvertent importation can occur through several means, including infested fruits or plant parts, on nursery stock of fruit trees, or infested plants of other species that serve as hosts.

Notwithstanding these generalizations, there are several external feeding pests of tropical fruits that can be of quarantine importance in that they are often intercepted at ports of entry. One of the most common external feeders on tropical fruits is the redbanded thrips *Selenothrips rubrocinctus* (Giard) (Thysanoptera: Thripidae). This is a tropical–subtropical species that is widely distributed in Asia, Africa, Australasia, the Pacific Islands, North America, Central America, the West Indies, and South America (Denmark and Wolfenbarger, 1999). The redbanded thrips is reported from many plant species, of which the fruit crops, cacao, mango, and avocado are among the most important. Another insect commonly intercepted at ports of entry and a serious pest is the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell), (Hemiptera: Pseudococcidae). This pest is widely distributed in the tropics and present in all pineapple producing regions. This mealybug is known principally as a pest of pineapple but will also infest other fruits such as *Annona* (cherimoya, atemoya, sugarapple), banana, citrus and coffee. Besides the direct effect of the feeding of this species, it is also a vector of the pineapple wilt disease (Illingworth, 1931; Sether *et al.*, 1998). Interestingly, the control measures against this pest are directed at the varied ant species which form a mutualistic relationship with the mealybug and are essential for its survival (Beardsley *et al.*, 1982; Petty and Tustin, 1993). Another important pseudococcid pest frequently intercepted in quarantine is the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green. This is widely distributed in the tropics and has over 125 reported host plants (Ghose, 1972).

Miller *et al.* (2002) list 158 species of mealybugs that are invasive pest species. Among those occurring on tropical fruits and thus of quarantine concern are the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, the banana mealybug, *Pseudococcus elisae* Borschenius and the vine mealybug, *Planococcus ficus* (Signoret). The last of these attacks grapes as well as figs.

7.7 Control measures: an introduction

The appropriate level of protection for an importing country or region can be achieved by the application of a single phytosanitary measure, such as inspection or a quarantine treatment, or a combination of measures. System approaches integrate biological, physical and operational factors to meet quarantine requirements. The combination of specific phytosanitary measures that provides overlapping or redundant safeguards is distinctly different from the use of a single risk mitigative technique. Such combinations vary in complexity; however, all require the integration of two or more measures that act independently of each other, the cumulative effect achieving the desired level of phytosanitary protection (i.e., a systems approach).

Specific mitigations may be selected from a range of preharvest and postharvest options, and may include other safeguarding measures. Measures may be added or the strength of measures increased to compensate for uncertainty. At a minimum, for a measure to be considered for use in a systems approach, it must be: (1) clearly defined; (2) efficacious; (3) officially required (mandated); and (4) subject to monitoring and control by the responsible national plant protection organization. Systems approaches to risk mitigation have been specified in recent work plans for the importation of commodities, such as citrus from Chile and avocado from Mexico to the USA. For example, a system approach to mitigating risks involved with mango imports from Central or South America to the USA might combine a variety of measures, including some of the following:

(1) certification of pest-free areas, pest-free places of production, or areas of low pest prevalence for certain quarantine pests, such as fruit flies;
(2) programs (e.g., mechanical, chemical, cultural) to control pests within orchards;
(3) preclearance oversight by USDA-APHIS, or other government officials;
(4) packinghouse procedures (e.g., washing, brushing, inspection of fruit) to eliminate external pests;
(5) quarantine treatments to disinfest fruit of internal and external pests;
(6) consignments inspected and certified by importing country phytosanitary officials and APHIS, PPQ to be free of quarantine pests (in the case of the USA);
(7) fruit traceable to state of origin, packing facility, grower and orchard;
(8) consignments subject to sampling and inspection after arrival in the import country; and
(9) limits on distribution and transit within the import country.

7.8 Preharvest control measures

7.8.1 Pest-free areas

As a sole mitigative measure, the establishment of pest-free areas or pest-free places of production may be completely effective in satisfying an importing country's appropriate level of phytosanitary protection. This option has proven to be successful in practice, obviating the need for post-harvest commodity treatments to achieve probit-9-level security. Establishment and maintenance of pest-free areas or production sites should be in compliance with international standards, but the specifics are usually negotiated between the exporting and importing countries. Trapping (Fig. 7.2) or other effective detection methods are used to survey the area of pest populations. In surveys for fruit flies, such as *Anastrepha* spp., for which parapheromones are not available, minimal trap density in zones of high risk (areas having high probability of fly establishment or introduction) should be five traps per km^2 (e.g., McPhail) and these should be baited with protein hydrolysate. Trapping for other potential pests of concern may also be required in the absence of any postharvest treatment.

7.8.2 Areas of low pest prevalence

An area of low pest prevalence may comprise all of a country, part of a country, or all or parts of several countries, in which a particular pest species occurs at low population densities and which is or are subject to effective surveillance and

Fig. 7.2 Insect trap in a papaya field in Malaysia.

control or eradication measures. Procedures for the establishment and maintenance of areas of low pest prevalence should comply with international standards. For example, elements of an operational plan for establishment and maintenance of such areas might include a geographic description to delimit the area; specification of an upper limit of pest densities; means to document and verify all necessary procedures and maintain records; specification of phytosanitary procedures (e.g., survey, pest control); and controls of commodity movement to prevent pest entry or re-entry into the area. The international standards recommend that the exporting country consult with the importing country in the early stages of implementation of such programs to ensure that importing country requirements are met. In particular, target or threshold population densities defining an area of low pest prevalence should be established in consultation with the importing country. Any protocol for establishing and maintaining a pest-free area or area of low pest prevalence also should include a standardized pest-reporting procedure and emergency action plan to address target pest detections in the pest-free or low-prevalence zones.

7.8.3 Preharvest control program

Cultural, chemical, or mechanical means (e.g., orchard sanitation, pruning of dead and diseased branches, pre-harvest application of pesticides, fruit bagging) may be used to eliminate pests from orchards or prevent fruit infestation. Integrated pest management practices, including sanitation, scouting and timely pesticide applications, are essential components of commercial fruit production, the effectiveness of which can be augmented by additional practices. For fruit flies, in particular, sterile insect release and other controls may be employed as prophylactic measures or in response to pest detection. Simple physical barriers, such as paper or plastic bags, may be highly effective in protecting fruit from pests. For example, fruit bagging (Plate X, in the color section between pages 238 and 239) combined with protein bait sprays reduced fruit fly (*Bactrocera* and *Dacus* spp.) infestations in unspecified fruit by up to 98%.

7.8.4 Phytosanitary certification inspections and monitoring

Fruit should be sampled and inspected periodically during the growing season and after harvest. Orchards should be surveyed as often as twice per year, during which time 10% of the area of each orchard is inspected. At these times, a random sample of fruit from both tree and ground, in a specified number of trees (at orchard edges) per hectare, should be taken, inspected, and cut to detect a 0.00003 infestation rate (three infested fruit per 100 000). Results of surveys must be negative for larvae of fruit flies. Production areas also may be subject to periodic, unannounced inspections by certified officials to ensure that they meet stipulated requirements for the issuance of a phytosanitary certificate that would be required for each consignment. Statistical procedures are available to verify, to a specified confidence level, the pest-free status of an area, given negative survey or trapping results.

7.9 Postharvest control measures

7.9.1 Chemical control

Fumigants have been the primary quarantine treatments beginning with the development of MeBr fumigation in the early 1940s and EDB fumigation in the early 1950s. In addition to these two major fumigants, several others have been tested and used, including phosphine, ethyl formate, hydrogen cyanide, and acetaldehyde. Fumigants are easy to use and generally inexpensive, but their future is very uncertain because of the potential negative effects of residues on human health and the environment. The use of EDB is now banned and fumigation with MeBr may be discontinued because of health, safety, and environmental concerns, especially since it is a stratospheric ozone-depleting substance.

The effective concentration and treatment duration of fumigants are related to temperature and pressure. For example, MeBr concentrations for a two hours exposure of sweet cherries to control cherry fruit fly (*Rhagoletis indifferens*) and codling moth (*Cydia pomonella*) are 32 g m^{-3} at 21°C or above, 40 g m^{-3} at 16 to 20°C, 48 g m^{-3} at 10 to 15°C, and 64 g m^{-3} at 4.5 to 9°C. Vacuum or negative-pressure atmosphere fumigation improves the penetration, permitting the use of lower fumigant concentrations and treatment duration. Figure 7.3 shows a MeBr commercial chamber.

A concern with MeBr fumigation is that the research design for determining effective dosage may not have anticipated the standards applied in quarantine protocols. The USDA-APHIS treatment schedule for citrus potentially infested by *A. ludens* is 40 g m^{-3} at fruit temperatures between 21 and 29.4°C for two hours. However, this research is based on a study by Williamson *et al.* (1986) that was

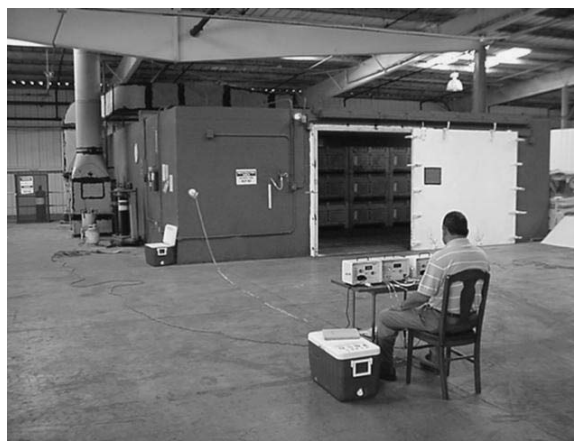


Fig. 7.3 Methyl bromide fumigation chamber at Edinburg, Texas (courtesy of USDA).

conducted only at 26.7°C, and only with eggs and early instars. Even in these tests at optimal temperatures and less resistant stages, Williamson *et al.* (1986) averaged about one survivor per 30 000 treated, i.e., below Probit 9 standards.

Fumigants cause several problems, including possible phytotoxicity depending on the type of crop and variety, seasonal conditions, humidity, temperature, fumigant concentration and duration of treatment. All insecticidal fumigants are toxic to humans and thus hazardous to apply; some act slowly such as MeBr and others act rapidly such as hydrogen cyanide. In addition, some fumigants, such as hydrogen cyanide, are flammable. Therefore, strict safety procedures are needed when designing fumigation chambers (Fig. 7.3), and methods.

Market quality is usually little affected by fumigants, but other associated problems and costs may occur. For example, lemons (*Citrus limon* Burm.) were evaluated for their tolerance to carbonyl sulfide (COS) fumigation and the influence of COS on market quality. At 70 mg L^{-1} no significant deleterious changes occurred in market quality up to a fumigation duration of eight hours and only a slight amount of peel injury was observed after 12 hours. However, longer fumigations lead to the presence of an offensive off-odor in the juice as well as to increasing rind injury. A test of the sensitivity of the Medfly (*C. capitata*) to COS indicated that long fumigation times (more than eight hours) will likely be required to achieve a degree of mortality sufficient for quarantine purposes for this insect. The tolerance of lemons to COS observed in this study suggests that COS is suitable for use as a quarantine treatment for this commodity. Its adoption, however, for this purpose will be hampered by the relatively high cost of application.

Of the few possible, alternative chemical fumigant treatments, Yokoyama *et al.* (1993) have suggested high compression and hydrogen phosphide fumigation for control of Hessian fly in a multiple-quarantine treatment and to maintain quarantine security levels required by regulatory agencies for hay exported to Japan.

In addition to health and environmental impacts, the cost of fumigants is generally increasing while their supply is decreasing. For example, the cost of MeBr increased by 75% from 2000 to 2001 and by 60 to 70% each year during the next four years (Pest Fog Sales Corporation, Pers. Com, G. T. Nakagawa, pers. comm.).

7.9.2 Low temperatures

Low temperatures have been used as effective quarantine treatments for fruit flies (especially the Medfly) for several years in a wide range of fruits including citrus and grapes. Although eggs and larvae of fruit flies are killed at temperatures below 10°C, the long exposure time at this temperature is too long to be practical and only temperatures below 3°C are practical and therefore commonly used (Armstrong and Couey, 1989). Cold quarantine treatments (or cold sterilization) are allowed for control of the pests *C. capitata*, *A. ludens*, *Eutetranychus orientalis*, *B. tryoni*, *B. cucurbitae*, *Curculio caryae*, *Conopomorpha sinensis*, *Cryptophlebia*

leucotreta and *Pterandrus rosa*. For example, the protocol for the control of Medfly consists of exposure of the commodity to 0°C or lower for ten days, 0.6°C or lower for 11 days, 1.1°C or lower for 12 days, 1.7°C or lower for 14 days, and 2.2°C or lower for 16 days.

Low temperature quarantine treatments are commonly and effectively used in crops that resist chilling, and those commodities with extended storage duration. For example, kiwifruit was reported to hold its quality extremely well in cold storage at 0 and 1°C, in treatments that were demonstrated to achieve 99.9968% (probit 9) mortality. Thus, cold treatments are a viable alternative to MeBr fumigation for this fruit (Delima, 1992). 'Eureka' and 'Lisbon' lemons artificially infested with immature life stages of *B. tryoni* or *C. capitata* and stored at 1°C for up to 32 days had excellent control of these pests and had no adverse effects on fruit quality (Jessup *et al.*, 1993).

7.9.3 Heat treatments

Hot water treatments

Hot water immersion (Plate XI between pages 238 and 239 and Fig. 7.4) can be an efficient quarantine treatment, especially for fruit flies. This treatment is currently the most commonly used quarantine treatment for mango, and also used in papaya to disinfest against fruit flies. The USDA Animal and Plant Health Inspection Service (APHIS) approved the hot water immersion quarantine treatment for Tephritidae fruit flies in mangoes in 1987, and therefore hot water treatments have been used in several countries as quarantine treatments for mango and papaya fruits. Large commercial hot water treatment facilities (Fig. 7.4) are routinely used to treat mangoes with hot water immersion at a temperature of 46.1 to 46.5°C for 65 to 120 minutes, depending on fruit weight and variety for export to the US and some other countries. These facilities generally consist of a series of hot water tanks, a rack system for loading of field bins filled with mangoes, and a crane for loading and unloading the racks into the hot water (Figure 7.4). There are approximately 75 hot water treatment facilities in Mexico, 5 in Ecuador, 6 in Guatemala, 11 in Peru and 10 in Brazil.

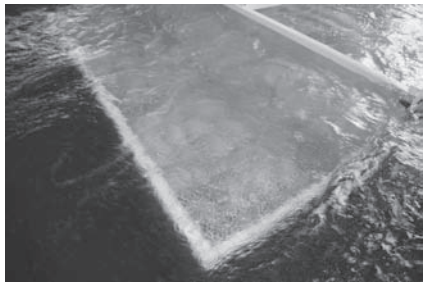
According to USDA APHIS requirements, for rounded varieties (Tommy Atkins, Kent, Haden, Keitt), the treatment for fruit flies requires heating in 46.1°C water for 75 to 110 minutes, depending on the weight of the mango. Fruit up to 500 g are treated for 75 minutes, fruit weighing 501 to 700 g are treated for 90 minutes and mangoes 701 to 900 g (only approved for Mexico and Central America) are treated for 110 minutes. For flat, elongated varieties (Frances, Ataulfo, Manila), fruit up to 375 grams are heated 65 minutes and fruit 375 to 570 grams are heated for 75 minutes. There are strict requirements for the water temperature during the first few minutes of treatment, and the hot water system must be certified each year before it is first used.

Hot water treatment immersion is not effective as a quarantine treatment for all quarantine pests. For example, mango seed weevils in the variety 'Alfonso' from India were not killed when infested mango fruit were immersed in water at

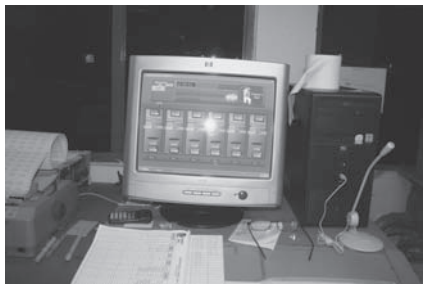

(a)


(e)


(b)


(f)


(c)


(g)


(d)


(h)

Fig. 7.4 Commercial hot water treatment in Mexico, (a) hot water tank, (b) preparation for treatment, (c) fruit to be loaded in the tank, (d) half way in the tank, (e) almost completely in the tank, (f) fruit in hot water, (g) treatment control, (h) fruit entering the restricted area of the packinghouse after the treatment.

48–52°C for up to 90 minutes, and 54–70°C for up to 5 minutes (Shukla and Tandon, 1985). However, more research is needed to evaluate the effectiveness of hot water immersion treatment for other quarantine pests.

Hydro-cooling is commonly used immediately following the hot water treatment if ten minutes are added to the heat treatment time, or fruit may be hydro-cooled after a waiting period of at least 30 minutes at ambient temperature. The hydro-cooler water must be no colder than 21.1°C, according to APHIS. Hydro-cooling mango fruit after hot water treatment decreases the pulp temperature much more rapidly (Shellie and Mangan, 2002a;b) and has been demonstrated to slow the metabolic rate of the fruit (Yahia and Campos, 2000). This cooling treatment often reduces possible heat damage to fruit, when compared with non-cooled fruit.

Hydro-cooler water must be properly sanitized with chlorine or other sanitizers to prevent the possible spread of human pathogens such as *Salmonella enterica* as was observed in 2000; sickening 15 people and causing the death of two (Sivapalasingam *et al.*, 2003). In the described example, the initial source of water used for the hydro-cooler was found to be contaminated with *Salmonella* and *E. coli* species. When hot water treated fruit are placed into the cool hydro-cooler water, cool water can be pulled inside the fruit, internalizing contamination if present in the water.

Vapor heat or forced hot air

Forced hot-air and vapor heat treatments are widely used for several fruits. While hot water immersion quarantine treatments are relatively easy to engineer, forced hot-air and vapor heat treatment equipments require more engineering and somewhat more complex computer programs to operate and monitor the treatment parameters and equipment. Costs of equipment for these treatments are also greater, as much as three times that of hot water immersion to treat the same quantity of fruit based on operational costs (Armstrong and Mangan, 2007). These cost considerations are in part based on the amount of commodity treated, or throughput. The more throughputs, the lower the treatment costs. Other cost factors include facilities, labor, refrigeration, power and shipping (Armstrong and Mangan, 2007).

Vapor heat, the oldest heat quarantine treatment, consists of heating the host fruit by moving hot air saturated with water vapor over the fruit surface. Vapor heat treatment (VHT) is a high humidity air treatment. For example, when the fruit is at dew point temperature or lower temperature, the air will condense on the fruit surface and the condensate will conduct heat energy from the surface into the center of the fruit flesh. Heat is transferred from the air to the commodity by condensation of the water vapor (heat of condensation) on the relatively cooler fruit surface (Armstrong and Mangan, 2007). Fruit may be heated over time to a target temperature which may be the end of the heat treatment, or fruit may be held for a specific time (holding time) that is required to kill the insect pests. Treatments usually take three to four hours from start to end of heating.

One of the first uses of vapor heat was in Mexico in 1913 to control the Mexican fruit fly (Hansen and Johnson, 2007). Vapor heat treatment is used for mangoes exported from Australia, Thailand, the Philippines and Taiwan, particularly for the Japanese market. An old vapor heat treatment for 'Manila' mangoes from Mexico, that requires a six-hour hold time at a core temperature of 43.3°C, is still on the approved list but currently not commercially used.

'Marsh' and 'Ruby Red' grapefruit tolerated a high-temperature, forced-air, vapor heat treatment of 43.5°C for 260 minutes, a treatment applied for the control of the Caribbean fruit fly (*A. suspensa*), where fruit did not develop symptoms of quality deterioration during subsequent storage (Miller and McDonald, 1991). With 'Marsh' grapefruit, 99% and 96% were sound, whereas with 'Ruby Red' 98% and 94% were sound after storage at 10°C for 28 days or 10°C for 28 days plus 7 days at 21°C, respectively. Differences in means for percentage of sound fruit were not significant for cultivar or vapor heat treatment. After the final storage period, there was significantly more aging observed on 'Ruby Red' than on 'Marsh' grapefruit, and vapor heat did not affect aging of 'Ruby Red' but increased aging of 'Marsh' grapefruit. Decay was reduced to almost 2% in vapor heat-treated fruit from 5% for non-treated fruit. The efficacy of thiabendazole to control stem-end rot increased on vapor heat-treated fruit compared with non-treated fruit. After the final inspection, the appearance of 'Marsh' grapefruit was fresher than that of 'Ruby Red' grapefruit, but the appearance of vapor heat-treated and non-treated fruit was similar.

Forced hot-air, also known as high-temperature forced air, is a modification of the vapor heat treatment developed by Armstrong *et al.* (1989) to kill Medfly, melon fly and oriental fruit fly eggs and larvae in papaya. It is essentially the same as vapor heat except that the fruit surfaces are dry during forced hot-air treatment. Improvements in temperature and moisture monitoring and air delivery have advanced forced hot-air treatments (Hallman and Armstrong, 1994), leaving forced hot-air treatments to be developed for commodities previously treated with vapor heat and also for new commodities (Hansen and Johnson, 2007). Forced hot-air treatment appears to be as effective in controlling internal pests as vapor heat, and provides better fruit quality (Laidlaw *et al.*, 1996), becoming the treatment of choice for many fruit previously treated with vapor heat (Table 7.2). The fruit skin temperature remains cooler during forced hot air treatments than during vapor heat treatment, while the tissue just below the skin heats to lethal temperatures because of the occurrence of evaporative cooling on the fruit surface during forced hot air treatment at lower relative humidity (Shellie and Mangan, 2000).

High temperature forced air (HTFA) is approved for use on citrus from Hawaii, grapefruit and mango from Mexico, mountain papaya from Chile, and papaya from Hawaii and Belize. The HTFA treatment for the control of fruit flies in papaya consists of exposure of the fruit until the temperature of the seed cavity first reaches 41°C and then 47.2°C over a period of about six hours. An approved treatment for the disinfestation of grapefruit of Mexican fruit fly consists of a high-temperature forced-air procedure that requires a stepped heating profile of

Table 7.2 Use of forced hot-air and vapor heat treatments for mango fruit in different countries

Exporting country	Importing country	Treatment and parameters
Australia	Japan	Vapor heat to 47°C core temperature, 15 min hold ('Kensington' mangoes)
Cook Islands	New Zealand	Forced hot-air to center temperature 47.2°C, 20 min hold period (only used commercially for papayas)
Fiji	New Zealand, Australia	Forced-hot air to center temp. of 47.2°C, 20 min holding period
Mexico	USA	Forced hot-air to center temp. of 48°C, 2 min hold
New Caledonia	New Zealand	Forced hot-air to center temp. of 47°C, 20 min hold period
Philippines	Australia, USA, Japan, New Zealand, Korea	Vapor heat with 46°C, 10 minute hold ('Carabao' mango)
Taiwan	USA	Vapor heat to center temperature of 46.5°C, 30 min hold period ('Irwin' and 'Haden' mangoes)
Thailand	Japan	Vapor heat to center temperature of 46.5°C, 10 min hold period ('Nang Klang Wun' mangoes)
		Vapor heat to 47°C core temperature, 10 min hold ('Nam Doc Mai', 'Pimsen Dang', 'Rad' mangoes)
Tonga	New Zealand	Forced hot-air to center temp. of 47.2°C, 20 min hold period
USA (Hawaii)	New Zealand, USA (Mainland)	Forced hot-air to center temperature of 47.2°C in >4 hours

Source: Adapted from Armstrong and Mangan, 2007

40°C for 120 minutes followed by 50°C for 90 minutes and 52°C until fruit center temperatures reach 48°C. This treatment was further shortened by using a hot forced air procedure that required the maintenance of fruit center temperatures at 44°C for 100 minutes, providing that the fruit reach the temperature in 90 minutes.

Heat injury and its control

Possible reduction in fruit quality as the result of using heat treatments depends on several factors (Yahia, 2006; 1997a). Despite its effectiveness as a quarantine control method, hot water immersion can damage the quality of fruit, in particular mangoes (Yahia and Campos, 2000). Small mango fruit are generally damaged more readily by heat compared to large fruit, in part because they heat more quickly. Grading by weight or size before heat treatments, with shorter treatment

times for smaller fruit, can reduce heat damage. Paull and Armstrong (1994) reported that the temperature and immersion time affects potential damage of mango fruit such as skin scalding, lenticel spotting, and retention of unripe starchy areas in mango flesh (stem-end cavity). The damages vary by cultivar. Some of the factors that have been shown to reduce fruit injury by heat include delaying treatment for 24 hours after harvest, and treatment of more mature fruit (Esguerra and Lizada, 1990; Esguerra *et al.*, 1990; Jacobi and Wong, 1990; Jacobi *et al.*, 1995). Spalding *et al.* (1988) reported that immersion in hot water at 46°C for 60–90 min, followed by storage for three days at 13°C and ripening at 24°C did not damage the market quality (ripening time, pH, total titratable acidity, ascorbic acid, soluble solids content) of 'Tommy Atkins' or 'Keitt' mangoes. However, lenticels were darker on 'Tommy Atkins' fruit immersed in water at 46°C for 120 minutes, on 'Keitt' immersed in water for 90 minutes at 46°C, and on both cultivars immersed for 60 minutes at 49°C. Anthracnose decay was reduced in 'Keitt', and stem-end rot, caused by *Diplodia natalensis* or *Phomopsis citri*, was reduced on both cultivars immersed in water at 46°C or 49°C. Immersion of 'Oro' mangoes for 75 minutes at 46.1°C caused no fruit damage (Sharp *et al.*, 1989a). 'Kent', 'Tommy Atkins' and 'Keitt' mangoes immersed for 90 minutes and then refrigerated at 11.1°C for 7, 11 or 14 days were not damaged. 'Haden' mangoes immersed for 90 minutes at 46°C and then held at 24°C were acceptable (Sharp *et al.*, 1989a). Treatment of 'Ataulfo' mangoes with 46°C water for 75 to 90 minutes did not cause visible damage, but fruit needed to be stored at 11.1°C after treatment to slow ripening to allow time for marketing before ripening (Sharp *et al.*, 1998b).

The effects of hot water treatment and storage temperature (4°C, 13°C or 22°C) on the quality and impedance of outer and inner mesocarp of mango fruit were assessed in two experiments during storage (Nyanjage *et al.*, 2001), impedance being a potential non-destructive measure of tissue damage following heat treatment. Fruit were subjected to equivalent heat units at 36.5°C for 60 minutes plus 46.5°C for 43 minutes or 46.5°C for 90 minutes by hot water treatments on the assumption of cumulative heat effects and a base temperature of 12–13°C. Fruit reflectance decreased whereas chroma and hue angle values increased over storage time and also with increase in storage temperature. The yellow color increased with a rise in storage temperature in hot water treated mangoes. Impedance of all fruits decreased with increase in frequency, storage temperature and storage time. The impedance of hot water treated mangoes was lower than that of non-treated fruit eight hours after immersion, recovered almost to control levels on day five at 4°C or 13°C, and decreased gradually after five days at 13°C. Impedance of all mangoes stored at 22°C decreased continuously during storage, and was higher in the inner mesocarp than in the outer pulp. Impedance of hot water treated fruit was poorly correlated with soluble solids content and chroma but well correlated with reflectance of fruit pulp at 22°C. A hot water treatment of 'Kensington' mangoes of 47°C fruit core temperature held for 15 minutes resulted in: (1) increased weight loss (50%), (2) decreased fruit softness (15%), (3) disrupted starch hydrolysis and interacted with maturity to reduce the skin yellowness (40–51%) of early harvested fruit (Jacobi *et al.*, 2001). Immature fruit were found to be more susceptible to hot

water treatment-induced skin scalding, starch layer and starch spot injuries and disease. Talcott *et al.* (2005) evaluated mature, green mangos subjected to a simulated hot water immersion at 50°C for 60 minutes and subsequent storage at 5°C and 20°C. Fruit held at 5°C were transferred to 20°C after eight days of storage to complete ripening, whereby symptoms of chilling injury were observed. Storage temperature during ripening and not the hot water treatment was the major factor contributing to changes in polyphenolic content, with antioxidant capacity unaffected. Major polyphenolics identified were free gallic acid and four gallotannins that collectively increased in concentration by 34% as the fruit ripened, also independent of postharvest and storage treatments. Carotenoid concentrations were highest in hot water-treated fruit stored at 20°C, whereas storage at 5°C initially delayed ripening. Despite appreciable differences in fruit quality during quarantine treatment or low temperature storage, only minor differences in antioxidant phytochemicals were observed.

Mango fruit weighing > 700 g were not allowed into the USA from Mexico for lack of a quarantine treatment against fruit flies, and therefore immersion in water at 46.1°C for 110 minutes was suggested to provide Probit 9 level quarantine security against Mexican fruit fly for fruit weighing more than 700 g and up to 900 g without adversely affecting fruit market quality (Shellie and Mangan, 2002b). The treatment is currently used commercially. Guava fruit infested with third instars of the Caribbean fruit fly immersed in hot water at 46.1°C for 35 minutes and hydro-cooled until fruit center temperatures returned to 24°C had only slight reduction in quality of fruit held at 10°C (Gould and Sharp, 1992). The fruit quality and ripening response of 'Brazilian' bananas (*Musa* sp., group AAB) were determined following hot water immersion treatments for surface disinfestations (Wall, 2004). Summer-harvested fruit were exposed to 47, 49, or 51°C water for 10, 15, and 20 minutes and ripened at 20°C, and winter-harvested fruit were immersed in 48, 49, or 50°C water for 5, 10, and 15 minutes, stored for 12 days at 14°C, and ripened at 22°C. The hot water exposure time had a greater effect than the water temperature on banana fruit ripening. Non-treated bananas ripened after 13 to 15 days, and ripening was delayed by two to seven days when fruit was exposed for 15 or 20 minutes to hot water. Hot water treatments did not inhibit pulp softening, but peels tended to be firmer for bananas immersed in 49 to 51°C water than control fruit. Heat-treated bananas were no different from control fruit in soluble solids content or titratable acidity; however, the conversion of starch to sugars was reduced at higher temperatures and exposure times. Bananas exposed for 20 minutes to hot water had delayed respiratory peaks and ethylene production, especially at 51°C. Mild peel injury was observed on fruit exposed to higher temperatures (49 to 51°C) for longer durations (15 or 20 minutes).

Fruit damage using hot air treatments have also been evaluated. Early, mid-, and late-season grapefruits were treated with hot air at 46, 48, and 50°C for three, five, or seven hours to determine the effects of time and temperature on market quality. Early and late-season fruit were more easily damaged by the higher temperatures than midseason fruit (McGuire and Reeder, 1992). Increased times at the lower temperatures had less of a deleterious effect on weight loss, loss of

firmness and color, and susceptibility to scalding injury and fungal decay than did shorter times at the higher temperatures. Their regression equations predicted that three hours at 48°C or two hours at 49°C would not adversely affect market quality of early and midseason fruit. However, the authors have suggested that it may not be possible to raise the treatment temperature for late-season fruit above 47.5°C without damaging the quality of juice from these fruit based on taste testing.

Vapor heat at 43.5°C and 100% RH for 5 h reduced peel pitting five-fold compared to control fruit after five weeks of storage (four weeks at 10°C + one week at 21°C) in freshly harvested Florida grapefruit and did not cause peel discoloration or rind breakdown (Miller *et al.*, 1991b). There was no difference in volume between treated and non-treated fruit after 1 week of storage or in weight loss, peel color, total soluble solids content, acidity, and pH after five weeks, and fruit were slightly less firm after VH treatment and remained less firm throughout storage, compared with control fruit. This study suggested that vapor heat is a potentially viable alternative quarantine treatment for control of the Caribbean fruit fly (*A. suspensa*) because it is not phytotoxic to grapefruit and has been reported effective for disinfestation of this pest in grapefruit. In a later work, Miller and McDonald (1992) demonstrated that de-greened 'Marsh' and 'Ruby Red' grapefruit, exposed to vapor heat treatment (43.5°C for 240 minutes), had 45% reduced incidence of aging after four weeks at 16°C plus one week at 21°C, had little effect on change in peel color during treatment or subsequent storage, and no effect on total soluble solids, titratable acidity, and pH. Early season de-greened 'Dancy' tangerines subjected to high-temperature, moist, forced-air (HTMFA) treatments using air at 45, 46, or 48°C for 0, 1, 2, 3, or 4 hours, and evaluated immediately after treatment and weekly during three weeks at 4°C had phytotoxic symptoms, such as inferior flavor and darkened flavedo tissue in fruit treated at 46 or 48°C, while fruit heated with 45°C forced moist air had flavedo color, percent juice yield, soluble solids concentration, and flavor ratings similar to those for control fruit (Shellie *et al.*, 1993).

Damage factors have also been evaluated with forced air treatments. 'MacFadyen' grapefruit infested artificially with late third instars of Mexican fruit fly and treated with forced hot air until the fruit-center temperature was 48°C with an incremental air-temperature increase, with humidity controlled to maintain a dew point that was 2°C lower than the fruit surface temperature, had no fruit damage resulting from scald by condensation and by desiccation (Mangan and Ingle, 1994). The treatment did not significantly affect fruit appearance or flavor quality ratings, although ratings for flavor and overall preference were lower for treated late-season, commercially stored fruit. The flavor of 'Valencia' oranges exposed to moist-forced air (MFA) at 47 or 50°C was rated significantly inferior to those exposed to 46°C for 1, 2, 3, or 4 hours (Shellie and Mangan, 1994). The degree minutes that accumulated in the center of the fruit between two and four hours and the maximum fruit center temperature during the heat treatment were associated with inferior fruit flavor. Oranges exposed to the moist-forced air at 46°C for up to four hours could not be distinguished from the non-heated fruit, and therefore these authors have suggested that 46°C is a promising quarantine treatment for 'Valencia' oranges. A number of

volatile compounds that contribute to orange flavor were quantified following high-temperature forced-air treatment to determine if a relationship exists between the flavor loss that is observed following this treatment and the volatile composition of the juice (Obenland *et al.*, 1999). α-pinene, β-myrcene, and limonene were reduced in amount by 60%, 58%, and 34%, respectively, over the course of the five-hours treatment, but the influence of heat on the amount of decanal was less clear, although in one of the two fruit lots there was little change. Ethanol content was reduced by 70% after the initial hour of treatment and then steadily increased to exceed the initial amount during the remaining four hours of treatment. Taste evaluations of the fruit showed a reduction of flavor quality following four hours or more of treatment. Acidity and soluble solids content were nearly unchanged by treatment. Therefore, this work has suggested that alterations in the volatile constituents of oranges by high temperature forced-air treatment may be an important reason behind the negative impact of this treatment on flavor quality. Freshly harvested mangoes treated with forced air at 51.5°C for 125 minutes then stored for one, two, or three weeks at 12°C, followed by 21°C until soft-ripe, lost one per cent more fresh weight than non-treated fruit and developed trace amounts of peel pitting, but had no different total soluble solids content than non-treated fruit, and peel color at the soft-ripe stage was similar (Miller *et al.*, 1991a). Treated fruit generally reached the soft-ripe stage about one day earlier than non-treated fruit regardless of storage duration and had a lower incidence and severity of stem-end rot and anthracnose. The authors have suggested that the trace of pitting on treated fruit likely will not influence consumer acceptance.

Studies have been conducted to compare the effect of different heat treatments. The market quality and condition of grapefruit was compared after three heat treatments for quarantine control of Caribbean fruit flies (McGuire, 1991b). Treatment by forced air at 48°C for three hours was compared with immersions in water at either a constant 48°C for two hours or with a gradual increase to 48°C lasting three hours. The immersion at a constant 48°C significantly increased weight loss and promoted injury and decay while reducing firmness and color intensity after four weeks of storage. By more slowly heating fruit in the gradient water immersion, weight, firmness, and natural color were retained, and injury was substantially reduced, but the incidence of decay remained high. No loss in quality resulted from treatment by forced hot air. These heat treatments had little effect on juice characteristics, although acidity was slightly reduced by each method of application. In taste tests, juice from fruit treated in water that was gradually raised to 48°C was preferred over that of fruit treated at a constant 48°C. Four heat treatments for quarantine control of Caribbean fruit flies in mango did not affect fruit quality but variably controlled two postharvest diseases (McGuire, 1991a). Immersion of fruit in water at a constant temperature of 46°C for 90–115 minutes significantly reduced anthracnose (*Collectrichum gloeosporioides*) on three cultivars by 60–87%, stem-end rot (*Diplodia natalensis*) by 61–88%, and treatment by forced air at 46°C for 195 minutes or at 48°C for 150 minutes reduced anthracnose on two cultivars, but an immersion for 150 minutes in which the water temperature gradually rose to 48°C, following a gradient identical to that created by forced air treatment, had no effect on disease severity. Although all heat

treatments led to increased weight loss during two weeks of storage, fruit treated by gradient hot water immersion were least affected. Hot air treatment and gradient hot water immersion slightly accelerated the softening of fruit, and all heat treatments hastened the development of yellow pigmentation. Therefore, the author has recommended the immersion in water at a constant temperature of 46°C for the disinfestation of mangoes because it controls disease without reducing market quality, and has indicated that forced air treatment at 48°C is tolerated by the fruit and is more effective than forced air at 46°C for disease control. The effect of postharvest hot water treatments (HWT) as an alternative to vapor-heat insect disinfestation of 'Kensington' mango on fruit quality was studied by Jacobi *et al.* (1995). Fruit were given 46°C HWT for 30 minutes at a fruit core temperature of 45°C either 24 hours after harvest or after various conditioning treatments of 4 to 24 hours at 39.1°C in air, and fruit were compared to non-treated fruit after a subsequent seven days at 22°C. The HWT increased fruit softening and reduced chlorophyll fluorescence and disease incidence. The longer conditioning times produced softer fruit. Conditioning reduced damage to the fruit caused by HWT, and preconditioning for eight hours or longer resulted in < 1% of fruit being damaged as shown by cavities, skin scald, and starch layer formation. The quantitatively measured higher mesocarp starch content paralleled the visible starch layer injury. Skin yellowing increased in response to HWTs that were not damaging to the fruit.

Several treatments have been developed to reduce or eliminate heat injury. Conditioning fruit at 40°C for up to 16 hours before hot water treatment accelerated fruit ripening, as reflected in higher total soluble solids and lower titratable acidity levels (Jacobi *et al.*, 2001). As fruit maturity increased, the tolerance to hot water treatment-induced skin scalding and the retention of starch layers and starch spots increased and susceptibility to lenticel spotting decreased. A conditioning treatment of either 22 or 40°C before hot water treatment could prevent the appearance of cavities at all maturity levels. The 40°C conditioning temperature was found to be more effective in increasing fruit heat tolerance than the 22°C treatment; the longer the time of conditioning at 40°C, the more effective the treatment. Therefore, for maximum fruit quality, it was recommended that mature fruit are selected and conditioned before hot water treatment to reduce the risk of heat damage. It is proposed that increased sugar concentration in the mesocarp increased the level of fruit heat tolerance (Jacobi *et al.*, 2002). Changes in carbohydrate metabolism of 'Kensington' mango fruit from two major production regions in Queensland, Australia, were measured after conditioning fruit with hot air at 40°C for 0, 2, 4, 8, and 16 h or at 22°C for 16 hours (control) followed by hot-water treatment at either 45°C fruit-core temperature for 30 minutes or 47°C fruit-core temperature held for 15 minutes. Advancing physiological maturity of 'Kensington' mango fruit was correlated with increased starch concentration within the mesocarp. An α-amylase inhibitor was present in unripe 'Kensington' mesocarp. α-Amylase activity was promoted by conditioning fruit at 40°C for eight hours, and this enhanced enzyme activity persisted until the fruit was ripe. Consequently, starch degradation was accelerated and the concentration of total soluble solids was higher in fruit

conditioned at 40°C for eight hours than in fruit left at the lower temperature of 22°C for 16 hours or not conditioned. Immediately on removal of fruit from hot-water treatment, activities of α-amylase and phosphorylase were inhibited. This inhibition was correlated with higher starch concentration and starch layer and starch spot injuries in these fruit. A positive correlation was also found between increased sucrose concentration and greater starch loss in 40°C conditioned 'Kensington' fruit. Immersion of guavas for 35 minutes in water at 46.1°C slowed softening, sweetening, and color development of fruit, delayed ripening by two days, increased susceptibility to chilling injury, decay, and weight loss in storage, but overall loss of quality was minimal (McGuire, 1997a). Waxing fruit within 90 minutes of heat treatment exacerbated chilling injury, further delayed ripening with a concomitant increase in the percentage of fruit not ripening, and caused fruit to remain greener. Waxed fruit had a lower acidity and soluble solids content and did not appear to ripen normally. Although heating did not appreciably affect the percentage of fruit that failed to ripen, the combination of heating and nearly immediate waxing increased the proportion of not ripened fruit to 45%. Heat and wax treatments, alone or in combination, caused CO_2 levels to increase significantly before the initiation of ripening, but waxing also reduced the O_2 content of fruit at this time. Before ripening, O_2 levels were inversely correlated with injury, firmness, date and percentage of fruit ripening, and pH, and directly correlated with peel color and the concentration of acids and sugars in the pulp. Therefore, delaying the waxing of heat-treated guavas or reconditioning them for 24 hours at 20°C before cold storage promoted normal ripening and helped to maintain the quality of heat-treated fruit. Various cooling methods following heat treatment were investigated to reduce injury to carambola fruit caused by heat stress (Miller and McDonald, 2000). Cooling fruit with ice water (IW) caused more severe degradation to carambola quality compared with ambient air (AA), ambient water (AW) or refrigerated air (RA). Cooling fruit after heat treatment with RA at the targeted storage temperature (10°C) resulted in the least damage to carambola. RA, AA, and AW caused similar degradation such as peel pitting, bronzing, decay and weight loss, but AA cooled fruit were the least firm, and AW cooled fruit had the least preferred flavor. At the end of seven days of storage, fruit heated with hot water (HW) were less yellow than vapor heat (VH)-treated fruit, and fruit cooled by AA were more yellow than those cooled by other methods. There was no difference in peel color, total soluble solids, titratable acidity, pH, or mastication texture of pulp among treatment combinations. Therefore, carambolas cooled with refrigerated air had the least injury compared with other cooling methods evaluated. The tolerance of six nectarine cultivars to hot water treatment was evaluated and the effect of addition of NaCl to the treatment solution was tested as a means of reducing treatment damage (Obenland and Aung, 1997). Hot water immersion was extremely damaging to all cultivars tested, although differences in tolerance existed. Treatment at 50°C for 25 minutes, a treatment for fruit fly disinfestation, rendered the fruit of all cultivars unmarketable. NaCl at a concentration of 200 mM reduced the injury rating in all six cultivars tested from severe damage to slight damage, although the latter was still too severe for the fruit

to be marketable. At the less injurious temperature of 46°C, the slight amount of injury caused by hot water treatment was eliminated in the presence of NaCl. Flesh firmness was not altered by NaCl in the treatment solution, but firmness was slightly greater in fruit treated at 50°C as compared with 46°C. This study concluded that NaCl reduced damage by effectively reducing the amount of water entering the fruit during treatment and may be useful in reducing the damage from hot water immersion treatments used for surface disinfestation. Chilling injury symptoms were reduced when 'Sharwil' avocados were held at 37 to 38°C for 17 to 18 hours and then air-cooled at 20°C for four hours before storage at 1.1°C for 14 days or more, but non-heated fruit developed severe surface discoloration and pitting (Sanxter *et al.*, 1994). Chilling injury symptoms were reduced further when the heated fruit were stored in perforated polyethylene bags during storage at 1.1°C, and no treatment equaled or surpassed the quality of fruit in non-treated controls. Hot water (HW) has been shown to cause severe injury to grapefruit. Grapefruit preharvest-treated with gibberellic acid (GA) or not treated, were postharvest-treated with vapor heat or HW such that the surfaces of fruit were exposed to the same rate of temperature increases and treatment durations, and quality attributes were then compared with ambient air (AA) and ambient water (AW) controls after storage (Miller and McDonald, 1997). After four weeks' storage at 10°C plus one week at 20°C, scald affected 5% of HW and 20% of VH-treated fruit. No scald developed on control fruit, and at the end of storage, mass loss for HW and VH fruit was approximately 5%. HW-treated fruit had a five times higher incidence of aging than VH fruit; however, control fruit showed significantly more aging than all heat-treated fruit. Gibberellic acid and the heat treatments reduced decay relative to the control, and GA-treated fruit remained greener during storage than control fruit. These findings indicate that VH and HW treatments at the temperatures and durations sufficient to control the Caribbean fruit fly will likely cause peel injury to 'Marsh' grapefruit produced in Florida, regardless of treatment with GA.

Although pre-treatment conditioning and post-treatment cooling can reduce damage to the commodity, it also reduces mortality of the target pest. Hence such treatments cannot be applied in combination with quarantine schedules that have been designed to kill pests with heat. For example, live *Anastrepha* larvae were found in mangoes imported from Mexico (Scruton 2000). As a consequence, the post-treatment cooling application was prohibited (Heather and Hallman, 2008). Similarly, gradual heating to reach the target core temperature results in much higher survival rates of the pest (Yin *et al.*, 2006) compared to the short ramp-up times used in the development of treatment schedules. Thus conditioning treatments should not be applied until their effect on the target pest has been thoroughly tested.

7.9.4 Modified (MA) and controlled (CA) atmospheres

Some commodities are capable of anaerobic metabolism, but insects generally have a lesser capacity, and this can be used to control these insects (Table 7.3). Modified or controlled atmospheres (insecticidal atmospheres with very low O_2

Table 7.3 Atmospheric conditions used to control various fruit flies

Fruit flies	Stage	Commodity	O_2/CO_2 kPa	Temperature °C	Duration	Reference
Anastrepha suspensa	5 d larvae	Citrus	0/0	22–23	60 h	Benschoter *et al.* (1981)
Anastrepha suspensa	Eggs & larvae	*In vitro*	10–50/20–80	10 & 15.6	7 d	Benschoter (1987)
Cydia pomonella	Larvae	Walnut	Var/98	39–45	33–48 h	Gaunce *et al.* (1982)
Platynota stultana	All	Grape	Var/45	0	4.1 d	Mitcham *et al.* (1997)
Frankliniella occidentalis	All	Grapes	Var/45	0	5.8	Mitcham *et al.* (1997)
Tetranychus pacificus	All	Grapes	Var/45	0	8.1 d	Mitcham *et al.* (1997)

and/or very high CO_2 pressures, with or without the addition of other gases (such as CO) have been tested as quarantine systems (Yahia, 1998; 2008). Insecticidal atmospheres have been used successfully as quarantine systems for some dried horticultural commodities such as raisins and nuts (Yahia, 2008), but do not seem to be tolerated by many fresh horticultural crops (Yahia, 1997a;b;c; 1998; 2006; 2008; Yahia and Vazquez, 1993) (Table 7.4).

'Sunrise' papaya fruit exposed to a continuous flow of an atmosphere containing < 0.4% O_2 (balance N_2) for 0 to 5 d at 20°C had decay and some fruit had developed off-flavors after three days in low O_2 plus three days in air at 20°C (Yahia *et al.*, 1992). The intolerance of the fruit to low O_2 correlates with an increase in the activity of pyruvate decarboxylase and lactate dehydrogenase but not with the activity of alcohol dehydrogenase. Therefore, the authors suggested that insecticidal O_2 (< 0.4%) atmospheres can be used as a quarantine insect

Table 7.4 Tolerance of some horticultural commodities to different atmospheres intended to control quarantined pests

Commodity	Temperature, °C	Atmosphere, kPa		Tolerance, days	Reference
		O_2	CO_2		
Avocado	20	0.25	80	3	Ke *et al.* (1995)
	20	0.1–0.4	50–75	1	Yahia and Carrillo-López (1993)
Dates	22–28	4–7	60–85	145	Navarro *et al.* (1998)
Grapes	5	0.5	35–45	6	Ahumada *et al.* (1996)
Grapefruit	10	0.05	0	21	Shellie *et al.* (1997a)
	46	1	20	0.13	Shellie *et al.* (1997b)
Kiwifruit	40	0.4	20	0.3	Lay-Yee and Whiting (1996)
Mango	20	0.2–0.3	50	5	Yahia (1993; 1994)
	20	2	70–80	5	Yahia *et al.* (1989)
	20	0.5		4	Yahia and Tiznado-Hernandez (1993)
	43	0	50	160 min	Yahia and Ortega-Zaleta (1999)
	48	0.8	67	160 min	Yahia *et al.* (1997)
	12	1	30–50	3	Leon *et al.* (1997)
Orange	5	0.02		16	Ke and Kader (1989 1990)
	5	0.25		23	Ke and Kader (1989; 1990)
	10	0.02		15	Ke and Kader (1989; 1990)
	5	8.4	60	5	Ke and Kader (1989; 1990)
Papaya	20	0.2–0.4		2–3	Yahia (1995) Yahia *et al.* (1989; 1992)

control treatment in papaya for periods less than three days at 20°C without the risk of significant fruit injury. Avocado and guava have been shown to be very sensitive to these atmospheres (Yahia, 1997a;b; 1998; 2006; 2008), and mango is very tolerant (Yahia, 1997c; 1998; 2006; 2008; Yahia and Vazquez, 1993).

Non SO_2-fumigated 'Thompson Seedless' table grapes were stored at 5 or 20°C for 6 and 4.5 days, respectively, in air or one of four controlled atmospheres (CA) ; 0.5% O_2 + 35% CO_2; 0.5% O_2 + 45% CO_2; 0.5% O_2 + 55% CO_2; or 100% CO_2, and fruit were evaluated for weight loss, berry firmness, soluble solids concentration (SSC), titratable acidity, berry shattering, rachis browning, berry browning, and anaerobic volatiles (Ahumada *et al.*, 1996). Fruit quality was not affected at 5°C with the exception of greater rachis browning in fruit treated with 0.5% O_2 + 45% CO_2. At 20°C, CA treatments maintained greener rachis compared to the air control; however, SSC was reduced in the fruit treated with 55% and 100% CO_2. At both temperatures, CA induced the production of high levels of acetaldehyde and ethanol. Ethanol concentrations were two-thirds lower at 5°C than at 20°C. Consumer preference was negatively affected by some CA treatments for grapes kept at 20°C, but not by any of the treatments at 5°C.

7.9.5 Irradiation

Food irradiation is a process by which products are exposed to ionizing radiation to sterilize or kill insects. In 1986, the US Food and Drug Administration (FDA) approved the use of radiation treatments of up to 1 kGy (100 krad) on fruits and vegetables. Radiation may be provided by gamma rays from cobalt-60 or cesium-137 sources, electrons generated from machine sources (e-beam), or by X-rays. Absorbed dose is measured as the quantity of radiation imparted per unit of mass of specified materials. The unit of absorbed dose is the gray (Gy) where 1 gray is equivalent to 1 joule per kilogram.

It has been known for several decades that irradiation is effective at killing, sterilizing or preventing further development of a wide variety of insect pests of quarantine importance on perishable fruits and vegetables. Research has shown that the dose required for sterilization of most insects is below 0.50 kGy. Until relatively recently, the only irradiation treatment approved for quarantine use for the US market was for the movement of papaya fruit from Hawaii to the mainland US. The protocol required the papayas be treated in Hawaii with 150 Grays of ionizing radiation for control of fruit fly pests. However, this protocol, which was approved in 1989, was not used until 1995, when two Cobalt-60 irradiation facilities were built. Subsequently, an X-ray facility was opened at Keaau in 2000 and at one point 50% of all papayas exported from Hawaii were irradiated. But because of maintenance problems those numbers have fallen to around 10–15% (Heather and Hallman, 2008), which highlights one of the potential challenges to irradiation treatment.

In May of 1996, the USDA APHIS published a policy statement in the Federal Register regarding their position concerning the use of irradiation as a treatment for quarantine pests in plants. Generic dosages were proposed and later accepted

for various fruit fly species as shown in Table 7.4. The dosages were generic in the sense that the prescribed dose was deemed appropriate regardless of the commodity. Where more than one fruit fly species is present, the dose would be that for the most tolerant species. This generic approach was a departure from traditional quarantine treatment protocols approved by USDA which have been both insect species and commodity specific. Irradiation doses that are needed to kill the insect are higher than those tolerated by fruits, and therefore the other unique feature of irradiation treatments is that they are generally designed to cause sterility or developmental incompetence in insects, not to kill them outright. In the case of fruit flies, APHIS has established the criterion for a successful dose as the non-emergence of adults to prevent such adults from triggering control strategies if detected in traps within areas free of established fruit fly populations. Additional research may support changes to these minimum doses in the future (Torres-Rivera and Hallman, 2007).

USDA-APHIS approved the use of irradiation to treat fruit for importation into the United States in 2002, but it was only in 2007 that India began shipping irradiated fruit to the US. Currently, Mexico is shipping irradiated guava and mango to the US (Figs. 7.5 and 7.6). Several countries are developing Work Plans with APHIS to initiate bilateral trade in products irradiated for phytosanitary purposes (Follett and Griffin, 2006). Irradiation has been used since 2004 to disinfest mangoes shipped from Australia to New Zealand of fruit flies without insurmountable incident (Torres-Rivera and Hallman, 2007).

According to APHIS, live stages of pests found in a commodity following a Plant Protection and Quarantine (PPQ) prescribed and approved irradiation

Fig. 7.5 Control unit of irradiated plant in Mexico (courtesy of Mr Cesar Moreno, Sterigenics Gamma México).

Fig. 7.6 Irradiated guava in Mexico (courtesy of Mr Cesar Moreno, Sterigenics Gamma México).

treatment will be presumed by PPQ to have been effectively treated unless evidence exists to indicate that the integrity of the treatment was inadequate. This means that when irradiation is used as a quarantine treatment, there must be a good degree of trust between the trading partners.

Although gamma rays, high energy electrons, and X-rays all have similar effects, gamma rays are most commonly used in food irradiation because of their ability to deeply penetrate pallet loads of food. Gamma irradiation equipment irradiates packaged or bulk commodities by exposing the product to gamma energy from cobalt-60 in closed chambers, which range in size from single modular pallet irradiators to large contract irradiation facilities. The actual cost of food irradiation is influenced by dose requirements, the food's tolerance of radiation, handling conditions (packaging and stacking requirements), construction costs, financing arrangements, and other variables particular to the situation (Forsythe and Evangelou, 1993). Irradiation is a capital-intensive technology requiring a substantial initial investment, ranging from US$1 million to US$3 million. A major capital cost includes the radiation source (cobalt-60), hardware (irradiator, totes, conveyors, control systems), land (0.2–0.5 Ha), radiation shields, and warehouse (preferably with cold storage). Operating costs include salaries (for fixed and variable labor, must be well trained), utilities, maintenance, taxes/insurance, cobalt-60 replenishment, etc. Radiation plants are costly and would be more economical if used essentially year-round. However, fresh fruit and vegetable production is seasonal, which would require facilities to be, at a minimum, shared among commodities with somewhat different harvest schedules.

Certain policies are required to ensure system integrity in the application of irradiation as a phytosanitary treatment. These policies focus on pretreatment, treatment and post-treatment conditions as well as required documentation and monitoring. Before treatment, packers and treatment facilities must maintain records concerning the sources of commodities; how untreated commodities are stored and handled in the irradiation facility and packaging requirements. During treatment, the absorbed dose must be measured and monitored, including dose mapping of the minimum and maximum dose utilizing calibrated dosimeters. After treatment, pests may continue to live and develop. Therefore, confidence in the adequacy of irradiation treatments rests with the assurance that the treatment is efficacious against the pest under specific conditions and has been properly conducted and the commodity safeguarded. This requires strict treatment procedures and well designed and closely monitored systems for treatment delivery and safeguards that assure system integrity. Following treatment, packages must be marked and labeled with treatment lot numbers to allow trace back if needed.

Consumption of foods irradiated at doses up to 10 kG has been considered safe by the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the International Atomic Energy Agency. While consumers have concerns associated with the safety of irradiation technology and its effects on food, research indicates that properly irradiated food does not pose a risk to consumers (Thorne, 1983, OTA, 1985). Studies have shown that consumer acceptance of irradiated produce in the US is increasing (Morrison, 1992), but serious social and public policy issues remain. Some produce companies have shied away from irradiation because they fear a backlash from consumers, but attitudes appear to be shifting.

Fruits differ in their tolerance to irradiation (Table 7.5). For example, the tolerance of mango and guava fruits is generally good, but there are differences between varieties and stages of maturity. The greater the dose that can be tolerated by the fruit, the less expensive the treatment process. If the commodity is treated in a palletized form or in totes (a packing arrangement feasible with cobalt-60 or cesium-137 irradiation sources) in order for the fruit in the center of the load to receive 150 Grays, the product on the outside may receive two to six times higher dosage (300 to 900 Grays), causing damage to some fruits. The higher the dosimetry ratio (lowest to highest dose administered to a batch of fruit), the more flexibility the operator has, resulting in potentially lower costs to the shipper. By disassembling the pallet and treating product in boxes, the range of doses received by the product can be much smaller, but the cost of treatment is higher as compared to a pallet irradiator due to the additional labor involved. This is also the case for a tote irradiator since these boxes must also be de-palletized.

Fruit damage by irradiation is a function of cultivar, irradiation dose and fruit maturity/ripeness at the time of treatment (Boag *et al.*, 1990; Singh, 1990). Symptoms of irradiation stress on fruits include accelerated softening, uneven ripening, and surface damage. Irradiation stress is additive to other stresses

Table 7.5 Approved irradiation doses for the control of some quarantined pests

Scientific name	Common name	Dose (Gray)
Anastrepha ludens	Mexican fruit fly	70
Anastrepha obliqua	West Indian fruit fly	70
Anastrepha serpentina	Sapote fruit fly	100
Anastrepha suspensa	Caribbean fruit fly	70
Aspidiotus destructor	Coconut scale	150
Bactrocera jarvisi	Jarvis fruit fly	100
Bactrocera tryoni	Queensland fruit fly	100
Brevipalpus chilensis	False red spider mite	300
Conotrachelus nenuphar	Plum curculio	92
Copitarsia decolora	No common name	100
Croptophlebia ombrodelta	Litchi fruit moth	250
Cryptophlebia illepida	Koa seedworm	250
Cylas formicarius elegantulus	Sweetpotato weevil	150
Cydia pomonella	Codling moth	200
Euscepes postfasciatus	West Indian sweetpotato weevil	150
Grapholita molesta	Oriental fruit moth	200
Omphisa anastomosalis	Sweetpotato vine borer	150
Pseudaulacaspis pentagona	White peach scale	150
Rhagoletis pomonella	Apple maggot	60
Sternochetus mangiferae (Fabricus)	Mango seed weevil	300
Fruit flies of the family *Tephritidae* not listed above		150
Plant pests of the class *Insecta* not listed above, except pupae and adults of the order Lepidoptera		400

(physical, chilling, water), which should be avoided to minimize the negative effects of ionizing radiation. Mango fruit softening was not produced as a result of irradiation in the range of 0.1 to 1.2 kGy (Boag *et al.*, 1990). Fruit which were partially ripe and in their climacteric were largely unaffected by irradiation; that were 25% to 50% ripe Haden mangoes showed no change in the rate of ripening when treated at 250 Grays (Akamine and Goo, 1979). Because utilizing a higher upper limit of dose results in easier logistics and lower cost to the grower, there is a danger that fruit might be exposed to higher doses than they can tolerate while still meeting the minimum phytosanitary dose. This could result in quality issues if practiced. It will depend on the tolerance of mango fruit cultivars to irradiation.

Several irradiation facilities treat large quantities of fruit and are important in global quarantine programs. In Hawaii, an irradiation facility is available with an electron beam/X-ray source which has similar penetration to gamma sources. Product is stacked three boxes deep on a metal carrier and moved through the facility making two passes by the source. This keeps the max/min dose received down to 1.5 for most products. The facility is currently only used at 30% to 50% capacity. Another older irradiation facility in Palo Alto still operates near Mexico City. Many tests were conducted at this facility in collaboration with USDA and FAO over a long period of time with several fruits, including mango and citrus, but this facility is not currently irradiating fruit for commercial purposes. A relatively new facility has been established, the Sterigenics facility, in the state of Hidalgo, near Mexico City. Sterigenics Inc. is treating guava fruit (Figs. 7.5 and 7.6) and planning to treat other fruits including mangoes. The Sterigenics facility in Mexico has the capacity to treat approximately ten tons of fruit per hour when the entire product is treated at the same settings. The fruit in this system are loaded into totes for movement past the radioactive source. The aluminum totes are 59 cm × 92 cm × 142 cm high. Each tote has a false floor that allows the product to be easily loaded into the top of the tote. The floor is lowered as more and more product is added. The false floor is stainless steel and is raised and lowered by pistons. Forty-five totes are exposed to irradiation at one time and they rotate in a serpentine fashion through the maze so that each side of the tote is treated equally. The shortest cycle time through the irradiator is 1.5 min per position (1.5 × 45 positions = 90 min) for a total treatment time of 90 min minimum.

A joint study in 1997 by the International Atomic Energy Agency, the World Health Organization and the Food and Agriculture Organization of the United Nations concluded that there is no safety basis for limiting the amount of irradiation that can be applied to food for human consumption (Hallman, 2000). The use of irradiation with doses of up to 1.0 kilogray (KGy) has been approved by the Food and Drug Administration. Irradiation doses that can kill insects, often in excess of 1KGy, may also damage the commodity, and therefore quarantine treatments have been proposed using sublethal doses that eliminate viability of the insects rather than killing them outright. However, this does result in regulatory problems in that it requires inspectors to accept shipments infested with living pests. Although consumer acceptance of the use of irradiation in foods has slightly improved, its economic feasibility is still one of the major problems facing its commercial application, especially in developing countries.

One of the more feasible and potentially useful applications of irradiation in fruits and vegetables is probably for disinfestation as a quarantine treatment. Moy (1993) has reported that all immature stages of a fruit fly will become effectively unviable upon being irradiated at a minimum dose of 150 Gy, the dose level approved by the USDA in January 1989 for treating Hawaiian papayas as a quarantine procedure. This is also well below the dose level approved in April 1986 by the US Food and Drug Administration for irradiating fresh foods for disinfestation and delaying maturation. Low doses (0.15 to 1.0 KGy) of gamma irradiation have been reported to disinfest fruit flies from several fruits such as

mangoes, papayas, bananas, litchis, and also other insects such as mango seed weevils. Irradiation is an accepted treatment to control quarantine pests in ten fruits and five vegetables for export from Hawaii to the US mainland (Follet, 2004). A generic dose of 150 Gy has been proposed for tephritid fruit flies. Contrary to the 150 Gy dose, approved irradiation quarantine treatment doses for Med fly, melon fly, and oriental fruit fly in Hawaii are 210–250 Gy (Follet, 2004). Work by Follet (2004) showed that 250–300 Gy can control Hawaii's sweet potato pests. Approved irradiation quarantine treatment doses for *Bactrocera cucurbitae* (Coquillet), melon fly; *Ceratitis capitata* (Wiedemann), Medfly; and *Bactrocera dorsalis* (Hendel), oriental fruit fly, infesting fruits and vegetables for export from Hawaii to the continental United States are 210, 225 and 250 Gy, respectively (Follett and Armstrong, 2004). Irradiation at 250 Gy, as a minimum dose, has been approved as a quarantine treatment for the export of tropical fruits grown in Hawaii (Boylston *et al.*, 2002). In early 1995, the Hawaii Department of Agriculture was granted a special permit from USDA-APHIS allowing untreated Hawaiian fruits to be irradiated in the US mainland (Moy and Wong, 2002). In April 1995, the first shipment of Hawaiian fruit was irradiated at a minimum quarantine dose of 250 Gy in an Isomedix plant near Chicago, and then distributed to supermarkets in Illinois and Ohio (Moy and Wong, 2002). A commercial e-beam/converted X-ray facility was installed by Titan Corp in Hawaii and was operational by late July 2000, making Hawaii the first place in the world to use irradiation as a quarantine treatment of fruits (Moy and Wong, 2002).

Ten citrus cultivars grown in Florida, including the orange (Ambersweet, Hamlin, Navel, Pineapple, and Valencia), and five mandarin hybrids (Fallglo, Minneola, Murcott, Sunburst, and Temple), were exposed to irradiation at 0, 150, 300 and 450 Gy, and stored for 14 days at 1°C or 5°C plus three days at 20°C, to determine dose tolerance based on fruit injury (Miller *et al.*, 2000). Softening of 'Valencia', 'Minneola', 'Murcott', and 'Temple' was dose-dependent, but that of other cultivars was unaffected. Only 'Ambersweet', 'Valencia', 'Minneola', and 'Murcott' did not develop peel pitting at 150 Gy or higher. Total soluble solids of 'Ambersweet' and 'Sunburst' declined slightly with increasing dose. Titratable acidity of oranges was not affected, but that of 'Sunburst' and 'Temple' juice was slightly reduced by irradiation at 450 Gy. Juice flavor of 'Hamlin', 'Navel', 'Valencia', and 'Minneola', and pulp flavor of 'Hamlin', 'Valencia', 'Fallglo', 'Minneola', and 'Murcott' was less acceptable after irradiation at 300 or 450 Gy. The appearance of all cultivars was negatively affected by the loss of glossiness with the 450 Gy dose. Less than 1% of fruit decayed and irradiation treatment had no effect on decay. Therefore, this study indicates that the effects of irradiation on citrus fruits are highly variable and both cultivar-dependent and dose-dependent.

Grapefruit harvested from gibberellic acid (GA) treated trees were irradiated at 300 or 600 Gy, and evaluated for quality after treatment and simulated commercial storage (Miller and McDonald, 1996). A condition, not observed before with grapefruit, was described by these authors and termed 'spongy fruit', which increased as irradiation dosage increased, consisted in peel pitting after five weeks' storage, which increased from < 2% to 11% and 25% as irradiation

dose increased from 0 to 300 and 600 Gy. The majority of pitting at 300 Gy was slight, and incidence of decay (mostly green mold) was reduced with thiabendazole (TBZ), and mean decay among all treatments was <1%. Total soluble solids, titratable acidity and the resulting ratio were not affected by GA or irradiation. There was a general decline in the sensory preference for juice flavor, and pulp flavor and texture as irradiation dose increased. These authors have concluded that GA-treated grapefruit will tolerate irradiation dosage of 300 Gy without serious damage; however, at dosage of 600 Gy, serious peel damage detrimental to fruit quality will likely develop during storage.

Aroma and flavor of papayas, rambutans, and 'Kau' oranges irradiated at 0 (control) and 750 (irradiated) Gy and stored for two and nine days tended to be more intense in the irradiated fruit, but the effects on specific sensory attributes were dependent on the specific fruit (Boylston *et al.*, 2002). Firmness decreased as a result of irradiation and storage, though it was significant only in rambutans. The color of rambutans and oranges were significantly affected by irradiation. Irradiation did not contribute to significant changes in the ascorbic acid and carotenoid contents, pH, titratable acidity, and total soluble solids.

The World Trade Organization does not permit its member countries to reject any quarantine treatment, including ionizing radiation, which is safe and effective. Unlike traditional inspection protocols which are based on absence of living pests, shipments are permitted based on certification that the commodity has received the appropriate phytosanitary dosage. There has been some interest in developing a test for verifying that the pest or the commodity has been treated based on the attributes of radiation induced pathology. Nation *et al.* (1995a;b) noted that Caribbean fruit flies irradiated as larvae fail to melanize in the pre-pupal stage and proposed a phenoloxidase assay. However, the test is not reliable for third instars. Similarly, Yulo-Nazarea *et al.* (1991) working with the oriental fruit fly and Lescano *et al.* (1994) working with the Queensland fruit fly, found detectable protein differences between irradiated and non-irradiated larvae. However, those studies involved lethal doses and were only demonstrated between dead and living insects. Thomas and Hallman (2000) found a difference in larval storage protein metabolism between survivors of a sublethal dosage and non-irradiated control larvae. But inasmuch as the difference was quantitative rather than giving a simple positive or negative, its applicability as a definitive assay is limited. At quarantine level doses, fruit fly larvae will not survive to emerge as adults. Perhaps the most reliable attribute of irradiated fruit fly larvae is the failure to complete pupal ecdysis. Hallman and Thomas (1999) proposed an assay based on this phenomenon for measuring irradiation efficacy in temperate fruit flies which have a diapause, thus obviating a score based on adult emergence. But its applicability as a quarantine assay may not be practical in that it requires the inspector to hold the larvae for pupariation and metamorphosis, during which time the perishable commodity will be losing its value.

Besides fruit flies, pests in the orders Coleoptera and Homoptera may be controlled with doses < 100 Gy but many Lepidoptera and mites (Acari) require about 300 Gy (Hallman 2000). Because the radiation damage at the subcellular level is traceable to chromosome breakage and subsequent interruption of cell

division typically manifest as tissue atrophy (Olive 1998), it is not overly surprising that Lepidopterans and Acari are more resistant to radiation. The Lepidoptera and Arachnida have holokinetic chromosomes and as a consequence, fragments are not left behind during mitosis as they are in those insects such as Diptera which have the kinteochore localized to the centromere.

This resistance to radiation becomes problematic, however, in that as the effective absorbed dosage necessary to achieve quarantine security increases, the minimum applied dosage increases exponentially. As a practical matter a commercial shipment would be irradiated in pallet-load quantities and the difference in the received dosage between the center and the perimeter of such a load can be as much as three-fold. Such doses become damaging to the commodity and even if effective against the pest are unacceptable as a commercial application.

7.9.6 Microwave or radio frequency

The microwave region of the electromagnetic spectrum is from 1 to 100 GHz, between infrared and FM radio, and is close to the radio frequency range. Radio frequency (RF) waves are at the lower frequency range of the electromagnetic spectrum, with longer wavelengths. Accepted frequencies for industrial purposes are 13.56, 27.12, and 40.68 MHz (Tang *et al.*, 2000). RF energy generates internal heat by agitating molecules in the fruit with a very rapid change in charge within the electrical field. RF heating is very fast, can penetrate deep, and can sometimes heat insects more than fruit.

Rapid heating by microwave or radio frequency energy can reduce the potential for fruit damage, which can commonly occur with slower heating deinfestation treatments (Varith *et al.*, 2006). The concept of high-temperature-short-time treatment is possible to shorten the treatment time while retaining fruit fly control at the Probit 9 level. The high-temperature-short-time concept is extensively used in food processing to minimize thermal degradation of food quality (Stumbo, 1973; Holdsworth 1997). Tang *et al.* (2000) proposed high-temperature-short-time thermal quarantine methods using RF energy to control codling moth in in-shell walnuts at 50 to 54°C. Microwave heating was tested on mangoes for control of mango seed weevil in the late 1960s, but fruit appeared 'cooked' after treatment. More recently, Varith and Kiatsiriroat (2004) studied microwave heating on 'Chokanan' mango with a 2450 MHz/800 Watt microwave oven and found an increase of internal temperature up to 46°C within 40 seconds. Heat distribution within the fruit depended on orientation, microwave power and treatment time. The horizontally positioned mango treated with 50% microwave power yielded better heat distribution than the vertical one.

Varith *et al.* (2006) compared a combination microwave followed by vapor heat treatment with the standard vapor heat treatment (heat with 55°C air until center temperature reaches 47°C and hold for 18 minutes) on 'Namdokmai Si Thong' mangoes. Mango fruit were exposed to 50% power using a 2450 MHz/800 Watt microwave oven. The mango was first placed horizontally within the oven and rotated while being heated with MW power of 400 W for 40 seconds. Secondly, the mango was placed vertically and the radiation focused on the cheek, the thickest part

of the fruit. The final process was a vapor heat treatment (saturated steam) at 55°C. After treatment, the fruit were hydrocooled with 25°C water for 30 minutes. It took two minutes to raise the core temperature of the mango to 47°C with microwave heating. When a hold time of seven minutes in 55°C vapor heated air was added, 100% mortality of oriental fruit fly eggs was achieved. Only 96% mortality was obtained with the vapor heat treatment at 55°C and an 18 minute hold when the fruit core reached 47°C. The combination treatment caused no skin browning while the vapor heat treatment did. Also, internal damage was much reduced with the combination treatment, with only slight internal tissue collapse at the apex of the pit. While these treatments have so far been accomplished on a very small scale utilizing single fruit treatments, the results indicated some promise for this treatment approach.

When multiple fresh fruit are treated with microwave heating in a batch, they must be immersed in a saline solution to prevent burning due to concentration of electrical energy at the contact points with other fruits. Small-scale studies with RF heating of fresh fruit in a saline solution have shown some promise for guava and some other crops (Monzon *et al.*, 2006; 2007). Combinations of RF heating with hot water immersion have also been explored for oranges (Birla *et al.*, 2005). Hot water assisted RF was tested for control of Medfly in oranges, using heat exposures previously demonstrated to provide 100% mortality (Gazit *et al.*, 2004). Fruit were pre-heated in 35°C water (a non-damaging temperature) for 45 minutes prior to RF heating to 48°C and holding the fruit at that temperature for 15 minutes. This treatment controlled Medfly without affecting fruit quality (Birla *et al.*, 2005). A similar treatment was better tolerated by sweet cherries than a hot water treatment alone. However, a similar approach with apple fruit resulted in excessive fruit damage.

The practical implications for implementation of RF or microwave treatments are difficult for fresh fruits in large scale systems due to the potential for large temperature variations in the treated load. This limitation is further complicated by the requirement for treating fresh fruit in a saline solution thus requiring unique engineering solutions that have not yet been developed.

As a quarantine treatment, RF heating would most likely find success with dry products where the infesting pests have a higher moisture content than the host, consequently absorbing more energy, especially at lower frequencies (10–100 MHz) (Vincent *et al.*, 2002). Also, dried products are more tolerant of RF heating than fresh commodities. Thermoelectric energy delivered in pulsed electrical fields has also shown some promise for both antimicrobial and disinfestation of insects (Hallman and Zhang, 1997). But though efficacious, the technique has not yet developed past the experimental stage.

7.9.7 Combination treatments

Fumigation and heat

High temperatures (> 21°C) are effective in decreasing fumigant concentration and treatment duration periods because of increased penetration and insecticidal

activity. In addition, increased fumigant temperature increases the metabolic activity of the pest organism, increasing the rate of fumigant uptake and thus resulting in shorter treatment duration.

MA and CA at high temperatures

This treatment combines the stress of heat with that of atmospheric stress due to reduced oxygen and/or elevated carbon dioxide concentrations (Neven and Mitcham, 1996; Yahia, 1998; 2008). Reduced oxygen and elevated carbon dioxide atmospheres have been known to be effective in killing various insect pests for many years, but were generally applied at ambient or lower temperatures (Yahia, 1998; 2009). Killing time is faster at elevated temperatures. Along with the forced hot-air, nitrogen is used to replace oxygen, and carbon dioxide is added. The mechanism of control is to increase the respiratory demand of the insects with the heat treatment while at the same time modifying the atmosphere, both of which contribute to the death of the insect. Treatment times with CA at high temperatures can be one-half those with heat treatments alone. This effectiveness is the result of reducing the availability of O_2 during a heating stress that hinders the insects' ability to support elevated metabolic demands due to the heat load. There is also evidence that a heat treatment under anoxic conditions reduces the production of heat shock proteins in insects (Thomas and Shellie, 2000), and elevated CO_2 atmospheres may interfere with the insects' ability to produce ATP (Friedlander, 1993; Zhou *et al.*, 2000; 2001). Heat treatments combined with an anoxic environment can provide quarantine security more rapidly than a heat treatment or an MA/CA treatment alone (Lay-Yee and Whiting, 1996; Moss and Jang, 1991; Neven and Mitcham, 1996; Sonderstrom *et al.*, 1992; Whiting *et al.*, 1991; 1992; 1995; 1996;Whtitng and Hoy, 1997; Yocum and Denlinger, 1994).

Several treatments combining MA or CA with heat (Ortega and Yahia, 2000; Yahia and Ortega, 2000, Neven and Mitcham, 1996) have been studied for different crops. Treatments with CA in combination with forced hot air have been tested for control of Mexican fruit fly and West Indian fruit fly in 'Manila' mangoes (Yahia and Ortega, 2000; Ortega-Zaleta and Yahia, 2000). 'Manila' mangoes tolerated treatment with 0% O_2 and 50% CO_2 at <44°C and 50% RH for 160 minutes (Ortega-Zaleta and Yahia, 2000), but injury occurred at 44°C and increased with increasing temperature. However, treatment at <44°C was not fully effective to control the two fruit fly pests (Yahia and Ortega, 2000). High-temperature CA treatments were approved in 2008 by USDA APHIS for export of US nectarines, sweet cherries and apples to control codling moth (*Cydia pomonella*), oriental fruit moth (*Grapholita molesta*) and western cherry fruit fly (*Rhagoletis indifferens*) (Neven and Rehfield-Ray, 2006), but are not yet approved for product imported into the US. Some chambers have been constructed to treat different crops (Fig. 7.7).

Insecticidal CA treatments have also been investigated at room temperature (20–25°C) as insects might be controlled within a period of 48 to 72 hours (Yahia, 1998). If this approach can work, then a significant amount of energy (for heating and for cooling after the heating process) can be saved.

(a)

(b)

(c)

Fig. 7.7 (a) A commercial pallet-configured heated controlled atmosphere chamber at the USDA-ARS Laboratory, Parlier, CA, (b) loading a commercial bin-configured CATT chamber at a packinghouse in George, WA, (c) commercial bin-configured CATT chamber at a packinghouse in George, WA (courtesy of Dr Lisa Neven, USDA-ARS, Wapato, WA, USA).

Another combined treatment was investigated by Lay-Yee and Whiting (1996), who examined the response of 'Hayward' kiwifruit to high-temperature CA treatments for control of two-spotted spider mite (*Tetranychus urticae* Koch). 'Hayward' kiwifruit were subjected to 40°C for seven or ten hours in 20% CO_2 (treatments identified as giving 100% mortality for non-diapausing and diapausing two-spotted spider mites, respectively) or in air, and following treatment, fruit were cooled in ambient water or ambient air, stored at 0°C in air for eight weeks, then held at 20°C overnight and assessed for quality. Relative to non-treated controls, no significant damage was observed with fruit subjected to 40°C air treatments. No significant damage was observed with fruit treated for seven hours with 20% CO_2 followed by hydro-cooling. However, the treatment without hydro-cooling and ten hours treatments with hydro-cooling showed only slight damage, while the ten hours CA without hydro-cooling had moderate fruit damage. Following storage, flesh firmness of fruit treated at 40°C in air for ten hours or in 20% CO_2 CA for seven and ten hours, with and without hydro-cooling, was lower than that of non-treated controls.

Soderstrom *et al.* (1992) investigated the potential of a combination heat and CA treatment for the disinfestation of diapausing codling moth in walnuts, and found that treatments of 98% CO_2 at 45°C provided Probit 9 security level of codling moth control in 3.6 hours. This duration of treatment is comparable to traditional MeBr fumigation. The temperatures used in this study were based on walnut drying temperatures determined not to adversely affect walnut quality. However, although this treatment looks promising, the walnut industry is reluctant to pursue this alternative, citing excessive costs of treatment and concerns over volume and time constraints. But with increasing costs of MeBr fumigation, these concerns may change.

Fruit and insects were subjected to heat treatments with and without CA (1% O_2, 15% CO_2) with a linear heating rate of 12°C to final treatment temperatures of either 44 or 46°C for 4 or 3 h, dew point being controlled to 2°C above the surface temperature of the fruit, air speed maintained between 1.5 and 2.0 m s^{-1}, and following treatment, all fruit that were to be evaluated for quality were stored under standard CA conditions (1% O_2, 1% CO_2, 0°C) for 0, 45 or 90 d (Neven and Drake, 2000). No significant differences were detected in fruit quality for fruit heated in air or under CA conditions, and in all heat-treated fruit, quality after cold storage was as good as or better than fruit that were not heat-treated.

Traditionally, heat treatments have been conducted to reach the target temperature as quickly as possible (Mangan and Ingle 1994; Mangan *et al.*, 1998, Shellie *et al.*, 1993; 1996; Shellie and Mangan, 1994; Armstrong, 1994; Sharp, 1994; Hallman and Armstrong, 1994). This strategy seems to work effectively for tropical and subtropical fruits, but not for temperate tree fruits (Neven *et al.*, 1996). Some studies have shown that short term, high temperature treatments of apples prior to long term cold storage improve quality and lengthen shelf life (Klein, 1994; Klein and Lurie, 1992; Klein *et al.*, 1990). Studies on apples and pears indicated the rate of heating directly impacted fruit quality (Neven and Drake, 2000). By controlling the rate of heating, the fruit can compensate for the heat load.

Some researchers have indicated that the slower the rate of heating, and the lower the final treatment temperature, the longer the total treatment needs to control the insect pest (Neven and Rehfield 1995; Neven *et al.*, 1996; Neven, 2000). Although intuitively one would expect that tropical insect pests would be more tolerant of high temperatures than their temperate zone counterparts, this is not necessarily the case. The lipid layer of the insect cuticle plays a critical function in preventing dessication. At higher temperatures the cuticle becomes permeable depending on the mix of polar lipids and typically there is a transition temperature at which permeability increases exponentially. Desert species often have a higher transition temperature than insects from humid environments but the generality does not always hold. For many insects the transition occurs in the 40–50°C range and this often determines the lethal temperature and therefore the target for quarantine treatments. For tephritid fruit flies the lethal temperatures are in the 40–45°C range.

All living organisms, including fresh harvested fruits and vegetables, produce heat shock proteins in response to thermal stress (Craig, 1985) and this response is the primary basis for resistance to temperature based quarantine treatments. This response explains why the rate of heating, the so called 'ramp-up', is more critical than the total thermal units applied in determining efficacy. For example, Jang (1992) found that the heat shock protein response was triggered within 30 minutes by exposure to temperatures of 33 to 41°C and that this synthesis conferred thermotolerance, but above 41°C the protein synthesis was inhibited. Thus a pest develops resistance most efficiently with a slow ramp-up. The commodity responds in a similar manner and thus pre-treatments at less severe temperatures can confer treatment protection to the fruit, but of course, such pre-treatments also cause the pest to be more tolerant (Chen *et al.*, 1991) and should be avoided.

Irradiation and heat

Only limited studies have tested combinations of irradiation and heat treatments. 'Marisol' clementines from Spain were irradiated with gamma rays at doses of 0.3 and 0.5 kGy, with and without a hot water treatment (53°C for 5 minutes), stored at 17°C and 46% RH for three weeks, and compared to un-irradiated control clementines for shelf life, texture, color, pH, degree-Brix, vitamin C content and organoleptic quality (Abdellaoui *et al.*, 1995). Irradiated clementines were found to be less firm than control fruit, minor changes occurred in the pH of control and irradiated clementines during storage, and the total soluble solids content decreased for the irradiated clementines but, little difference was noted between the hot water treated clementines irradiated at a dose of 0.5 kGy and the control. Vitamin C content of treated clementines was higher than that of the control fruit. Sensory evaluation studies indicated that the organoleptic quality of the irradiated clementines was maintained during storage. However, the hot water treated irradiated fruits reached the limit of acceptability after two weeks of storage and were judged unacceptable from the third week. Therefore, these authors have concluded that irradiation at doses of 0.3 and 0.5 kGy, as an alternative process to quarantine treatment, preserved the quality of clementines but that the hot water treatment combined with irradiation was detrimental to their shelf life under the chosen experimental conditions.

'Marsh' grapefruit was treated with vapor heat (two hours at 38°C), and fungicidal treatments of thiabendazole (TBZ) (4 gm L^{-1}) and TBZ (1 gm L^{-1}) plus imazalil (1 gm L^{-1}) prior to irradiation at 0.5 or 1.0 kGy (Miller and McDonald, 1998). Vapor heat reduced the severity and incidence of peel injury by approximately 50% without adversely affecting other quality attributes. The fungicide did not reduce peel injury.

Radio frequency and hot water

Radio frequency-hot water treatments for postharvest control of codling moth in 'Bing' sweet cherries were examined as a potential alternative method by Hansen *et al.* (2005). 'Bing' sweet cherries were each infested with a codling moth larva, submerged in a 38°C water bath for six minutes pretreatment, then exposed to various temperatures generated by radio frequency and held at different temperatures and durations: 50°C for six minutes, 51.6°C for four minutes, 53.3°C for 0.5 minutes, and 54.4°C for 0.5 minutes. Insect mortality was evaluated 24 h after treatment and fruit quality was evaluated after treatment and after 7 and 14 days of storage at 1°C. No larvae survived at the 50 and 51.6°C treatments. Fruit color of non-infested cherries was darkened as temperature increased. Stem color was severely impacted after seven days of storage, even in a warm water bath of 38°C for six minutes, as was fruit firmness at the same treatment. Fruit quality loss increased after 14 days of storage, compared to after seven days of storage. The amount of pitting and bruising of cherries increased with temperature and again this increase was more evident after 14 days of storage.

7.9.8 Comparative studies between different treatments

'Galia' melons (*Cucumis melo* L. var. reticulatus) from four harvests in two years were irradiated at doses up to 1 kGy, and hot-water dipping at 53°C for one minute was studied as a treatment for protection from decay in combination with irradiation (Lalaguna, 1998). Irradiation had no effect on vitamin C content, titratable acidity, soluble solids content, moisture content, or decay after 14 days of storage at 23°C. Hot-water dipping reduced decay, but did not accentuate sensitivity to irradiation.

Comparative effects of gamma irradiation and MeBr fumigation were determined for fresh chestnut on mortality of pests and quality stability by Kwon *et al.* (2004). Chestnut was exposed to both irradiation at 0–10 kGy and MeBr fumigation in commercial conditions, and then stored at 5°C for six months. Pests with quarantine importance for chestnut showed 100% mortality by MeBr at the third day after fumigation and by irradiation at 0.5 kGy in about four weeks. Sprouting was controlled for six months with treatments with 0.25 kGy or more and of MeBr, but rotting rate dramatically increased from two months after fumigation. Irradiation over 1 kGy as well as fumigation significantly caused changes in the color of stored chestnut. Considering the cumulative mortality of chestnut pests, these authors recommended irradiation at 0.5 kGy as an alternative to MeBr fumigation for both quarantine and sprout control purposes. In another study by Kwon (2005) gamma irradiation (up to 10 kGy) was evaluated

as an alternative to MeBr fumigation for the control of *Curculio sikkimensis* in fresh chestnuts. A 100% mortality was achieved by MeBr on the third day after fumigation and by irradiation at 0.5 kGy in about three weeks at 23°C. Respiration rates of samples one day after treatments increased in proportion to irradiation dose. Respiration pattern of MeBr group was equal to that of the 10 kGy-group. Both MeBr and irradiation at 0.25 kGy or higher showed 100% inhibition of sprouting during storage at 5°C for six months. Flesh firmness was significantly reduced by MeBr or irradiation over 5 kGy one day after treatments. MeBr fumigation resulted in appreciable decrease in flesh weight, reducing sugar and ascorbic acid contents, as compared to irradiated samples. Therefore, Kwon (2005) has indicated that irradiation at 0.5 kGy was effective as an alternative to MeBr in controlling pests while maintaining overall quality of fresh chestnuts during storage.

Forced, hot-air (48.5°C for 3–4 hours) treatment of papaya fruit, a developed quarantine treatment for fruit flies, did not significantly reduce incidence of postharvest diseases when compared with fungicide or hot-water treatments. However, when combined with thiabendazole (TBZ) (4 g a.i. L^{-1}) or hot-water immersion (49°C for 20 minutes), the incidence of most postharvest diseases was reduced (Nishijima *et al.*, 1992). Although disease incidences were not significantly affected by the sequence of hot-air or hot-water application, de-greening, along with pitting and scalding symptoms increased when hot-water treatment preceded hot-air treatment, but these symptoms did not occur when hot-air treatment preceded hot-water treatment. The hot-air treatment was associated with an increase in the incidence of internal lumpiness (hardened lumps of flesh in ripe fruit) when compared with untreated fruit.

'Arkin' carambolas fruit were subjected to the fruit fly quarantine treatments of hot-water immersion at 43.3 to 43.6°C for 55 or 70 minutes, 46.0 to 46.3°C for 35 or 45 minutes, or 49.0 to 49.3°C for 25 or 35 minutes, or vapor heat at 43.3 to 43.6°C for 90 to 120 minutes, 46.0 to 46.3°C for 60 or 90 minutes, or 49.0 to 49.3°C for 45 or 60 minutes (Hallman, 1991). The 49.0 to 49.3°C treatments resulted in excessive damage to the carambolas two to four days after treatment, but there were no statistically significant differences in the variables measured among the other treatments and control; however, heat-treated carambolas appeared duller in color than control fruits. Overall, fruit treated at 46.0 to 46.3°C lost significantly more weight than that treated at 43.3 to 43.6°C.

'Navel' orange was exposed to moist, forced air at 46°C for up to 4.5 hours or 50°C for two hours, or immersed for three hours in water at 46°C, and evaluated for quality attributes after four weeks of storage at 7°C and one week at 23°C (Shellie and Mangan, 1998). The flavor of oranges immersed in hot water was rated significantly inferior to fruit heated in air and fruit that were not heated. Oranges immersed in hot water also developed a higher incidence of decay during storage than oranges heated in air or non-heated control fruit. The flavor of oranges exposed to moist, forced air at 46°C for up to 4.5 hours was rated by sensory panelists as similar to non-heated controls, even though heated fruit had a significantly lower amount of titratable acidity and a higher ratio of sugar to acid.

Fruit exposed to high-temperature forced air developed less decay during subsequent storage than non-heated control fruit. Therefore, these authors have concluded that Texas 'N33' navel oranges tolerated exposure to forced air at 46°C for up to 4.5 hours without deleterious effects on fruit market quality.

Litchi fruit were subjected to either 15 days at 1.1°C or to gamma irradiation from a Co-60 source at dosages of 100, 200, or 300 Gy (McGuire, 1997b). Cold-treated 'Mauritius' fruit lost some color intensity externally and internally, and the pale flesh had a greener hue. The pericarp of 'Brewster' fruit was injured to a greater extent by cold treatment than that of 'Mauritius', and the pulp of treated fruit had lower concentrations of acids and soluble solids. Cold treatment increased decay susceptibility of both cultivars. 'Mauritius' fruit were also more susceptible to decay following irradiation at 300 Gy and six days of storage at 5°C. Both cultivars lost firmness after this treatment. The pericarp of irradiated 'Mauritius' fruit became more orange, whereas the flesh of both cultivars became greener. Irradiated 'Brewster' fruit were less acidic and contained less soluble solids, but sensory evaluations could not differentiate between irradiated and non-treated fruit regardless of cultivar. Loss of quality was minimal with either cold or irradiation treatment, and both were recommended to be acceptable for litchis requiring quarantine treatment for eradication of exotic pests.

Canistel fruit were subjected to cold storage (1 or 3°C for 17 days) and hot-water immersion (46°C for 90 minutes or 48°C for 65 minutes) treatments known to kill immature Caribbean fruit flies (*A. suspensa*) in other fruit (Hallman, 1995). Cold storage did not cause appreciable loss in canistel quality compared with fruit stored at the normal 10°C, and unripe canistels immersed in both hot water treatments developed dark blotches on the peel and a 2–3-mm-thick layer under the peel that did not soften.

Hot-water immersion and irradiation quarantine treatments are used to disinfest litchi of fruit flies and other pests before export from Hawaii to the US mainland. Follett and Sanxter (2003) compared the quality of the fruit exposed to each of these treatments. One day after harvest, 'Kaimana' litchi fruit were subjected to (1) hot-water immersion at 49°C for 20 minutes, (2) irradiation treatment at a minimum absorbed dose of 400 Gy, or (3) left untreated as controls, and fruit were then stored at 2 or 5°C in perforated plastic bags, and quality attributes were evaluated after eight days (Follett and Sanxter, 2003). Litchi fruit treated with hot-water immersion were darker (lower lightness) and less intensely colored (lower chroma) than irradiated or untreated fruits at both storage temperatures. Fruit stored at 2°C were darker (lower lightness) than fruit stored at 5°C, but fruit held at 5°C had greater weight loss. External appearance of fruit treated with hot-water immersion was rated as unacceptable, whereas irradiated and non-treated fruit were rated as acceptable. Taste of fruit was rated as acceptable in all treatments. In another experiment by the same authors, litchi fruit were subjected to 1) hot-water immersion at 48, 48.5, or 49°C for 20 minutes or (2) irradiation at 400 Gy, or (3) left untreated as controls, and fruit were then stored at 4°C in perforated plastic bags, and external appearance of the pericarp was evaluated after one, two, five, seven, eight and nine days. Pericarp darkening was more rapid for litchi fruit

treated with hot-water immersion than for irradiated or control fruit, and the degree of quality loss increased with increasing hot-water immersion temperature. Overall, under these experimental conditions, the authors have concluded that irradiation was superior to hot-water immersion as a quarantine treatment on the basis of fruit quality maintenance.

Follett and Sanxter (2002) evaluated the effect of hot-water immersion and irradiation quarantine treatments on the quality of longan fruit. One day after harvest, longan fruit (cvs. 'Chompoo' and 'Biew Kiew') were subjected to hot-water immersion at 49°C for 20 minutes, to irradiation treatment at a minimum absorbed dose of 400 Gy, or left untreated as controls, and fruit were then stored at 10°C in perforated plastic bags, and quality attributes were evaluated after 7, 14, and 21 days. 'Chompoo' and 'Biew Kiew' fruit treated by hot-water immersion were darker (lower L*) and less intensely colored (lower C*) than irradiated or untreated fruits after 14 days of posttreatment storage. For both cultivars, external appearance of fruit treated by hot-water immersion was rated as unacceptable after 14 and 21 days of posttreatment storage, whereas irradiated and non-treated fruit were rated as acceptable on all days. *Penicillium* mold contributed to the unacceptable external appearance ratings after 21 days for fruit that were treated by hot-water immersion. Taste of fruit treated with hot-water immersion in both cultivars was rated as unacceptable after 21 days of storage, whereas irradiated fruit remained acceptable. Overall, these authors concluded that under these experimental conditions, irradiation was superior to hot-water immersion as a quarantine treatment based upon the maintenance of fruit quality.

Irradiation of rambutan with 250 Gy is an Animal Plant Health Inspection Service (APHIS)-approved quarantine treatment, but a hot forced-air treatment has also been proposed for eliminating fruit fly pests, and therefore Follett and Sanxter (2000) evaluated the effect of each of these treatments on the quality of rambutan. Two days after harvest, rambutan fruit (cultivars R134 and R167) were subjected to: (1) hot forced-air at a seed surface temperature of 47.2°C, (2) irradiation treatment at 250 Gy, or (3) left untreated as controls. Fruit were then stored at 10°C in perforated plastic bags, and quality attributes were evaluated after 4, 8 and 12 days. 'R134' fruit treated with hot forced-air were significantly darker (lower L*) and less intensely colored (lower C*) than irradiated or non-treated fruit after four and eight days of posttreatment storage; the external appearance was unacceptable after four days of storage, whereas irradiated fruit remained acceptable through eight days of storage. Differences between treatments were less pronounced for 'R167'. 'R167' fruit treated with hot forced-air had lower L* and C* values and less acceptable external appearance ratings than did irradiated fruit at 4, 8 and 12 days post-treatment, but differences were not statistically significant. For both cultivars, external appearance of fruit in all treatments was unacceptable after 12 days of storage, whereas taste was rated as acceptable for all treatments on each day. These authors have concluded that under these experimental conditions, irradiation was superior to hot forced-air as a quarantine treatment on the basis of fruit quality maintenance.

7.9.9 Postharvest safeguards and packinghouse procedures

Containers of harvested fruit should be covered with tarpaulins or other covers and moved to the packinghouse in a fruit fly-proof conveyance in a timely manner (e.g., within three hours of harvest). Upon arrival at the packinghouse, a random sample of fruit per lot should be taken to be inspected for external pests and cut to reveal internal pests, each sample to be of sufficient size to detect a 0.00003 infestation rate. In the packinghouse, fruit should undergo mechanical brushing or other treatment to remove external pests. Fruit should then be immersed in a water bath containing surfactant and, perhaps, a surface sterilant, such as chlorine bleach (e.g., NaOCl). Surfactants, such as common dishwashing detergent, may show a high degree of insecticidal activity with minimal risk of phytotoxicity. All fruit should be inspected prior to packing. Consignments should be transported in sealed, refrigerated vehicles.

7.10 Conclusions

International trade of tropical and subtropical fruits has become increasingly important, but limitations imposed by phytosanitary restrictions continue to limit markets and increase postharvest costs. Phytosanitary or quarantine treatments are often required to disinfest host commodities of economically important arthropod pests before they are moved through market channels to areas where the pest does not occur within or between countries. This chapter describes some of the important quarantine pests, as well as some of the treatments that can be used for their control and the effects of the commercially developed or potential insect quarantine treatments and systems on the quality of tropical and subtropical fruits. The increase in the demand and thus in the export of fresh tropical and subtropical fruits, and the increased restrictions on the use of chemical fumigants as quarantine treatments has increased research activity to develop different physical quarantine treatments and systems. As a result, several quarantine treatments and systems have been developed using low and high temperatures, modified and controlled atmospheres, irradiation, radio frequency, and combinations of some of these. Quarantine treatments and systems should control the insect pests without negatively affecting the quality of the crop. Concerns about the safety of food supply, along with concerns about the impact of agricultural chemicals on the environment, are increasing public and scientific interest in the development of non-chemical quarantine treatments. The challenge for future research is to develop non-chemical, low cost quarantine treatments and systems that do not harm the consumer nor the environment, and that can be applied either in permanent installations or aboard transport ships. Successful quarantine can be implemented using individual treatments, but most probably, the development of various, specific combinations of treatments will be most effective.

7.11 References

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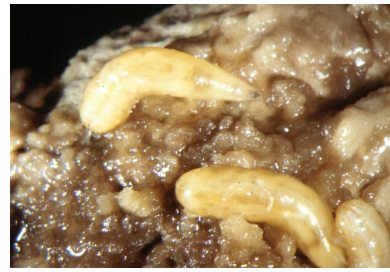
(a)



(b)

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(f)

(g)

(h)

(i)

Plate IX (Chapter 7) Some examples of quarantined pests. (a) Medfly; (b) larvae of *Anastrepha ludens*; (c) adults of *A. ludens* on grapefruit; (d) *A. Striata*; (e) *Bactrocera dorsalis*; (f) *B. cucurbitae*; (g) *B. tryoni* on citrus; (h) *B. tryoni* on avocado; (i) Mexican fruit fly (courtesy of USDA).

(a)

(b)

(c)

(d)

Plate X (Chapter 7) Bagging of fruit in South-East Asia.

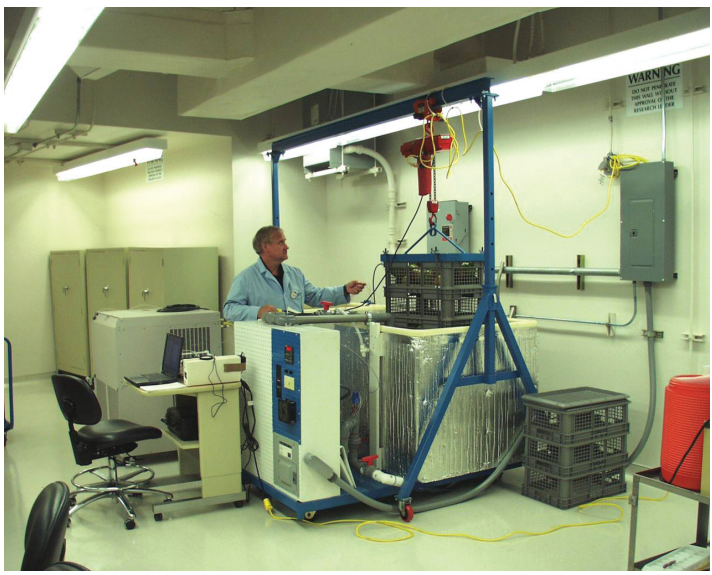

Plate XI (Chapter 7) Hot water dip testing at the Weslaco, Texas USDA Laboratory (courtesy of USDA).

8

Microbial safety of tropical and subtropical fruits

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Abstract: This chapter describes past outbreaks, potential routes of contamination for specific pathogens, potential interventions, and operational procedures associated with tropical and subtropical fruits. Various preharvest sources can result in contamination of fruits. Survival and growth of pathogens on whole and fresh-cut fruits are variable depending on the biotic and abiotic factors such as indigenous microorganisms, storage temperature and humidity, etc. The effectiveness of chemical, physical, and biological treatments designed to eliminate pathogens on these fruits can be limited by the surface topography, hydrophobicity, organic material present on fruits, and other confounding factors. Integrated food safety management programs, and pathogen prevention and decontamination approaches in the supply chain must be utilized to make tropical and subtropical fruits safe for consumption.

Key words: tropical fruit, subtropical fruits, outbreaks, interventions, *E. coli* O157:H7, *Salmonella*, norovirus, hepatitis A, incidence, route of contamination, prevention.

8.1 Introduction

As global trade increases, so does the import and export of fresh produce from one country to another. There are many benefits to consumers from this increased trade, which include increased choices among fruits and vegetables, year-round availability of tropical fruits and vegetables, and increased nutritional benefits that these fruits have for human health. However, the lack of confidence and traceability of fruits grown in different countries and regions is a daunting food safety challenge. To realize the nutritional and culinary benefits of tropical fruit, there is a critical need to understand the microbiological hazards associated with these commodities, and the intervention and prevention options targeting

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microbial contamination. This chapter addresses some of the most common microbial foodborne pathogens associated with tropical and subtropical fruits, the routes of contamination for these fruits, how these microorganisms may survive in or on tropical fruits, and strategies to control and minimize the risk of contamination by these pathogens. This chapter is limited to examining microbial contamination that has caused foodborne illness in the past. There are certainly chemicals (toxins in fruits, pesticides) that can be harmful and cause serious health effects in humans. However, those issues are outside the scope of this chapter.

8.2 Foodborne pathogens associated with tropical and subtropical fruit

This chapter provides information on selected foodborne pathogens and disease associated with the consumption of tropical and subtropical fruits. The descriptions below are by no means comprehensive, but give a brief introduction to foodborne pathogens that have previously contaminated tropical and subtropical fruits.

8.2.1 *Escherichia coli* O157:H7

Escherichia coli O157:H7 is a prominent example of enterohemorrhagic *E. coli* (EHEC), and as such is a principal causative agent for hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Its ability to produce shiga-toxin, which results in HUS, distinguishes it from other pathogenic *E. coli. E. coli* O157:H7 is a member of the *Enterobacteriaceae* (gram-negative rods) with all of their relevant properties. The main reservoir of *E. coli* O157:H7 is in the intestinal tracts of ruminant animals (cattle, sheep, deer and goats), and the pathogen is shed into the environment through animal feces (Thorpe *et al.*, 2002). The infectious dose of this organism appears to be low, 1 to 100 cells (Mead and Griffin, 1998). The onset of symptoms can be variable, between one and eight days after infection. Symptoms of *E. coli* O157:H7 infections are characterized by watery diarrhea, possibly becoming bloody diarrhea (hemorrhagic colitis) within 48 hours. Most patients tend to recover without supportive therapy, but 5–10% of patients progress to develop HUS, affecting their kidneys, gastrointestinal tract, brain, lungs and pancreas (Thorpe *et al.*, 2002). Of the patients who develop HUS, 30% will develop post-infection sequelae including proteinura or other non-renal sequelae (Paton and Paton, 1998). HUS is the leading cause of acute pediatric renal failure in the U.S. These symptoms can lead to death in some cases.

8.2.2 *Shigella*

The genus *Shigella* consists of four species that are genotypically and phenotypically closely related to *E. coli.* The incubation period is similar to that

of *E. coli* O157:H7 (one to seven days), with symptoms usually presenting themselves within three days (Maurelli and Lampel, 1998). These symptoms include watery diarrhea that can transition to dysentery (bloody stool with rectal pain, cramps and fever). The severity of symptoms which patients encounter is dependent on the *Shigella* species causing the infection. *S. dysenteriae* produces the more severe illness, and *S. sonnei* produces the mildest. *Shigella* spp. have low infectious doses similar to EHEC (Maurelli and Lampel, 1998). Contamination of foodstuffs with *Shigella* spp. is commonly traced backed to food handling in unhygienic environments or water contaminated with feces.

8.2.3 *Salmonella*

The bacterium *Salmonella*, like *E. coli* O157:H7, is a member of the *Enterobacteriaceae*. There are 2463 serotypes in two *Salmonella* species: *S. enterica*, which contains five subspecies (I, II, III, IV, and VI) and *S. bongori* (Brenner, 2000). Serotypes isolated from humans mainly reside in *S. enterica* subspecies I. Approximately 150 serotypes of *Salmonella* have been associated with human illness (Doyle and Cliver, 1991). Symptoms of non-typhoidal *Salmonella* infections usually appear between 8 and 72 hours (with a mean of 24 hours) after exposure to the pathogen, and include nausea, vomiting, abdominal pain, headaches, and diarrhea (D'Aoust, 1998). The infectious dose of non-typhoidal *Salmonella* in foods is variable and can depend on the serovar, characteristics of the food (e.g., pH, fat content) and host susceptibility. Reports describe a dose range from 10^1 to 10^7 cells being associated with outbreaks (Jay *et al.*, 1996). *Salmonella* is thought to have more non-host environmental reservoirs than *E. coli*, possibly increasing its potential to cause outbreaks on tropical fruits (Winfield and Groisman, 2003). *Salmonella* inhabit, at least transitorily, the gastrointestinal tracts of a wide variety of animals, including humans. Birds, reptiles, rodents and insects are well-recognized as potential carriers of the pathogen, and can serve as a vehicle for the transfer of the pathogen from animal feces to foods or to pre-harvest environments in fields (Beuchat, 1996).

8.2.4 Norovirus and Hepatitis A

Noroviruses belong to the *Caliciviridae* family of viruses. They are small, non-enveloped viruses, have a spherical shape with a size ranging from 28–35 nm (Koopmans *et al.*, 2002). The genome of the caliciviruses is composed of single-stranded RNA, with between 7.3 and 7.6 kB of genetic material. Noroviruses are classified into five separate genetic groups (GI, GII, GIII, GIV and GV), with the majority of human viruses occurring in groups GI and GII (Hirneisen *et al.*, 2009). The majority of norovirus outbreaks are associated with eating fresh produce, salads, or sandwiches – foods that require handling without an intervention designed to kill pathogens. Multiple norovirus outbreaks have been reported for cruise ships, nursing homes, hospitals, military bases and college campuses,

though this may be biased by the fact that these locations are more likely to report outbreaks. Noroviruses have a very low infectious dose, as few as ten virons, and can be disseminated through aerosolized vomitus, person-to-person contact, and food (Hirneisen *et al.*, 2009). Persons infected with norovirus can shed viruses for up to ten days after infection (Atmar *et al.*, 2008). Symptoms from norovirus infections include acute diarrhea, nausea, vomiting, abdominal cramping, fever, and some headaches, lasting for between one and two days. Diarrhea is more prevalent in adults, with vomiting more prevalent in children. Exposure to norovirus does not provide long-lasting immunity to subsequent infections (Koopmans *et al.*, 2002).

Hepatitis A virus (HAV) belongs to the *Picornaviridae* family and is a non-enveloped virus. The single-stranded RNA genome is 7.5 kB. There are four genotypes of HAV that infect humans, with most human strains belonging to genotypes I or III (Cuthbert, 2001). HAV is disseminated through foods or water contaminated with fecal matter. The incubation period of the disease is 15 to 50 days between exposure and onset of symptoms. Symptoms include jaundice, dark urine, diarrhea and symptoms resembling flu, and can last for variable periods of time, from two weeks to three months (Cuthbert, 2001). Similar to norovirus, the infectious dose of HAV can be very low (10 to 100 virions). Unlike norovirus, immunity to HAV can be developed upon exposure to virus, providing lifelong protection (Hirneisen *et al.*, 2009). An effective vaccine for HAV is available. HAV outbreaks are usually linked to foods contaminated by infected food handlers or foods contaminated before distribution.

8.3 Outbreaks of foodborne illness attributed to tropical and subtropical fruits

Worldwide, the proportion of foodborne outbreaks attributed to fresh produce has increased over the past 20 years. Foodborne outbreaks attributed to produce in the United States have increased from less than 1% in the 1970s to 6% during the 1990s (Lynch *et al.*, 2009). In Australia, 4% of the total number of foodborne outbreaks from 2001–2005 were attributed to fresh produce (Lynch *et al.*, 2009). Tropical fruits and, in some cases, unpasteurized juices made from these fruits, have become contaminated with bacterial and viral pathogens and caused foodborne outbreaks.

8.3.1 Outbreaks associated with mangoes and papayas

Mangoes and papayas contaminated with *Salmonella* spp. have been implicated in several foodborne outbreaks. The largest and most severe outbreak occurred in the United States in 1999, when mangoes contaminated with *Salmonella* Newport were responsible for sickening 78 people, 15 who required hospitalization and two individuals who died (Sivapalasingam *et al.*, 2003). The mangoes were traced back to a Brazilian farm, where unchlorinated water, used to cool the fruit after

they were dipped in hot water, may have been contaminated with *S.* Newport. Hot water dips are used to kill the larvae of the Mediterranean fruit fly present in mangoes. *Salmonella* Saintpaul was identified in an outbreak in 2001 associated with contaminated mangoes (Beatty *et al.*, 2001), sickening 26 individuals in the U.S. At least one contaminated mango purchased was traced back to Peru, where again, after immersion in hot water to kill fruit fly larvae, the fruits were cooled in unchlorinated water contaminated with *S.* Saintpaul (Beatty *et al.*, 2001). Smaller outbreaks involving mangoes contaminated with *S.* Oranienburg and *S.* Saintpaul sickened 9 and 17 individuals in the US, respectively (Hanning *et al.*, 2009).

In 2006 and 2007, 27 people in the Australian states of Queensland and Western Australia became ill from eating papayas contaminated with *S.* Litchfield (Gibbs *et al.*, 2009). Papayas tested from the grocery stores where the fruit was purchased were positive for *S.* Litchfield. Similar to outbreaks associated with contaminated mangoes, river water used to rinse the fruits in fungicide and low levels of chlorine (5 parts per million) was thought to be the source of contamination (Gibbs *et al.*, 2009). Although river water did not test positive for *S.* Litchfield, other *Salmonella* serotypes were found (*S.* Chester, *S.* Eastborne, and *S.* Poona).

8.3.2 Outbreaks associated with citrus fruits

Unpasteurized orange juice has been the source of several outbreaks of Hepatitis A. Some of these outbreaks have been traced to the infected workers who contaminated juices during preparation at restaurants or hotels. A hepatitis A outbreak in orange juice in 1962 in the US was also traced back to an infected kitchen worker in a Missouri hospital (Eisenstein *et al.*, 1962). A large outbreak among 351 tourists staying at a Red Sea resort in Egypt was linked to a commercially prepared unpasteurized orange juice (Frank *et al.*, 2007). Subsequent investigation of the manufacturing facility identified significant hygiene deficiencies.

Salmonella spp. have been the most common bacterial foodborne pathogen associated with unpasteurized orange juice. *S.* Typhi outbreaks in 1944 and 1989 in orange juice sickened 18 and 67 people, respectively (Duncan *et al.*, 1946; Birkhead *et al.*, 1992). The orange juice was also prepared and distributed in an unhygienic manner where potentially infected kitchen workers' hands were in contact with juice being served to customers. An outbreak of *S.* Gaminara and *S.* Hartford in unpasteurized orange juice led to 62 cases of confirmed illness at a theme park in the US (Cook *et al.*, 1998), although estimates are that up to 630 people may have been affected (Parrish *et al.*, 1997). The processing facility was thought to have inadequate physical security, where orange handling and washing was performed in an unenclosed structure where *Salmonella*-carrying toads and frogs may have infiltrated the facility and contaminated the juice. Several *Salmonella* serovars were isolated from bottled unpasteurized juice packaged at this facility (Parrish, 1998). An outbreak of *S.* Muenchen associated with the consumption of unpasteurized orange juice in 1999 in the western U.S. and Canada sickened 350 individuals (CDC, 1999b), and was thought to have resulted

from unhygienic conditions where the juice was mixed and bottled (Vojdani *et al.*, 2008). In this case, even though procedures were in place to yield a 5-log reduction of *Salmonella* without pasteurization, contamination still occurred and was not reduced by cleaning the outside surface of oranges. Non-compliance with an existing HACCP (Hazard Analysis of Critical Control Points) plan at another facility led to an outbreak of *S.* Typhimurium and *S.* Saintpaul in unpasteurized orange juice, resulting in 153 cases of illness spread over 23 US states (Vojdani *et al.*, 2008). The juice contained high counts of generic *E. coli*, an indicator of fecal contamination, a condition which would require alternate processing treatments (Jain *et al.*, 2009). Sanitation of equipment was found to be sub-standard and may have led to cross contamination between products. Further discussion of potential disinfection methods for subtropical and tropical fruits will be addressed later in this chapter.

8.3.3 Outbreaks associated with melons

Melons have also been a frequent source of outbreaks in the US over the past 15 years (see Table 8.1). As with oranges, *Salmonella* has been the enteric pathogenic bacterium most frequently associated with contaminated watermelons and cantaloupes (also referred to as muskmelon and rockmelons). Extensive

Table 8.1 Documented outbreaks of salmonellosis associated with the consumption of contaminated cantaloupes and watermelon

Type of melon	Year	Number of cases	Serotype	Location
Watermelon	1950	6	*S.* Bareilly	Minnesota
Watermelon	1955	17	*S.* Miami	Massachussetts
Watermelon	1979	18	*S.* Oranienburg	Illinois
Cantaloupe	1990	235	*S.* Chester	30 US states
Cantaloupe	1991	> 400	*S.* Poona	23 US states and Canada
Watermelon	1991	39	*S.* Javiana	Michigan
Cantaloupe	1997	24	*S.* Saphra	California
Cantaloupe	1998	22	*S.* Oranienburg	Ontario, Canada
Watermelon	1999	82	*S.* Enteriditis	California
Cantaloupe	2000	46	*S.* Poona	6 US states
Cantaloupe	2001	50	*S.* Poona	5 US states
Watermelon	2002	22	*Salmonella* spp.	Washington
Cantaloupe	2002	58	*S.* Poona	10 US states 4 Canadian provinces
Watermelon	2002	20	*S.* Newport	New York
Cantaloupe	2003	58	*S.* Muenchen	multiple US states
Cantaloupe	2007	30	*S.* Litchfield	New Jersey
Cantaloupe	2007	11	*S.* Litchfield	California
Cantaloupe	2008	51	*S.* Litchfield	16 US states and Canada

treatment of these outbreaks can be found in reviews by Castillo *et al*. (2008) and Hanning *et al*. (2009). *Samonella* Miami was the causative agent of an outbreak of salmonellosis in 17 individuals who consumed a sliced watermelon, and most likely originated from a knife blade when the melon was cut (Gayler *et al.*, 1955). Pre-cut watermelons contaminated with *S.* Oranienburg sickened six people in 1979 (Castillo *et al.*, 2008). Watermelons which were cut before washing were thought to be responsible for an outbreak of 39 infections in children, caused by *S.* Javiana in Michigan (Blostein *et al.*, 1993). Contaminated watermelons, honeydew and muskmelons were responsible for 82 cases of salmonellosis due to *S*. Enteritidis, most likely introduced by an ill food handler at a school in California (Hanning *et al.*, 2009; CDC, 1999a). Watermelons contaminated with *S.* Newport were also responsible for an outbreak of 20 cases of salmonellosis in New York state (CDC, 2006), where the source of contamination was thought to have originated in a restaurant or delicatessen.

In the early 1990s, two major outbreaks of salmonellosis were associated with the consumption of cantaloupes. Cantaloupes contaminated with *S.* Chester led to 245 cases of illness in 30 US states (CDC, 1991b). Flesh of melons was thought to have been contaminated by transferring the pathogen from the exterior of the fruit to interior flesh of the melon during cutting (Castillo *et al.*, 2008). The second major outbreak of salmonellosis associated with contaminated cantaloupes occurred in multiple states in the US and Canada, affecting 400 individuals, and was attributed to *Salmonella* Poona (CDC, 1991b). The contaminated melons in this outbreak were traced back to the lower Rio Grande Valley in the state of Texas. In a 1995 outbreak with 24 cases of salmonellosis, cantaloupes imported to the US from Mexico and contaminated with *S.* Saphra were traced back to the specific growing region of Altamirano in the state of Guerrero (Mohle-Boetani *et al.*, 1999). Contaminated cantaloupes from Mexico were also implicated in three separate outbreaks of infections caused by *S.* Poona between 2000–2002 (CDC, 2002a). The potential sources of contamination could have been irrigation water in fields, washwater, or packing equipment. The voluntary *Melon Quality Control Plan* for US growers and packers was initiated in response to salmonellosis outbreaks in the early 1990s, recommending that chlorinated water be used in all processing steps associated with melons (Hanning *et al.*, 2009). Growers outside of the US may not be obligated to follows these guidelines. In 2008, cantaloupes from Honduras were implicated in an outbreak of 51 infections in a multi-state outbreak of *S.* Litchfield (CDC, 2008a), leading to a USFDA (United States Food and Drug Administration)-issued import alert that all cantaloupes from a specific company be detained.

Honeydew melons have also been implicated in causing foodborne illness. Honeydew melons contaminated with *S.* Newport were implicated in a multi-state outbreak in the US with no single source of contamination identified (CDC, 2003). Contaminated honeydew melons (along with other fruits) were implicated in an outbreak of *S.* Litchfield in New Jersey in 2007, where cut melons contaminated with *Salmonella* may have been held at abusive temperature conditions (allowing *Salmonella* to grow), and unsanitary kitchen practices may have contributed to this outbreak (CDC, 2008b).

Norovirus was responsible for an outbreak in South Carolina in the U.S. involving 41 people, where honeydew melons from a delicatessen were implicated (CDC, 1998). Noroviruses were also responsible for outbreaks associated with honeydew and cantaloupe melons in 2001, leading to 23 and 42 cases of illness in Illinois and Minnesota, respectively, in the US, and presumably contaminated through handling by infected food handlers (CDC, 2001; CDC, 2002b). Norovirus contamination is usually thought to occur through improper food handling, but it is unclear if the surface or flesh of melons supports the enhanced survival of the virus.

Reports of melon-associated outbreaks outside of North America have been very limited. It is unclear whether this represents a true difference in risk or simply an underreporting of cases. Recently, a multi-state salmonellosis outbreak involving 36 cases in Australia was linked to cantaloupes associated with *S*. Saintpaul (Munnoch *et al.*, 2009). Trace-back investigations revealed *Salmonella* spp. were detected in various irrigation water locations, dust on packing boxes, and on floors in packing sheds, but no isolate matched that of the *S.* Saintpaul outbreak strain (Munnoch *et al.*, 2009).

8.3.4 Other tropical fruits associated with outbreaks

Several frozen products from tropical fruits have been implicated in foodborne outbreaks. In 1991, contaminated frozen coconut milk imported to the U.S. was also implicated as the source of three *Vibrio cholera* infections in Maryland (CDC, 1991a). *Vibrio cholera* O1 strains in the coconut milk were not killed by home preparation (improper heating) of the milk. The use of dried and fresh coconut as a food ingredient has long been recognized as a potential source of *Salmonella* (Wilson and Mackenzie, 1955; Schaffner *et al.*, 1967; Meedeniya, 1969). Fruit shakes made with frozen mamey contaminated with *Salmonella* Typhi caused at least 16 people to become ill with typhoid fever (Katz *et al.*, 2002). Frozen mamey was imported from Guatemala and Honduras. Inspection of two Guatemalan plants where mamey was processed, frozen and packaged indicated that one plant was not following good manufacturing practices (GMPs) and it was then closed (Katz *et al.*, 2002). The other plant improved sanitation and record keeping practices, and implemented a pasteurization program to remain functional (Katz *et al.*, 2002).

Pineapples and products made from avocados have also been implicated in foodborne outbreaks. Pineapples contaminated with *E. coli* O11:H43 resulted in an outbreak, but the source and number of cases of illness were not determined (Sivapalasingam *et al.*, 2004). Guacamole contaminated with *Shigella boydii* caused 40 cases of shigellosis in California in 1998 (CDC, 1998). *Shigella sonnei* caused illness in three individuals after the consumption of tainted guacamole in 2002. *Campylobacter jejuni* was the causative agent in an outbreak sickening 50 people who also consumed contaminated guacamole (CDC, 2002). *Salmonella* Typhimurium in guacamole sickened 11 people in 2003 in Oregon (CDC, 2003). All of these outbreaks occurred in food preparation settings (home or restaurant), so the greatest likelihood for contamination was during handling and processing by contaminated food workers and handlers.

8.4 Routes of contamination of tropical fruits

As seen on other tree fruits, stem scar areas provide protection to bacterial and potential foodborne pathogens. Treatments to reduce and inactivate bacterial cells are usually less effective on stem scar areas compared to other sections of fruits. The formation of bacterial biofilms and bacterial aggregates on the surface of fruits and leaves may allow them to persist for longer durations, or become protected from specific sanitizing techniques. Microorganisms have been shown to enter fruits through various natural anatomical pathways, especially stem scar/calyx (Samish and Etinger-Tulczynska, 1963; Samish *et al.*, 1963; Zhuang *et al.*, 1995). Microbes also enter through injured tissues where damage has occurred to the natural structure, such as punctures, wounds, cuts, and splits. Uptake of contaminated water through natural structures as well as damaged tissues is a major cause of pathogen internalization in produce. Likewise, the presence of market diseases resulting from preharvest and postharvest microbiological bacterial and fungal infections (e.g., bacterial soft rot; rhizopus rot, gray rot), may lead to increased incidence of enteric pathogens as secondary infections (Richards and Beuchat, 2005b).

8.4.1 Routes of contamination of mangoes

Salmonella contamination of mangoes has been associated with the treatment of mangoes for tephritid fruit fly, specifically Mediterranean fruit fly, contamination. The US Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) requires mangoes to be immersed in water at 46.1°C (115°F) for 65 to 90 min (depending on the weight of the mangoes) to kill fruit fly larvae (Penteado *et al.*, 2004). After this treatment, mangoes are typically cooled quickly by immersion in colder water (not higher than 22°C, 70°F) to decrease quality losses. Immersing a warm piece of fruit into a colder liquid allows gases inside the fruit to contract, creating a partial vacuum inside the fruit and drawing fluid into the pores of the fruit (Solomon *et al.*, 2009). If the cooling water has not been appropriately treated, then microbial pathogens like *Salmonella* and *E. coli* O157:H7 may be drawn into the fruit. Penetado *et al.* (2004) showed that mangoes, immersed in water at 46°C for 90 min and then immersed in colder water (22°C) containing a high population of *Samonella* Enteriditis for ten minutes, internalized *Salmonella* into the flesh of the fruit. Entry of *Salmonella* into the fruit occurred significantly more frequently from the stem end of the fruit rather than the blossom end. Bordini *et al.* (2007) performed a similar experiment using a lower population of *Salmonella* than Penetado *et al.* (2004) and also determined that *Salmonella* was more likely to internalize to the flesh of mangoes through the stem end of the fruit. These authors further determined that *Salmonella* grew on the rind, stem end, middle side, and blossom end of the fruit at 22°C, but not surprisingly, increased only slightly when held at 8°C in these same sections of fruit. These investigations point to the critical need of wash water to be chlorinated to kill potential foodborne pathogens from internalizing into fruit, and for refrigerated temperature conditions

to control the growth of pathogens under postharvest conditions (see section 8.5.1). The likelihood of internalization of enteric pathogens into tropical fruits is decreased if the temperature of the fruit is lower than the temperature of the water used in processing. The survival and growth of *E. coli* O157:H7 and *Salmonella* on fresh cut mangoes stored at various temperatures has also been evaluated (Strawn and Danyluk, 2010a). Populations of *E. coli* O157:H7 increased by 1.1 log CFU g^{-1} on fresh cut mangoes over seven days at 23°C, but the largest increase (1.8 log CFU g^{-1}) in counts occurred after day one before populations began to decline. *E. coli* O157:H7 population declined by 3.1 log CFU g^{-1} and and 2.3 log CFU g^{-1} when stored for 28 days at 12°C and 180 days at −20°C, respectively. Populations of *E. coli* O157:H7 remained relatively unchanged when stored at 4°C for 28 days (Strawn and Danyluk, 2010b). In contrast to *E. coli* O157:H7, populations of *Salmonella* on fresh cut mangoes increased by 3 log CFU g^{-1} on day one but then declined slightly over seven days at 23°C (Strawn and Danyluk, 2010b). *Salmonella* populations increased by 1.2 log CFU g^{-1} after three days of storage at 12°C, but eventually declined by 3.9 log CFU g^{-1} over a 28-day storage period. In this same work, populations of *Salmonella* stored at 4°C declined by 1.3 log CFU g^{-1} over 28 days.

8.4.2 Routes of contamination of citrus

Since outbreaks have occurred with unpasteurized orange juice, internalization experiments, similar to those performed on mangoes, were conducted with oranges. Experiments conducted with dye showed that internalization occurred in 3.3–4.2% of fruit when warm oranges were transferred to cooler water, but no internalization occurred when fruit was immersed in water at the same temperature or warmer than the fruit (Eblen *et al.*, 2004). These authors showed that three percent of oranges (6/200) inoculated with *Salmonella* and cooled from 37°C to 24°C internalized the pathogen. For oranges inoculated with *E. coli* O157:H7, 2.5% of oranges (5/200) internalized the bacteria when cooled from 37°C to 4°C. They also reported that oranges which suffered larger puncture wounds were more likely to internalize *Salmonella* than those with smaller wounds. Both *Salmonella* Hartford and *E. coli* O157:H7 were able to grow inside oranges when injected into the section portion of oranges and held at 24°C, but no growth was observed at 4°C.

Pao and Brown (1998) evaluated the impact of normal packing operations on the microbiological burden of market oranges. The average total microbial loads on oranges entering packinghouses were 4 log CFU cm^{-2}. After undergoing dumping, washing, brushing, rinsing, water elimination, wax application, drying, and hand packing, microbial loads decreased to 2.1 log CFU cm^{-2}. These procedures also reduced the presence of fecal coliforms, yeasts, molds and aciduric organisms.

8.4.3 Routes of contamination of melons

Several investigations have attempted to address the source of pathogens in the melon-growing regions of Sonora, Mexico and in the Lower Rio Grande Valley in

the US (Espinoza-Medina *et al.*, 2006; Materon *et al.*, 2007). Irrigation water, in-field cantaloupes, packed cantaloupes, soil, ground water, chlorinated water and workers' hands were all tested for the presence of *Salmonella* on farms in Sonara, Mexico (Espinoza-Medina *et al.*, 2006). The pathogen was most frequently found in cantaloupes in the field (26% of samples), in irrigation water (24% of samples), and on packed cantaloupes (21% of samples). Interestingly, *Salmonella* was detected on the hands of packinghouse workers (17% of samples), but not on the hands of in-field workers (0%). Irrigation water at several of these farms was carried through open canals with open access to animals and other potential contaminants (Espinoza-Medina *et al.*, 2006). Only one of 21 samples of chlorinated water tested positive for *Salmonella* contamination. This study indicated that *Salmonella* can certainly survive after packing, although the level of chlorination in the water is unknown in these studies. Materon *et al.* (2007) found the presence of *Salmonella* spp., *E. coli* O157:H7 and *Listeria* spp. in irrigation water canals in the Lower Rio Grande Valley in the U.S. *E. coli* O157:H7 was not detected on cantaloupes prior to or after harvesting, or on the hands of field workers; however, *Salmonella* spp. and *Listeria* spp. were detected on these fruits and on the hands of field workers (Materon *et al.*, 2007). In this study, cantaloupes coming into six melon packinghouses were also analyzed. In five of the six packinghouses, populations of *Salmonella* were significantly higher on fruits entering the packinghouse than after the fruits were rinsed and packed. As with the Espinoza-Medina *et al.* (2006) study, the concentration of chlorine used in these rinses and their duration are not specified. *Listeria* spp. populations on cantaloupes entering facilities were significantly higher than on those leaving the facility in three of the six cantaloupe packing sheds evaluated. However, no *E. coli* O157:H7 was detected on cantaloupes entering or leaving the packinghouse (Materon *et al.*, 2007). Gagliardi *et al.* (2003) found that melons in fields, irrigation furrows, standing water in fields, and raised row fields had high numbers of fecal coliforms, an indication of fecal contamination potentially containing foodborne pathogens. Primary washes used for cantaloupes in packinghouses were also identified to increase the number of fecal coliforms on cantaloupe rinds, but secondary washes lowered these counts significantly (Gagliardi *et al.*, 2003). At this particular packing shed, chlorinated drinking water was used for both primary and secondary rinses. In this same study, no salmonellae were found on the rinds of cantaloupes harvested in the spring, but only a small number of cantaloupes were examined in this study.

Foodborne outbreaks associated with melons indicated that contamination is associated with the rind of the melon and can be transferred to the internal flesh by cutting. Several studies have examined the survival of pathogens on the rind and in the flesh of melons. The surface of cantaloupes was determined to have more surface roughness, as measured by confocal laser scanning electron microscopy, than apples, avocados and oranges (Wang *et al.*, 2009). The increased surface roughness on cantaloupes provides more grooves and cavities for bacterial attachment than the surfaces of other fruits (Wang *et al.*, 2009). Storage temperature affected the survival of *E. coli* O157:H7 on the rinds (Del Rosario and Beuchat, 1995). When inoculated on cantaloupe and watermelon rinds stored

at 5°C, *E. coli* O157:H7 populations decreased by more than 2 log CFU cm^{-2} within three days, while populations increased by almost 2 log CFU/cm^2 at 25°C during the same period (Del Rosario and Beuchat, 1995). Several serotypes of *Salmonella* were shown to form biofilms on the surface of cantaloupe rinds within 2 h of being deposited; within 24 hours, *Salmonella* had migrated to the cracks of the netting on the rind (Annous *et al.*, 2005a). Other investigators have determined that humidity plays a role in the survival of *E. coli* and *Salmonella enterica* subsp. *enterica* on cantaloupe rinds (Stine *et al.*, 2005). *E. coli* and *Salmonella* was inactivated more slowly under humid conditions (86–90% relative humidity) than under dry conditions (45–48% humidity). In contrast, feline calicivirus, a non-pathogenic surrogate of norovirus, was inactivated more slowly under dry conditions than under humid conditions (Stine *et al.*, 2005).

The role of fungal infections, in association with *Salmonella* contamination, has also been evaluated on cantaloupes. Wounded tissues of cantaloupes infected with *Cladosporium cladosporioides* and *Penicillium expansum* both permitted the infiltration of *S.* Poona into the mesocarp areas of fruits (Richards and Beuchat, 2005a). However, the association of *S.* Poona with several molds (*Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Geotrichum candidum*, and *Penicillium expansum*) on the surface of intact and wounded cantaloupes did not enhance or inhibit the ability of the pathogen to persist on the surface (Richards and Beuchat, 2005b). Associations between *Salmonella* and molds provide increased opportunities for the pathogen to infiltrate into internal tissues of cantaloupes, but do not necessarily increase the ability to persist and survive on the surface of the fruit.

On fresh-cut cantaloupe and watermelon cubes, *E. coli* O157:H7 did not grow when stored at 5°C for 34 hours on either melon, which was anticipated since the minimum temperature for the growth of this microorganism is ≥ 8°C (Del Rosario and Beuchat, 1995). When stored at 25°C, counts increased by 3 log CFU cm^{-2} in cantaloupes and by 5 log CFU cm^{-2} on watermelon cubes over 34 hours. Populations of aerobic bacteria, yeast and molds, and *Pseudomonas* spp. on fresh cut pieces of cantaloupe, watermelon, and honeydew melons stored at 22°C (room temperature) for five hours were significantly increased over counts on fresh cut melon pieces immediately stored at 5°C (refrigeration temperature) (Ukuku and Sapers, 2007). *Salmonella* spp. populations increased significantly on fresh-cut cantaloupes and honeydews stored at 22°C for five hours when compared to populations on fresh-cut cantaloupes and honeydew melons immediately stored at 5°C; however, no increases were observed on fresh-cut watermelons under the same conditions (Ukuku and Sapers, 2007). *Salmonella* spp. counts on fresh-cut cantaloupes, honeydew and watermelon pieces increased by at least 1 log CFU g^{-1} when stored at 10°C for up to ten days (Ukuku and Sapers, 2007). These studies indicate the importance of temperature to control the potential growth of pathogenic bacteria on rinds and in fresh-cut melon cubes. *Campylobacter jejuni* counts decreased by more than 1 log CFU $cube^{-1}$ after storage between 25–29°C after six hours (Castillo and Escartin, 1994), again reflecting the fact that this microorganism typically does not grow in food, particularly at these temperatures.

8.4.4 Incidences of contamination of avocados

Avocado pulp (AP) can become contaminated in the same manner as cantaloupes during cutting. Pathogens could be transferred from the peel of the fruit to the interior flesh. A survey of samples from Queretaro City, Mexico, revealed that *Staphylococcus aureus*, *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* were found in 10.3%, 69%, 3.4% and 17.2% of guacamole prepared by street vendors, respectively. These incidences were higher than in restaurant-prepared guacamole: *Staphylococcus aureus* (4.3%), *E. coli* (54.3%), *Salmonella* spp. (0%) and *Listeria monocytogenes* (15.2%) (Arivizu-Medrano *et al.*, 2001). No *Salmonella* was found in restaurant-prepared guacamole, and *S. aureus* counts were much lower in restaurant-prepared guacamole (Arivizu-Medrano *et al.*, 2001). Since guacamole does not receive any heat treatment before serving, these studies indicate that pathogens can be present in avocado pulp and may not be killed by the low pH of lemon juice or diced tomatoes in guacamole. This same study reported that *Salmonella, E. coli* O157:H7 and *S. aureus* all increased by approximately 5 log CFU g^{-1} in avocado pulp stored at 22°C for 24 h. As expected, these findings indicate that the neutral pH of avocado pulp is not inhibitory to the growth of these pathogens, and that these bacteria can grow in the lipid-rich environment of avocado pulp, which is composed of 20% lipids and less than 5% carbohydrates (Arivizu-Medrano *et al.*, 2001). When stored at 4°C in avocado pulp, none of the three pathogens decreased by more than one log CFU g^{-1} over 15 days. These results show that avocado pulp can support the survival and growth of bacterial foodborne pathogens, and these pathogens can be found in guacamole from various preparations. A separate investigation revealed that populations of *L. monocytogenes* grew in avocado pulp stored at 4 and 22°C (Iturriaga *et al.*, 2002).

The conversion of avocado pulp into guacamole changes the ability of *L. monocytogenes* to grow in that fruit (Iturriaga *et al.*, 2002). Ingredients in processed guacamole include onions, tomatoes, jalapeno, chili, garlic, sugar, salt, sodium alginate, xanthan gum, and disodium dihydrogen phosphate, ascorbic and citric acid. Guacamole was then subjected to a 'quick freeze' process. The addition of these ingredients lowered the pH in avocado pulp from 6.7 to 5.2 in the processed guacamole. Refrigeration of avocado pulp-based products can inhibit the growth of pathogens, but psychotrophic pathogens (which have the ability to grow at cold temperatures) may still be able to grow after more than seven days. After processing, freezing guacamole may also help reduce pathogen counts in both avocado pulp and processed guacamole (Iturriaga *et al.*, 2002). Storage at −18°C for 58 weeks reduced *L. monocytogenes* populations by approximately 2 log MPN g^{-1} in avocado pulp and 3 log MPN g^{-1} in processed guacamole, but did not completely eliminate the pathogen. None of these interventions are sufficient to completely eliminate pathogens in guacamole.

8.4.5 Contamination of other tropical fruits

The survival of other pathogens has been examined on the surface of several other fruits. Fruits were immersed in suspensions of *E. coli*, *Salmonella* Salford and

Listeria innocua between 10^5–10^6 CFU ml^{-1} for five minutes, allowed to dry, and then analyzed or stored at temperatures used in commercial distribution (Behrsing *et al.*, 2003). On bananas stored at 18°C for 13 days, populations of *E. coli* fell below the detection limit immediately on day 0, although it is unclear if detection by enrichment techniques would have recovered the microorganism. Populations of *S.* Salford and *L. innocua* slightly declined from 1.78 to 1.63 and 1.07 to 0.95 log CFU cm^{-2}, respectively, from day 0 to day 13. Populations of *E. coli* on inoculated passionfruit stored at 10°C for six days declined from 1.32 log CFU cm^{-2} on day 0 to below the detection limit by day 6; populations of *S.* Salford and *L. innocua* declined from 0.6 and 1.42 CFU cm^{-2} to below the detection limit and 0.83 CFU cm^{-2} from day 0 to day 6, respectively (Behrsing *et al.*, 2003). Overall, these results show that enteric pathogens do not attach well on the surfaces of bananas and passionfruit. The poor attachment and subsequent poor survival of pathogens indicate that these surfaces may not retain enough liquid on the surface to allow persistence, and may not present enough niches to allow bacteria to colonize. Overall, the relatively smooth surfaces of bananas may not be as permissive to the survival of bacterial pathogens as the rough surfaces of cantaloupes and oranges.

Investigations into the survival of *E. coli* O157:H7 and *Salmonella* spp. on fresh-cut pineapples revealed that neither organism was able to grow when stored at 12°C or 23°C. The low pH of the pineapple (3.6) and the lack of available nutrients were presumed to inhibit the survival of both organisms (Strawn and Danyluk, 2010a). However, inoculated frozen-cut pineapples supported the survival of both *E. coli* O157:H7 and *Salmonella* spp. for up to 180 days, although populations of both pathogens declined by ca. 2.5 log CFU g^{-1} over this period (Strawn and Danyluk, 2010a).

8.5 Interventions to reduce contamination of tropical fruits

Various prevention or intervention strategies have been considered for reducing the frequency or level of contamination of tropical and semi-tropical fruits with pathogenic microorganisms. This includes pre- and postharvest approaches, as well as approaches for application with fresh-cut products. Preharvest strategies have relied primarily on the application of good agricultural practices (GAPs). These include both general guidance such as the USFDA GAPS guidance document (USFDA, 1998) and Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC, 2003), and commodity-specific guidance such as in the case of cantaloupes (UFFVA, 2005). These preharvest practices include advice on controlling contamination due to irrigation water, irrigation methods, farm workers, farm equipment, soil amendments, adjacent land use, etc.

One of the strategies used at harvest, during packing, and as part of subsequent production of fresh and fresh-cut product is the use of chemical sanitizers. Chemical sanitizers are commonly used on fruit surfaces, on food contact surfaces, and to prevent cross-contamination in dump tanks, flumes, and hydrocoolers. In many

cases, chemical sanitizers are added to preserve the quality of the water and to prevent cross-contamination through water. In general, the chemicals used in sanitizing surfaces of tropical fruits can reduce between 1 to 3 log CFU of total bacteria on fruit surfaces (Sapers, 2009), which is not sufficient to completely kill all potential pathogens attached to these surfaces. Studies have shown that surface topography and hydrophobicity of fruits significantly impacts the efficacy of sanitizers on pathogen removal and inactivation (Wang *et al.*, 2009). Other investigators have described a dual-phasic inactivation of *E. coli* O157:H7 on fruit surfaces, attributed to the surface topography of fruits (Wang *et al.*, 2006). This dual-phasic interaction describes an inactivation rate 0.4–0.6 log CFU cm^{-2} within the first minute of application of the sanitizer, followed by a much slower and less effective (0.02–0.04 log CFU cm^{-2}) inactivation rate in the next 14 minutes, using either acidified electrolyzed water or a peracetic acid based sanitizer (Wang *et al.*, 2006).

Commonly included sanitizers are halogen-based compounds (chlorine, bromine and iodine). Sodium or calcium hypochlorite are the most commonly used sanitzers on produce commodities because of their ease of use, availability and relative effectiveness. Other halogen compounds such as iodine and bromine are less widely used. In the United States, concentrations of hypochlorite range from 50–200 ppm for use with various produce commodities, and the typical time of exposure ranges between 0.5 and 2 minutes. To maintain active chlorine levels, it must be added continuously to the water to replace the chlorine lost to reactions with organic matter, certain chemicals and microorganisms. Free chlorine concentration should be frequently monitored, and additional chlorine added to the wash solution to maintain effective chlorine level (JIFSAN, 2002). Keeping organic matter low in wash solutions helps to maintain sufficient free chlorine or other sanitizer active components to kill bacteria and viruses in wash water and prevent cross-contamination (Hirneisen *et al.*, 2009; Materon, 2003; Mendonca *et al.*, 1994; Pao and Davis, 1999; Pao *et al.*, 1999, 2000; Ukuku *et al.*, 2005; Walter, 2009). The use of iodine and bromine as sanitizers is limited because their use changes the appearance of the fruit (Hernandez-Brenes, 2002).

There are a variety of alternative sanitizers that have been tested for their ability to reduce pathogen levels on fruits. Chlorine dioxide (ClO_2) can be used, although current USFDA regulations do not permit concentrations >3 ppm on produce surfaces (Sapers, 2009). Acidified sodium chlorite (ASC) can be used on produce at concentrations from 500–1200 ppm and followed by a potable water rinse (Sapers, 2009). Hydrogen peroxide, aqueous (liquid) ozone and peroxyacetic acid have all been effective sanitizers for produce with varying results based on times of exposure, concentration, and fruit commodity being evaluated (Sapers, 2009).

8.5.1 Chemical interventions to reduce microbial contamination of mangoes and coconuts

The addition of sodium hypochlorite and copper to water in which mangoes are hydrocooled reduced populations of *S.* Typhimurium and eliminated the potential for internalization of *Salmonella* into mangoes (Soto *et al.*, 2007). The addition of

5 mg L^{-1} hypochlorite and 8 mg L^{-1} copper ions reduced populations of *Salmonella* at 6 log CFU ml^{-1} in simulated 19-L hydrocooling tank (at 23°C) to a level where internalization of the pathogen into the mangoes was not observed (Soto *et al.*, 2007). Four mangoes in each trial were evaluated. In comparison, *Salmonella* present in the water without the antimicrobial treatment were able to infiltrate into mango pulp, with 2.5 log CFU g^{-1} recovered from the mango pulp. The number of fruit evaluated in this study was small, and the organic material from additional mangoes may affect the efficacy of the hypochlorite in these dump tanks, but this study shows that chlorination of water can limit the infiltration of *Salmonella* into mangoes. Washing with hot water and 100 ppm chlorine reduced the levels of microorganisms on the surface and stem scars of mangoes, but did not totally eliminate contamination that could be introduced into the fruit when sliced (Ngarmsak *et al.*, 2006). Chemical sanitizers were also effective in reducing *Listeria monocytogenes* populations on green coconuts (Walter *et al.*, 2009). Immersion of green coconuts in 200 ppm sodium hypochlorite and 80 ppm peroxyacetic acid reduced *L. monocytogenes* by 2.7 and 4.7 log CFU/coconut, respectively.

8.5.2 Chemical interventions to reduce microbial contamination on the surface of citrus fruit

The use of waxes on oranges reduced the number of microorganisms on the surface of oranges. Alkaline citrus waxes are used for a variety of reasons: to reduce water vapor loss, to enhance surface shine, or to transmit or carry an antimicrobial agent (Pao *et al.*, 1999). Waxes are usually applied by spraying but dips, drips and foams are also less commonly used. Application of waxes (pH 10) to oranges at 50°C for four minutes reduced *E. coli* counts by 4.7 log CFU cm^{-2} in mid-section areas, but only by 1 log CFU cm^{-2} around the stem scar area (Pao *et al.*, 1999). Similar results were observed when wax at pH 11 was applied for two minutes at the same temperature.

Chemical treatments have proven less effective on the surface of oranges than physical ones. Immersion of oranges in 100 ppm chlorine dioxide solutions reduced *E. coli* populations by 3.1 log CFU cm^{-2} in non-stem scar areas, but only by 1 log CFU cm^{-2} in stem scar areas (Pao and Davis, 1999). Chlorine dioxide at 100 ppm was more effective than the other antimicrobial solutions tested: 100 ppm chlorine, 200 ppm anionic sanitizer, 80 ppm peroxyacetic sanitizer, and 2% trisodium phosphate. The efficacy of these sanitizers may be limited by their ability to penetrate the surface of the oranges. An alkaline compound, sodium orthophenylphenate (SOPP) at pH 11.8, reduced *E. coli* counts between 2.9–3.5 log CFU cm^{-2}, regardless of any pre-wetting treatment (Pao *et al.*, 2000). The antimicrobial activity of SOPP may be attributed to the damage to Gram negative bacterial cell walls caused by alkalinity (Mendonca *et al.*, 1994).

8.5.3 Chemical interventions to reduce microbial contamination of melons

As with citrus fruits, chemical methods have proven to be less effective on melons than physical ones. A survey of four packinghouses in Texas revealed that melons

entering packinghouses had significantly higher counts of total aerobic bacteria, total and fecal coliforms and fungi than cantaloupes which were washed and then packed (Materon, 2003), indicating that washing with chlorinated water decreases microbial populations on cantaloupe rinds. Washing inoculated cantaloupes in 1000 ppm sodium hypohchlorite solutions or 5% hydrogen peroxide reduced *Salmonella* Stanley populations by 3.4 and 3.2 log CFU cm^{-2}, respectively (Ukuku and Sapers, 2001). However, these treatments were increasingly less effective three, five, and six days after inoculation, regardless of whether the inoculated melons were stored at 4 or 20°C. Similarly, *S.* Stanley was not transferred from rind to flesh of melons treated with hypochlorite or hydrogen peroxide on days 1 or 3 after inoculation and washing, but could be transferred three and five days after washing. In this same study, *S.* Stanley from melons washed in tap water only was transferred from rind to flesh on all days. These data indicate that chemical sanitizers can be effective in reducing attached *Salmonella* counts on melon rinds, but surviving cells potentially become more protected by biofilms in the rinds and netting of cantaloupes and more resistant to chemical disinfection the longer they are stored at refrigerated temperatures. Commercial preparations of acidified sodium chlorite (Sanova), at 850 and 1200 ppm, and a peracetic acid (PAA)-based sanitizer (Tsunami 200), at 20 and 40 ppm, reduced *S.* Poona counts on the surface of cantaloupes by 1.60–2.47 log CFU and 2.21–2.50 log CFU, respectively (Caldwell *et al.*, 2003). Comparatively, sodium hypochlorite solutions at 50 and 200 ppm only affected reductions of 0.35–0.70 log CFU *S.* Poona, indicating that acidified sodium chlorite and PAA sanitizers are more effective than chlorine solutions in reducing *Salmonella* populations on cantaloupe rinds (Caldwell *et al.*, 2003). Other researchers have supported the findings that acidified sodium chlorite (Sanova) at 1200 ppm was more effective than 180 ppm sodium hypochlorite and 80 ppm Tsunami (Fan *et al.*, 2009) in reducing *S.* Poona on cantaloupe rinds. This same study also showed that ASC was more effective than PAA or sodium hypochlorite in reducing total aerobic populations on cantaloupe rinds. Regulations for use of chemical disinfectants in wash water may differ from country to country, and attention should be paid to complying with these rules.

8.5.4 Limitations of chemical disinfectants

Chemical disinfectants are most effective when their concentration is monitored and maintained during postharvest fruit processing. Planktonic bacterial cells, those not attached to produce surfaces, are much more susceptible to the bactericidal effects of chemical sanitizers than bacteria which are protected in biofilms or in aggregates on produce surfaces (Solomon and Sharma, 2009). Exopolysaccharide (EPS) produced by bacterial biofilms on fruit surfaces, or organic material released by cut surfaces of fruits, may also serve to inactivate the disinfecting potential of chemical sanitizers. The ability of *Salmonella* cells to colonize surfaces in the same manner as non-pathogenic epiphytic bacteria (Beattie and Lindow, 1999) may also reduce the ability of chemical disinfectants to kill cells in these protected niches. Chemical disinfectants, including sodium or

calcium hypochlorite, are most effective in killing bacterial cells when the pH of solution is 4–7, when hypochlorous acid (HOCl) is the predominant chemical species (Marriott, 1999). Hypochlorite solutions are common as sanitizing agents because they are cost-efficient, have a broad spectrum of activity (gram positive and gram negative bacteria), and the concentration (free chlorine) is relatively easy to measure. As pH increases, the ratio of hypochlorite ion (OCl^-) to hypochlorous acid increases, decreasing the efficacy of the sanitizer (Marriott, 1999). The main limitation of hypochlorite solutions and similar compounds is that they are rapidly inactivated in the presence of organic matter, and they are corrosive to stainless steel and other metals. Chlorine dioxide (ClO_2) is more stable in the presence of organic matter and at higher pH values (up to 8.5) than hypochlorite over a shorter period of time. Overall, the use of chemical disinfectants in improving the safety of tropical fruits is most effective when integrated into an overall food safety management plan that emphasizes good agricultural practices, good manufacturing practices, and standard operating procedures that are designed to emphasize food safety and minimize the risk of contamination.

8.5.5 Physical and biological interventions to reduce contamination of tropical fruits

Other physical and biological treatments have been evaluated for their ability to reduce foodborne pathogens on whole and fresh-cut melons. Low dose gamma irradiation of fresh-cut cantaloupe, following immersion in 20°C water, reduced total microbial populations by 2.1 log CFU g^{-1} (Fan *et al.*, 2006). Surface pasteurization, the immersion of cantaloupes in 76°C water for three min in a commercial washing apparatus, has proven to be an effective treatment to kill *Salmonella* on the rinds and in the netting of the fruit (Annous *et al.*, 2004). Surface pasteurization reduced total aerobic populations and yeasts and molds on the surface of the cantaloupes stored for up to 20 days after treatment (Fan *et al.*, 2008). Surface pasteurization also resulted in reductions of more than 5 log CFU cm^{-2} *Salmonella* Poona on cantaloupes. Similar reductions in *E. coli* counts were observed during commercial surface pasteurization treatment. These treatments also reduced fungal spoilage in cantaloupes as compared to untreated cantaloupes, while maintaining firmness in cantaloupes. Combination of surface pasteurization with low-dose gamma irradiation (0.5 kGy) reduced microbial populations by 3.3 log CFU cm^{-2} (Fan *et al.*, 2006). Surface pasteurization treatments using a higher temperature of water (85°C) for 90 seconds resulted in a reduction in *Salmonella* populations of 4.7 log CFU cm^{-2} in another type of commercial pasteurization system, but also resulted in increased softness of the rind compared to treatments for 60 s (Solomon *et al.*, 2006). Temperature profiles conducted in this experiment showed a 40°C difference between the temperature at the rind versus the temperature in the flesh. Surface pasteurization of cantaloupes has proven to be more effective than chemical disinfection in reducing pathogenic microbial contamination of these fruits. Care must be taken to preserve the quality of the fruit during these treatments, and proper temperature controls must be used to

ensure that appropriate microbial reductions are observed. Currently this technology is being evaluated in field trials.

For oranges destined for juice production, immersion in water at 70°C for two minutes or 80°C for one minute achieved a 5 log CFU cm^{-2} of *E. coli* in non-stem scar areas (Pao and Davis, 1999) without affecting the flavor of the resulting juice. A 5 log CFU cm^{-2} reduction at stem scar areas on oranges was achieved by immersing fruit in water at 70°C for four minutes or 80°C for two minutes, but these conditions adversely affected the flavor of the resulting juice. Irradiation treatment has also been shown to reduce pathogen contamination on fresh-cut pineapples (Shasidahar, 2007). The use of high pressure processing technologies has been successfully applied to the processing of unpasteurized juices of tropical fruits, fresh-cut fruit, and homogenized products such as guacamole (Aleman *et al.*, 1994; Linton *et al.*, 1999a, 1999b; Bayindirli *et al.*, 2006)

Biological treatments, including bacteriophages, have been effective in reducing inoculated pathogens on fresh-cut melons. Bacteriophages are viruses that infect and lyse (kill) bacteria. They are naturally present in foods, and are not harmful or toxigenic to humans. A cocktail of bacteriophages specific for *Salmonella* Enteritidis applied to inoculated fresh-cut honeydew melons reduced *Salmonella* by approximately 3.5 log CFU when stored at 5 and 10°C after three days but were less effective when stored at 20°C (Leverentz *et al.*, 2001). Moreover, this work showed that the antimicrobial activity of bacteriophages takes place almost immediately once applied to inoculated melons. Bacteriophages specific for *Listeria monocytogenes* reduced populations of the pathogen by 2.0–4.6 log CFU/sample on inoculated honeydew melon slices compared to samples not treated with bacteriophages (Leverentz *et al.*, 2003). Bacteriophages applied to fresh-cut cantaloupes inoculated with *E. coli* O157:H7 stored at 4°C reduced the pathogen by 2.5 log CFU ml^{-1} compared to untreated controls (Sharma *et al.*, 2009). Bacteriophages are odorless and colorless, so their addition to food should provide a minimal impact to sensory and quality changes.

8.6 Food safety programs used to minimize microbial contamination of fruits

Interventions designed to improve the safety and quality of fruits are not the only method to limit pathogenic contamination on tropical fruits. Good Agricultural Practices (GAPs), Sanitation Standard Operating Procedures (SSOPs), and Good Manufacturing Practices (GMPs) must be implemented and monitored along with the Hazard Analysis and Critical Control Points (HACCP)-based food safety programs to minimize contamination associated with these commodities. These procedures and practices, when implemented properly with oversight and worker training, can help limit pathogenic contamination in harvesting and handling of fruits.

GAPs are outlined in the USFDA *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* (1998) which identifies eight principles

of food safety that must be considered in produce growing and harvesting operations (Gorny and Zagory, 2010).

1. Prevention of pathogen contamination is critical to ensure produce safety.
2. GAP should be implemented in areas in which produce growers and packers have a degree of control; while avoiding negatively impacting other areas of the food supply and/or environment.
3. Fresh produce can be easily contaminated. Human or animal feces can be a significant source of pathogen contamination.
4. The source and quality of water used in field application can have a major impact on produce contamination.
5. It is important to minimize the potential for pathogen contamination as much as possible, especially through careful management and application of manure or municipal biosolid wastes.
6. In order to minimize contamination on fresh produce, it is critical that workers be vigilant in their hygiene and sanitation practices during production, harvesting, sorting, packing and transport.
7. Concurrent to implementing GAP measures, it is critical that producers follow all applicable agricultural practice regulations and laws at the local, state, federal, and international levels, as mandated by the situation and regulations, or corresponding or similar laws, regulations or standards for operators outside the U.S. for agricultural practices.
8. Accountability at all levels of the agricultural environment is important to ensure food safety programs, like GAP, are rigorously and successfully followed.

In accordance with these eight principles, there are several practices at the preharvest and postharvest stages that can decrease the risks of contamination. Most tropical and subtropical fruits are perishable with short shelf life. Quality and safety of these fresh fruits can be strongly affected by harvesting and postharvesting practices. Harvesting fruits at their proper maturity is critical since after their separation from the parent plant they receive no more nutritional gains and their physiology changes from primarily growing to aging. Harvesting is labor-intensive and involves much handling, rendering the process susceptible to pathogen transfer via infected harvesting crew members. Workers should follow hygienic practices at all times, and those with transmissible diseases should not handle produce. Sanitary facilities must be provided for all field workers and visitors during harvesting.

Tropical and subtropical fruits, such as mangoes, papayas, watermelons, are susceptible to chilling injury at temperatures below 10°C. Therefore, cold storage is not advised in general for the intact fruits in order to maintain quality. However, for fresh-cut fruits, maintaining cold-chain integrity is critical to reducing food safety risks, since bacterial pathogens grow at a rapid, temperature-dependent rate despite the presence of large populations of native microorganisms and the lack of overt signs of quality deterioration.

GMPs provide a preventative food safety program designed to ensure food products are prepared, packaged, and stored under sanitary conditions, and are safe

for human consumption. In the USA, implementation of GMPs is required by law for processing food products including ready to eat fresh-cut products (USFDA, 2008). Although not mandatory for whole produce handling, following GMP guidelines can improve working conditions and produce safety. GMPs cover general provisions, employee hygiene, buildings, facilities and equipment, production and process controls, and describe defect action levels, as well as levels of natural or unavoidable defects in foods that present no health hazards for humans.

Personnel GMPs address the issue that food handlers can be a significant source of contamination. This includes all personnel that come in contact with food products or processing surfaces. Processing plant management train and supervise all personnel on how to properly handle foods and comply with GMP standards.

Cleaning and sanitation are some of the most important programs in any food processing plant or packing shed. Building, fixtures, and other physical facilities of the plant should be rigorously maintained in order to ensure sanitary conditions and prevent food adulteration. In order to protect against contamination, all food-contact surfaces must be cleaned frequently. Furthermore, regularly scheduled equipment cleaning and sanitizing assures food products are being processed under hygienic conditions. These should all be components of SSOPs.

8.6.1 Hazard Analysis and Critical Control Points (HACCP)

HACCP is a science-based, objective method used to improve food safety by concentrating on identifying hazards and controlling them at their source (Hurst, 2006). The first step in this process is to identify the scope of the problem, followed by composing a team to perform the HACCP analysis, and then describing the product for which the analysis is needed. A flow chart of the operation is then formulated. With fresh fruits, this product flow may consider the preharvest factors that may affect the chance of contamination. Implementing a HACCP program in processing fresh-cut, minimally processed, tropical fruits provides issues and challenges different from processed foods. The lack of a 'kill step' in minimally processed tropical fruits makes establishing critical control points (CCPs) more challenging (Hurst, 2006). The lack of a kill step places emphasis on establishing and maintaining microbiologically sound preharvest factors (GAPs), processing standards (SOPs and GMPs), and a sanitation regime (SSOPs) to ensure that critical control points are appropriate and realistic. Implementing these practices must take into account the local and regional context and realities where fruits are being harvested, processed and/or stored. Understanding these cultural factors will enhance the successful implementation of these practices (Piniero and Diaz, 2007).

8.7 Conclusions

The year-round availability of fresh and fresh-cut tropical fruits to consumers worldwide has increased the importance of ensuring their safety. There have been

numerous documented outbreaks with a variety of fruits and different pathogens. Fruits can become contaminated during field operations or fresh-cut processing through a variety of routes (washes, reptile intrusions, worker handling). Rough and uneven surfaces of fruits provide numerous colonization niches for foodborne pathogens. Chemical interventions are effective in reducing some but not all microbial contamination on the surface of fruits. Some physical methods may have the potential to improve the microbial safety of tropical fruits. Attention should be paid to using hygienic practices throughout the growing, handling, transportation and preparing of these commodities. An integrated approach to understanding the routes of contamination and practices to minimize this risk will benefit all growers, distributors and consumers of tropical and subtropical fruits.

8.8 References

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9

Biotechnology and molecular biology of tropical and subtropical fruits

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Abstract: This chapter discusses the current position on genetic transformation of tropical and subtropical fruits. Many of these plants are non-model systems, and research is driven mainly by their economic importance and the possibility of genetic improvement. The advances in the area of molecular biology are reviewed from the study of individual genes involved in specific biosynthetic pathways, massive sequencing of mRNA as ESTs (expressed sequence tags), to formal transcriptomic and whole genome sequencing projects. To a great extent, the genetic improvement of tropical and subtropical fruits will be determined by the advances in cell biology protocols for transformation and cell culture, and the knowledge of the genes involved.

Key words: genetic transformation, quality traits, transcriptome, genome sequencing.

9.1 Introduction

The improvement in the quality characteristics of tropical and subtropical fruits has been difficult to carry out by traditional breeding techniques due to the lack of germoplasm collections and the long life cycle of these species. In consequence, there are not many varieties for almost all the tropical and subtropical fruits, and genetic engineering has been suggested as an alternative for breeding. These techniques allow the introduction of specific genes conferring desirable characteristics to improve quality. Most importantly, these techniques do not have the limitation of the traditional breeding which can only utilize genetic material of related species to improve specific traits. Therefore, the cultivars already available

are an important source of genetic material. However, genetic engineering can be utilized after tissue culture techniques are developed, the role of specific genes is known, and the plant genomes are available.

Tissue culture techniques require knowledge of the organogenesis or induction of a whole plant from plant tissue and the embryogenesis, which is the induction of viable embryos from embryogenic tissues. Fortunately, for many of the tropical and subtropical fruit species analyzed in this chapter, protocols are available for both embryogenesis and organogenesis.

In addition, the genomes of some tropical and subtropical fruits are available for fruit improvement, and may allow transcriptional, biochemical and genetic studies. Besides complete genomes, several expression libraries providing EST collections from some fruits have been obtained and compared with those of model plants. These transcriptomes represent the genes that are being actively expressed at particular conditions in a specific type of cell or tissue, since it is comprised of the reverse-transcribed mRNA isolated at a particular time point. Therefore, fruit transcriptome represents the set of mRNAs expressed in specific tissues of the fruit.

The transcriptome is different to the genome since some genes generate multiple mRNAs by post-transcriptional processing, and only a subset of genes is expressed in a particular tissue. Therefore, in the absence of genomic information, transcriptome projects are very useful to obtain sequences related to flavor, color and fruit ripening, but also in human health since the biosynthetic pathways for vitamins, fatty acids, antioxidants and bioactive compounds are being revealed.

The ESTs produced during transcriptomic analysis represent the most abundant nucleotide sequence source from plant genomes. The information provided by ESTs can be exploited for gene discovery, genome annotation and comparative genomics. Some EST collections are currently available for several crop and model plant species; in this chapter those corresponding to tropical and subtropical fruits will be reviewed. Coding regions of genes as well as transcription factors involved in several quality characteristics, and postharvest shelf life, will also be considered.

Among the genes isolated and studied, there are those related to flavor, aroma, fruit color, softening and ripening. Furthermore, many genes are known to play a key role in the fruit response to biotic stress including fungi infection, bacterial infection and virus attack as well as to abiotic stresses including cold stress, which is the most common abiotic stress in these fruits, and to wounding, temperature, drought and salinity stresses.

Tissue culture techniques, knowledge about the molecular mechanisms underlying fruit quality traits, genes and transcriptomes available are all critical tools to improve quality characteristics of tropical and subtropical fruits. In this chapter these tools are analyzed and we shall see in the near future efforts to improve the quality characteristics of many tropical and subtropical fruits with the utilization of techniques derived from genetic engineering.

9.2 Genetic transformation, transcriptome sequencing, genome mapping and sequencing of fruits

Two reviews have been published, to the best of our knowledge, about the transformation of tropical fruits (Gomez-Lim and Litz, 2004; Petri and Burgos, 2005) as well as two edited volumes including chapters describing advances in the genetic engineering of banana, citrus, mango, papaya, pineapple, watermelon, avocado, grape and melon (Hui *et al.*, 2002; Pua and Davey, 2007). Another important reference reports advances in biotechnological protocols including somatic embryogenesis, organogenesis and development of transgenic plants in kiwifruit, cashew, mango, pistachio, coconut, date, atemoya, cherimoya, sugar apple, soursop, pineapple, papaya, mangosteen, avocado, fig, banana, guava, olive, carambola, passion fruit, grapefruit, lemon, lime, orange, longan, cacao and grape (Litz, 2005). In this chapter we will try to address the latest developments in tropical fruit genetic transformation although some of the earlier work will also be included (see Table 9.1). Transcriptomics and genomics will also be reviewed.

9.3 Acerola (*Malpighia emarginata* DC.)

Acerola fruits are known to contain a very large amount of ascorbic acid. Mezadri *et al.* (2006) reported 695 to 4827 mg 100 g^{-1} ascorbic acid in acerola. Ascorbic acid synthesis is performed through the Smirnoff–Wheeler pathway (Wheeler *et al.*, 1998). The pathway starts from glucose and includes the intermediates GDP-D-mannose, L-galactose, and L-galactono-1, 4-lactone, among others (Smirnoff *et al.*, 2001). Since the GDP-D-mannose pyrophosphorylase (GMP) (EC 2.7.7.13) catalyzes the interconversion of GDP-d-mannose from GTP and α-d-mannose-1-P (Smirnoff *et al.*, 2001), the *MgGMP* cDNA (GenBank DQ229168) was cloned and sequenced (Badejo *et al.*, 2007) as well as the complete gene (GenBank EU180071) (Badejo *et al.*, 2008). *MgGMP* mRNA was accumulated at high levels at the beginning of the ripening process, and decreased in mature fruits (Badejo *et al.*, 2007). Furthermore, the amount of ascorbic acid in the fruit correlated with *MgGMP* mRNA levels during the ripening process.

The MgGMP gene promoter was used to create transgenic tobacco plants expressing the β-glucuronidase (*uidA*) reporter gene and to compare its activity to the cauliflower mosaic 35S virus promoter (CaMV35S) activity. Transient expression of *uidA* was evaluated in tobacco protoplasts and the MgGMP promoter had higher activity than the CaMV35S promoter. Furthermore, tobacco transgenic plants expressing the *MgGMP* gene including its promoter showed levels of ascorbic acid that were twice as high as the tobacco isogenic line (Badejo *et al.*, 2008). These results clearly show that the amount of ascorbic acid in acerola is due to the higher level of activity of the *MgGMP* gene promoter.

A study done with other gene of the Smirnoff–Wheeler pathway, *MgPMM* (GenBank FJ380046), encoding phosphomannomutase (PMM) (EC 5.4.2.8) was reported (Badejo *et al.*, 2009). PMM catalyzes the interconversion of mannose-6-

Table 9.1 Different strategies to develop transgenic tropical and subtropical plants to improve fruit quality characteristics by the introduction of specific transgenes

Fruit	Transgene integrated	Transformation protocol	Plant tissue utilized	Engineered genetic trait	Reference
Avocado	CaMV35S::*nptII* CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Proembryogenic Masses	None	Cruz-Hernández *et al.*, 1998
	CaMV35S::*PDF1.2*	*Agrobacterium tumefaciens*	Embryogenic avocado cultures from Protoplast	Resistance to fungi attack	Raharjo *et al.*, 2008
	Cellulase promoter:*samK*	*Agrobacterium tumefaciens*	Embryogenic cultures	Longer postharvest life	Efendi, 2003 Litz *et al.*, 2007
Banana	Gelvin promoter::*uidA* Gelvin promoter::*ALS*	*Agrobacterium tumefaciens*	Suspension culture cells derived from shoot-tip	None	Ganapathi *et al.*, 2001
	CaMV35S::*nptII* CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Male flower derived embryogenic cell suspension culture	None	Huang *et al.*, 2007
	CaMV35S::*nptII* CaMV35S::*uidA*	*Agrobacterium Tumefaciens*	Embyogenic cell suspension from immature male flowers	None	Ghosh *et al.*, 2009
	Rd29A::*TPS-TPP*	*Agrobacterium Tumefaciens*	Meristematic banana tissues	Tolerance to salinity stress	Santamaria *et al.*, 2009
	UBQ3::*MSI-99*	*Agrobacterium tumefaciens*	Wounded meristems	Resistance to *Fusarium oxysporum* f.sp. cubense and *Mycosphaerella musicola*	Chakrabarti *et al.*, 2003
	CaMV35S:: Antimicrobial peptides	Particle bombardment	Embryogenic cells	Resistance against preharvest and postharvest diseases *Verticillium theobromae* or *Trachysphaera fructigena*	Sági *et al.*, 1995
	UBQ3::OcIΔD86	*Agrobacterium tumefaciens*	Embryogenic cells	Resistance to nematode *Radopholus similes*	Atkinson *et al.*, 2004

Citrus	CaMV35S::*egfp-nptII*	*Agrobacterium tumefaciens*	Segments cut from seedlings	None	Dutt and Grosser, 2009
	CaMV35S::CitPSY FMV34S::CitPDS CaMV35S::CitLCY-B	*Agrobacterium tumefaciens*	Epicotyl from etiolated seedlings	Color in the fruit and in the juice	Costa *et al.*, 2002
	CaMV35S::*CHS-AS** CaMV35S::*CHI-AS**	*Agrobacterium tumefaciens*	Epicotyl segments	Reduce bitter taste in the fruit and fruit juice	Koca *et al.*, 2009
Date	CaMV35S::*uidA* Act1-D::*uidA*	Particle bombardment	Embryogenic calli	None	Mousavi *et al.*, 2009
Fig	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Leaf	None	Yancheva *et al.*, 2005
Grape	pUBQ3::*SP* pUBQ3::*MSI99* pUBQ3::*MAG2*	Particle bombardment	Embryogenic cell cultures obtained from anthers or ovaries	Resistance to the attack of bacteria and fungi	Vidal *et al.*, 2003.
	pUBQ3::*MSI99*	Particle bombardment	Cell suspensions developed from anthers	Resistance to the attack of bacteria and fungi	Vidal *et al.*, 2006a
	CaMV35S- CaMV35S::*egfp-nptII*	*Agrobacterium tumefaciens*	Somatic embryos developed from young leaves	None	Li *et al.*, 2008
	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Leaves	None	Das *et al.*, 2002
	CaMV35S ::*VvWRKY2*	*Agrobacterium tumefaciens*	Leaves	Resistance to the attack of *Botrytis cinerea, Pythium* spp. and Alternaria tenuis	Mzid *et al.*, 2007
	CaMV35S Ω::*PGIP* CaMV35S ::*uidA* CaMV35S ::*nptII*	*Agrobacterium tumefaciens*	Pre-embryogenic calli	Resistance to the attack of *Xylella fastidiosa* and *Botrytis cinerea*	Aguero *et al.,* 2005

(Continued overleaf)

Table 9.1 Continued

Fruit	Transgene integrated	Transformation protocol	Plant tissue utilized	Engineered genetic trait	Reference
	CaMV35S Ω::*RCC2*	*Agrobacterium tumefaciens*	Somatic embryos	Higher resistance to the attack of *Uncinula necator*	Yamamoto *et al.*, 2000
	CaMV35S::*RIP* CaMV35S::*RCC2*	*Agrobacterium tumefaciens*	Leaves	Resistance to the attack of *Uncinula necator* and *Plasmopara viticola*	Bornhoff *et al.*, 2005
Guava	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Somatic embryos	None	Biswas *et al.*, 2007
Kiwifruit	CaMV35S::*VlmybA1-2*	*Agrobacterium tumefaciens*	Leaf and petioles	Improved color	Koshita *et al.*, 2008
	CaMV35S::*SSBIN* CaMV35S::*SSLAB* CaMV35S::*SSRIP*	*Agrobacterium tumefaciens*	Leaf and petioles	Resistance to fungi attack	Kobayashi *et al.*, 2000
Litchi	CaMV35S::*GFP4*	*Agrobacterium tumefaciens*	Leaf	None	Puchooa, 2004
Mango	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Somatic embryos	None	Mathews *et al.*, 1992
	CaMV35S::*ACCS-AS* CaMV35S::*ACCO-AS*	*Agrobacterium tumefaciens*	Somatic proembryos of mango	Longer postharvest shelf life	Cruz-Hernández *et al.*, 1997.
Melon	CaMV35S::*uidA* CaMV35S::*DHFR*	*Agrobacterium tumefaciens*	Cotyledon explants	None	Dong *et al.*, 1991
	CaMV35S::*CMACCO-AS*	*Agrobacterium tumefaciens*	Leaf from 10 days old seedlings	Longer postharvest shelf life	Nunez-Palenius *et al.*, 2007b
	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Somatic embryos	None	Akasaka-Kennedy *et al.*, 2004

	CaMV35S::*CMACCO-AS*	*Agrobacterium tumefaciens*	Leaf explants	Longer postharvest shelf life	Guis *et al.*, 2000
	CaMV35S::*uidA*	*Agrobacterium tumefaciens*	Cotyledons, hypocotyls and non-expanded true leaves	None	Nunez-Palenius *et al.*, 2007a
Olive	CaMV35S::*uidA*	Particle bombardment	Somatic embryos	None	Lambardi *et al.*, 1999
	pUBQ::*uidA*	Particle bombardment	Somatic embryos	None	Perez-Barranco *et al.*, 2009
	pUBQ::*uidA*	*Agrobacterium tumefaciens*	Embryogenic cultures	None	Torreblanca *et al.*, 2009
	CaMV35S::*OSM*	*Agrobacterium tumefaciens*	Somatic embryos	Resistance to fungi attack	D'Angeli and Altamura, 2007
Papaya	CaMV35S::*RP*	*Agrobacterium tumefaciens*	Embryogenic calli	Resistance to the papaya ringspot virus	Chen *et al.*, 2001
	CaMV35S::CBF1 CaMV35S::CBF3	*Agrobacterium tumefaciens*	Embryogenic cultures from hypocotyl segments	Improved resistance to cold stress	Dhekney *et al.*, 2007
	Ubq3::pmi	Particle bombardment	Embryogenic calli	None	Zhu *et al.*, 2005
	CaMV35S::*DmAMP1*	Particle bombardment	Somatic embryogenic cultures from seedling hypocotyls	Resistance to *Phytophthora palmivora*	Zhu *et al.*, 2007
	CaMV35S::*CPACCO-1*	Particle bombardment	Papaya embryogenic calli at the globular stage	Longer postharvest shelf life	Lopez-Gomez *et al.*, 2009
	CaMV35S:: *PRSVCP*	Particle bombardment	Somatic embryos	Resistance to ringspot virus	Lines *et al.*, 2002

(Continued overleaf)

Table 9.1 Continued

Fruit	Transgene integrated	Transformation protocol	Plant tissue utilized	Engineered genetic trait	Reference
	CaMV35S::*VST 1*	Particle bombardment	Somatic embryogenic cultures	Resistance to *Phytophthora palmivora*	Zhu *et al.*, 2004b
	CaMV35S:: *PRSVCP*	Particle bombardment	Somatic embryos	Resistance to papaya ringspot virus	Tennant *et al.*, 2002
	CaMV35S:: *PRSVCP*	*Agrobacterium tumefaciens*	Embryogenic tissues derived from immature zygotic embryos of papaya	Resistance to papaya ringspot virus	Bau *et al.*, 2003
	CaMV35S::KETc1.6His CaMV35S::KETc12.6His CaMV35S:: KETc7	Particle bombardment	Embryogenic papaya cells line at globular stage	Immunity against cysticer cosis in humans	Hernández *et al.*, 2007
	CaMV35S::*uidA- mgfp5*	Particle bombardment	Embryogenic calli	None	Zhu *et al.*, 2004a
Pineapple	Ubi 1::*bar* OCS-35S CaMV-rice actin I ::*Chitinase* CaMV35S:: *ap24*	*Agrobacterium tumefaciens*	Embryogenic calli	*Phytophthora nicotianae* var. parasitica	Yabor *et al.*, 2006
	CaMV35S:: *ACACS2*	*Agrobacterium tumefaciens*	Friable embryogenic cell cultures	Longer postharvest shelf life	Trusov and Botella, 2006
	CaMV35S:: *PINPPO1* CaMV35S:: *PINPPO1-AS*	Particle bombardment	Embryogenic calli	Reduction of blackheart	Ko *et al.*, 2006
	Ubi 1::*PAT*	Particle bombardment	Leaves from plant seedlings	Resistance to the herbicide bialaphos	Sripaoraya *et al.*, 2001
	Mas::*uidA*	*Agrobacterium tumefaciens*	Friable tissues containing cell clusters	None	Firoozabady *et al.*, 2006
	CaMV35S:: *MSI-99*	*Agrobacterium tumefaciens*	Leaf bases from shoots growing in vitro	Resistance to fungi attack	Mhatre *et al.*, 2009

Note: *AS means antisense direction

phosphate to mannose-1-phosphate and *MgPMM* mRNA levels correlated with the amount of ascorbic acid in acerola fruits and leaves (Badejo *et al.*, 2009). Accordingly, transgenic tobacco plants over expressing PMM showed levels of ascorbic acid that were twice as high as the isogenic line. These studies show that the particular concentration of ascorbic acid in acerola is due to the genetic expression of the genes encoding some of the Smirnoff–Wheeler pathway enzymes.

9.4 Avocado (*Persea americana* Mill.)

Avocado somatic embryogenesis has been successfully carried out (Pliego-Alfaro and Murashige, 1988; Witjaksono and Litz, 1999). A protocol to regenerate avocado plants *in vitro* including transient expression of the *uidA* gene has also been published (Ahmed *et al.*, 1998). In other studies, the introduction of the gene *nptII* conferring resistance to the kanamycin antibiotic and *uidA*, in proembryonic tissue of 'Thomas' avocado to create transgenic somatic embryos by *Agrobacterium* infection has been reported (Cruz-Hernández *et al.*, 1998). From these embryos, transformed plants expressing both cDNAs were obtained. Thus, the utilization of the mentioned protocols to improve fruit quality by the introduction of cDNAs conferring resistance to abiotic stress by genetic transformation was demonstrated (Litz and Litz, 2002).

Fungal infection in fruit reduces postharvest quality and increases losses. With the aim to improve the biotic resistance of avocado fruit, a construct containing a cDNA encoding the *Arabidopsis* antifungal plant defensin gene *PDF1.2* (GenBank NM123809) was introduced into embryogenic avocado cultures derived from 'Hass' protoplasts under the control of the CaMV35S promoter. The grafting technique of transgenic shoots into *in vitro* grown seedlings was used to obtain fully developed transgenic plants. Overexpression of this gene in avocado may provide a defense against anthracnose and other fungal pathogens (Raharjo *et al.*, 2008).

In order to manipulate the synthesis of ethylene in avocado, a construct containing the *SAMK* cDNA (GenBank NP523296) encoding S-adenosyl-L-methionine hydrolase (SAMK) (EC 3.3.1.2) originated from bacteriophage T3 and a fruit specific cellulase promoter was transformed into avocado somatic embryos. SAMK catalyzes the conversion of the S-adenosyl methionine metabolite, a precursor of 1-aminocyclopropane-1-carboxylic acid, into methylthioadenosine and homoserine (Good *et al.*, 1994). In this way, ethylene biosynthesis could be reduced and ripening could be delayed. Although the transformation was successfully carried out, the induction of plantlets from the transgenic somatic embryos was not possible. Therefore, the effects of the introduced *SAMK* into the avocado fruit ripening phenomena were more complex (Efendi, 2003). Other studies involving fully transgenic avocado plants expressing *SAMK* have been made by grafting the *in vitro* derived transgenic shoots onto three-week-old seedlings (Litz *et al.*, 2007).

Much of the avocado transcriptome sequencing has been derived mainly from the floral genome project (FGP) (http://fgp.huck.psu.edu) (Albert *et al.*, 2005; Wall *et al.*, 2009). Nearly 100 000 EST sequences from early development of

avocado flowers have been collected by the FGP (Albert *et al.*, 2005) and more are currently being developed. These sequences constitute a valuable resource of genes for specific biochemical pathways related to quality characteristics of fruits. In all, 16 558 avocado ESTs are deposited at the dbEST (EST database) release 052110 from NCBI (see Table 9.2).

The avocado haploid genome size has been estimated to be 883 Mb and contains 12 chromosomes (Arumuganathan and Early, 1991). A composite genetic map for avocado has recently been reported (Borrone *et al.*, 2009). Twelve linkage groups were generated from an F_1 population of 715 individuals derived from reciprocal crosses of two Florida cultivars 'Tonnage' and 'Simmonds' and 163 molecular markers. 'Tonnage' was heterozygous for 152 (93%) loci and 'Simmonds' for 64 (39%). The genetic map will enable the detection of quantitative trait loci (QTLs) affecting traits of economic importance in this fruit.

9.5 Banana (*Musa acuminata*)

Banana fruit genome manipulation to create polyploid organisms has been carried out using different techniques (Arvanitoyannis *et al.*, 2008). As in avocado, banana transgenic plants have been obtained by introducing genes into somatic banana embryos. Furthermore, stable integration of genes into the banana genome has been done by protoplast electroporation of new embryogenic cell suspensions (Sági *et al.*, 1998), particle bombardment of embryogenic cells (Sági *et al.*, 1995; Becker *et al.*, 2000), co-cultivation of wounded meristems with *Agrobacterium*

Table 9.2 Numbers of ESTs for tropical and subtropical fruits (June 25, 2010)

Fruit	No. of ESTs (dbEST)
Avocado	16 558
Banana	5524
Duncan grapefruit	8039
Orange	208 909
Lemon	1505
Coconut	6
Date	78
Grape	361 433
Kiwifruit	57 751
Longan fruit	66
Loquat	4
Mango	68
Mangosteen	149
Melon	35 547
Pistachio	1299
Olive	4860
Papaya	77 393
Pineapple	5659

(May *et al.*, 1995), and co-cultivation of embryogenic cell suspension culture developed from different banana cultivars with *Agrobacterium* (Ganapathi *et al.*, 2001 Huang *et al.*, 2007; Ghosh *et al.*, 2009). Thus, it has been possible to develop a banana plant expressing two genes from *Saccharomyces cerevisiae, TPS1* and *TPP* that encode enzymes for the biosynthesis of trehalose (trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively) under a drought-stress inducible promoter. The main goal was to develop a plant resistant to saline stress and it was found that salinity induced levels of trehalose in the transgenic plants that were twice as high compared with the isogenic lines. Furthermore, the transgenic plants showed better resistance to saline stress as measured by the effect on photosynthesis (Santamaria *et al.*, 2009).

Antimicrobial peptides have been used to increase the resistance of banana against fungi and bacteria. Transgenic banana plants resistant to *Fusarium oxysporum f. sp. cubense* and *Mycosphaerella musicola* have been developed by introducing a magainin analog, MSI-99 (a class of antimicrobial peptides isolated for the first time from *Xenopus laevis* skin) into the banana genome (Chakrabarti *et al.*, 2003). Also, transgenic plants resistant to *F. oxysporum f. sp. cubense* and *Mycospaerella fijiensis*, and fruits resistant to preharvest and postharvest diseases developed by *Verticillium theobromae* or *Trachysphaera fructigena* have been obtained (Sági *et al.*, 1998). Moreover, banana transgenic plants resistant to the nematode *Radopholus similis* have been obtained using the constitutive promoter of the ubiquitin maize gene. These plants express an altered rice protein cystatin (OcIΔD86), a cysteine protease inhibitor (Atkinson *et al.*, 2004). Also, transgenic banana plants resistant to the attack of *Xanthomonas spp*, the causal agent of bacterial wilt have been produced, by expressing synthetic cercosporins (Rajasekaran *et al.*, 2001), and plants resistant to *F. oxysporum* produced by expressing a human lysozyme gene (Pei *et al.*, 2005). In addition, transgenic banana plants have been created to be a vehicle of vaccines for humans. In this context, there are transgenic banana expressing the 's' gene of hepatitis B surface antigen (HBsAg) under the promoter of the ACC oxidase (Kumar *et al.*, 2005).

On the other hand, some banana genes encoding enzymes related to quality attributes of the fruit have been analyzed. The xyloglucan endotransglycosylase is a fruit ripening and softening-related enzyme and *XET1* mRNA (GenBank EF103136) was found to increase in pulp and peel of ripe banana fruit (Lu *et al.*, 2004). A second xyloglucan endotransglycosylase encoding cDNA, *XET2* (GenBank EF103137) was reported. The expression of three pectin methyl esterases, seven xyloglucan endotransglycosylase/hydrolase, a polygalacturonase, an expansin and pectate lyase genes were evaluated during banana ripening, where polygalacturonase gene and a pectin methylesterase were clearly up-regulated (Mbeguie-A-Mbeguie *et al.*, 2009). The α-amylase is an enzyme involved in starch degradation by depolymerizing the α-glucan chains released by the endo-hydrolytic enzymes. The expression levels of its transcript were induced by ethylene and that expression correlated with the decrease in starch content (do Nascimento *et al.*, 2006). The expression of the gene *SPS*, encoding a sucrose phosphate synthase, involved in sucrose formation, was found to be increased

dramatically during ripening and to be induced strongly by ethylene. In the promoter region of the *SPS* gene, several responsive elements were identified such as ethylene responsive, auxin responsive, low temperature responsive and light responsive elements (Choudhury *et al.*, 2008).

The isolation of 84 up-regulated unigenes during the late ripening stage of banana will allow us to have a better picture of the genes involved in the quality characteristics development of the fruit and to choose the best of them in order to improve the fruit quality by the DNA recombinant technology (Manrique-Trujillo, *et al.*, 2007).

Other reports on transcriptomic analysis in *Musa* leaves are related to drought stress. In Coemans *et al.* (2005), 10 196 tags were generated from leaf tissue by super-serial analysis of gene expression (SuperSAGE) to characterize global expression in this plant under drought stress. A total of 5292 unigenes were produced using this technology and the top 100 transcripts were annotated, being the most abundant a type 3-metallothionein, representing nearly 3% of total transcripts analyzed. The main discovery was the recovery of a full gene sequence of a novel NADPH: photochlorophyllide oxidoreductase, the key enzyme in chlorophyll biosynthesis (Coemans *et al.*, 2005). This technique could be useful to perform transcriptomics of banana fruits to uncover genes related to quality parameters desirable in postharvest.

There is another report (Davey *et al.*, 2009) to profile the response of banana leaf transcriptome to drought stress using genomic DNA-based probe-selection to improve the efficiency of detection of differentially expressed *Musa* transcripts. With the use of cross-hybridization to Affymetrix oligonucleotide Rice GeneChip® microarrays a total of 2910 banana gene homologues with a >2-fold difference in expression levels were identified. The differentially expressed genes included many functional classes related to biotic and abiotic stress responses. The results obtained highlight another strategy for gene discovery in non-model species. At the dbEST there are 5524 banana ESTs deposited to date.

9.6 Cherimoya (*Annona cherimola* Mill.)

No cherimoya transgenic plants have been created yet; however, there is a report (Prieto *et al.*, 2007) where the cDNA *AcPPO* (GenBank DQ990911) encoding polyphenol oxidase, an enzyme responsible for the marked susceptibility to browning of this fruit, has been cloned and studied. A strong induction of the *AcPPO* mRNA levels was reported for cherimoya after wounding, supporting the function of the gene in the fruit browning phenomena. At the GenBank there are some cDNAs reported to be differentially expressed under cold storage that were identified in cherimoya cv. 'Concha lisa'. These are the enolase cDNA (GenBank FJ664264) encoding an enzyme of the glycolitic and gluconeogenesis pathways; the *AcDPE2* gene (GenBank FJ664262) encoding a transglucosidase essential for the cytosolic metabolism of maltose in plant leaves at night and *AcPE* gene (GenBank FJ664260) encoding pectinesterase.

9.7 Citrus

In the case of the species belonging to the genus Citrus, several protocols to develop transgenic plants have been reported; the first one was published about 18 years ago (Moore *et al.*, 1992) and from there, many protocols utilizing *Agrobacterium*-mediated transformation have been published for different citrus species (Kaneyoshi *et al.*, 1994; Peña *et al.*, 1995a, Peña *et al.*, 1995b, Peña *et al.*, 1997, Peña *et al.*, 2001; Gutiérrez-E *et al.*, 1997; Bond and Roose, 1998; Cervera *et al.*, 1998; Cervera *et al.*, 2000; Pérez-Molphe-Balch and Ochoa-Alejo, 1998; Luth and Moore, 1999; Domínguez *et al.*, 2000; Yang *et al.*, 2000; Ghorbel *et al.*, 2001). However, the transformation of citrus plants is still inefficient and improved protocols to create transgenic plants in this genus are currently being developed.

A more versatile protocol was published (Kayim and Koc, 2005) in which transgenic plants were created from eight different citrus species: 'Milam' rough lemon (*Citrus jambhiri* Lush), 'Volkamer' lemon (*Citrus volkameriana* L.), 'Rangpur' lime (*Citrus limonia* L.), 'Hamlin' sweet orange (*Citrus sinensis* Osbeck), 'Duncan' grapefruit (*Citrus paradisi* Macf.), 'Sour' orange (*Citrus aurantium* L.), 'Cleopatra' mandarin (*Citrus reticulata* Blanco) and 'Carrizo' citrange (*Citrus sinensis* Osbeck × *Poncirus trifoliata* Raf.) by inducing plasmolysis in the tissue before *Agrobacterium* infection. The transformation efficiencies depended upon the citrus species; however, they were the highest reported for citrus to date.

There is a recent and improved protocol for genetic transformation of juvenile explants of 'Carrizo' (*Citrus sinensis* Osb. × *Poncirus trifoliata* Raf.), 'Duncan' (*Citrus paradisi* Macf.), 'Hamlin' (*Citrus sinensis* Osbeck) and 'Mexican Lime' (*Citrus aurantifolia* Swingle). This transformation by *Agrobacterium* includes a pre-incubation step with hormones and the use of acetosyringone in the media where *Agrobacterium* was cultured before infection (Dutt and Grosser, 2009).

9.7.1 Duncan grapefruit (*Citrus paradisi* Macf.)

A specific *Agrobacterium* transformation protocol has been developed for 'Duncan' grapefruit. The media composition in which the explants were cultured before transformation and the co-cultivation media, were the most important factors affecting the transformation efficiency (Costa *et al.*, 2002). The color in 'Duncan' grapefruit is due to the presence of carotenoids in the juice. Transgenic fruits under the CaMV35S or the figwort mosaic virus 34S (FMV34S) were created to express the genes encoding phytoene synthase, phytoene desaturase, or lycopene-β-cyclase that play a role in the carotenoid biosynthetic pathway. Although no analysis was carried out in fruits, the leaves of several transgenic plants showed an increased pigmentation (Costa *et al.*, 2002).

To obtain grapefruit with reduced naringin bitter taste, transgenic plants were produced. In these plants reduced levels of chalcone synthase and chalcone isomerase were obtained to inhibit two steps of the biosynthetic pathway of flavonoids. The approach was done by introducing constructs that expressed

chalcone synthase (*CHS*) and chalcone isomerase (*CHI*) cDNA in sense and antisense. Although the analysis were not carried out in fruits, at least one transgenic plant showed very low levels of naringin as compared with the controls. In general, the flavonoid profiles and composition percentages were similar in control and transgenic plants (Koca *et al.*, 2009). There are 8039 ESTs deposited at the dbEST release 052120 for *Citrus* × *paradisi*.

9.7.2 Orange (*Citrus sinensis* L.)

The cloning and characterization of genes *CsMYC2* (GenBank EF645810) and *CsMYB8* (GenBank EF537877) encoding transcription factors that regulate expression of genes for the enzymes of pathways for color development of orange was reported (Cultrone *et al.*, 2009). The expression of *CsMYC2* mRNA correlated with the expression of genes encoding enzymes of the anthocyanin biosynthetic pathway. Genes of this pathway are *CsCHS* (GenBank EU410483) encoding chalcone synthase, *CsANS* (GenBank AY581048) encoding anthocyanidin synthase and *CsUFGT* (GenBank CF972319) encoding UDP-glucose-flavonoid 3-O-glucosyltransferase. Therefore, manipulation of color in *C. sinensis* is possible by altering the temporal and spatial expression of *CsMYC2* (Cultrone *et al.*, 2009).

Another work reported the relationship between carotenoid accumulation and the expression of genes encoding enzymes for the biosynthesis of carotenoids during fruit maturation in three citrus varieties (Kato *et al.*, 2004). Carotenoids provide bright yellow, orange and red colors to flowers and fruits. Partial cDNA clones whose expression was evaluated are *CitPSY* (GenBank AB114656) encoding phytoene synthase, *CitPDS* (GenBank AB114657) encoding phytoene desaturase, *CitZDS* (GenBank AB114658) encoding xi-carotene desaturase, *CitCRTISO* (GenBank AB114659) encoding carotenoid isomerase, *CitLCYb* (GenBank AB114660) encoding lycopene beta-cyclase, *CitHYb* (GenBank AB114661) encoding beta-ring hydroxylase, *CitZEP* (GenBank AB114662) encoding zeaxanthin (zea) epoxidase, and *CitLCYe* (GenBank AB114663) encoding lycopene E-cyclase. A simultaneous increase in the expression of genes *CitPSY, CitPDS, CitZDS, CitLCYb, CitHYb* and *CitZEP* coincided with accumulation of *β*,*β*-xanthophyll in flavedo of Valencia orange. This work showed that carotenoid accumulation during citrus fruit maturation was highly regulated by the coordination among the expression of the carotenoid biosynthetic genes.

The orange (*Citrus sinensis* L. Osbeck var 'Ridge Pineapple') chloroplast genome (GenBank DQ864733) was sequenced to facilitate genetic improvement of this crop. Its length is 160 129 bp and it contains 140 genes, of which 87 are protein coding and 53 are structural RNAs (Bausher *et al.*, 2006). On the other hand, there are 208 909 ESTs deposited at the dbEST from NCBI.

9.7.3 Lemon (*Citrus limon*)

Lemon fruit is known to have a large variety of monoterpenoids in the glands of the fruit flavedo that together with other compounds are components of the lemon

essential oil. Monoterpenes play different roles in the defense of the fruit against the attack of insects and plant pathogens as well as pollinator attracters. In this regard, the manipulation of the amount of these compounds could improve the resistance of the fruit against biotic stress during postharvest. An important step towards this goal was the cloning of four cDNAs, *ClLIMS1* (GenBank AF514287) encoding d-limonene synthase 1, *ClLIMS1* (GenBank AF514289) d-limonene synthase 2, *ClTS* (GenBank AF514286) encoding gamma-terpinene synthase, and *ClPINS* (GenBank AF514288) encoding beta-pinene synthase. These enzymes are involved in the biosynthesis of monoterpenoids and they were expressed in *E. coli* and characterized. The recombinant enzymes were found to produce 10 out of 17 monoterpene skeletons present in lemon peel oil (Lücker *et al.*, 2002). There are 1505 ESTs deposited at the dbEST release 052110 for lemon.

9.8 Coconut (*Cocos nucifera* L.)

No transgenic coconut plants have been reported and in fact this plant is one of the most recalcitrant species for *in vitro* regeneration (Cueto *et al.*, 1997). Therefore, somatic embryogenesis has been used to regenerate embryos expressing transgenic. Analysis of the genes playing a role in somatic embryogenesis could be helpful to develop *in vitro* regeneration protocols. There is a report of the isolation of an orthologous locus of the somatic embryogenesis receptor-like kinase (SERK) named *CnSERK* (GenBank AY791293). SERK is a protein that plays a role in the first steps of somatic embryogenesis in several plants (Santos *et al.*, 2005; Yang and Zhang, 2010). *CnSERK* was expressed in embryogenic tissues before full embryo development, whereas there was no expression of *CnSERK* in non-embryogenic tissues. This gene could be used as a molecular maker to aid the development of protocols including somatic embryogenesis of coconut (Perez-Nunez *et al.*, 2009).

An important factor leading to postharvest losses is the biotic stress. In this regard, it is important to know that plants express defense proteins for fruit defense. Until now, there has been no suitable model to study coconut molecular responses to the attack of pathogens. Therefore, the defense response in coconut *calli* was induced with chitosan, a polymer known to induce defense responses in plants (Ebel and Mithöfer, 1998). Accumulation of hydrogen peroxide, induction of β-1,3-glucanase activity and activation of a 46 kDa MAPK (mitogen activated protein kinase) were reported (Lizama-Uc *et al.*, 2007). MAPKs are known to function in plant responses to different biotic and abiotic stresses, including wounding, pathogen infection, temperature stress, drought and salinity stress (Zhang *et al.*, 2006). Besides the above, in the chitosan treated coconut *calli*, expression of genes *CnLI* (GenBank AM076489) encoding a receptor-like kinase, *CnACT* (GenBank AM689520) encoding actin, and *CnCLM* (GenBank AM167527) encoding a mitochondrial alternative oxidase, was induced. The system utilized is a good experimental approach helpful to uncover the molecular basis of the coconut defense response (Lizama-Uc *et al.*, 2007).

9.9 Date (*Phoenix dactylifera* L.)

In the case of dates, transient expression of the *β*-glucuronidase (*uidA*) reporter gene by particle bombardment has been reported (Mousavi *et al.*, 2009). The protocol included the utilization of embryogenic callus, somatic embryos as well as leaf and root tissues derived from tissue culture plantlets. The best *uidA* expression was obtained with the rice actin promoter in all tissues except in roots. The highest frequency of expression was obtained in embryogenic callus. Although no transgenic plants were obtained, this effort undoubtedly will allow in the future the use of the DNA recombinant technology to develop transgenic date fruit with improved quality.

The chloroplast complete genome of *Phoenix dactylifera* (GenBank GU811709) has been completed recently. With a length of 158 455 bp, it contains 139 genes, of which 89 are protein coding and 48 encode structural RNAs. The chloroplast whole genome information is valuable for chloroplast genetic engineering.

9.10 Fig (*Ficus carica* L.)

In figs, the production of transgenic plants by *Agrobacterium*-mediated transformation from the common fig 'Brown Turkey' (fresh consumption) and 'Smyrna' (dry consumption) was reported (Yancheva *et al.*, 2005). The protocol included the introduction of a construct containing the *uidA* gene and the *nptII* gene conferring resistance to kanamycin in leaf by using the disarmed strain of *Agrobacterium tumefaciens* EHA105. Complete transgenic plants were regenerated from the explants. The transgene was detected in the fig genome by PCR, Southern blot or histochemical localization of *β*-glucuronidase (GUS) reporter activity in plant tissues. No reports, to our knowledge, are available in which the protocol described above has been used to develop fig with improved quality characteristics.

Fig maturation is usually not uniform and this is an important parameter to take into consideration to obtain good fruit quality. In order to reduce the maturation differences, olive oil, ethrel/ethephon or auxin are commonly applied to figs. To understand the molecular basis behind these treatments, four cDNAs *FcACS1* (GenBank DQ269492), *FcACS2* (GenBank DQ269493), *FcACS3* (GenBank DQ269494) and *Fc-ACO1* (GenBank DQ269495) encoding ACC synthases and ACC oxidase were cloned (Owino *et al.*, 2006). The effect of oil, propylene or auxin treatment on the steady state levels of the four mRNAs was tested. Some of the genes were either positively (*FcACS1* and *FcACO1*) or negatively (*Fc-ACS2*) regulated by ethylene. Other genes showed a more complex regulation, being activated by auxin and inhibited by ethylene (*FcACS3*). The knowledge gained in this study could be helpful in the designing of protocols to manipulate ethylene in the fruit and to improve fig maturation.

Fig fruit presents a fast softening phenomena during ripening, suggesting the activation of several cell wall enzymes during this stage of development. Genes *FcPG1* (GenBank AY487304) and *FcPG2* (GenBank AY487305) encoding

endo-polygalacturonases, *FcCEL1* (GenBank AY487304) encoding cellulase, *FcENG* (GenBank AY487306) encoding endo-glucanase, *FcGAL1* (GenBank AY487308) and *FcGAL2* (GenBank AY487309) encoding *β*-galactosidases, *FcXTH1* (GenBank AY487310), *FcXTH2* (GenBank AY487311) and *FcXTH3* (GenBank AY487312) encoding xyloglucan endotransglycosylases, *FcARABF1* (GenBank AY487314) encoding *α*-arabinofuranosidase and *FcEXP* (GenBank AY487313) encoding expansin were cloned and their expression evaluated during ripening of the fig (Owino *et al.*, 2004). Steady state levels of these 11 mRNAs showed that *FcEXP, FcGAL1, FcXTH1, FcXTH2* and *FcARABF1* are expressed in the immature stages, and *FcPG1, FcPG2, FcGAL2* and *FcCEL1* from the beginning of ripening until the overripe stage, whereas *FcXTH3* was found active only at the overripe stage. Studies of the regulation of these 11 genes could be helpful to design strategies to control the softening phenomena by developing transgenic fig with modified levels of some genes encoding cell wall enzymes, that will be useful to improve this species.

9.11 Grape (*Vitis vinifera* L.)

Grape has been extensively studied using molecular biology-derived tools (Roubelakis-Angelakis, 2009). The first attempt to create transgenic plants in *V. vinifera* was made using petiole explants. In this experiment, only transgenic buds were obtained (Mullins *et al.*, 1990). The selection markers kanamycin and hygromycin were identified using 32 *V. vinifera* cultivars and intraspecific hybrids (Peros *et al.*, 1998). Later on, the successful creation of transgenic grape by *Agrobacterium* infection of somatic embryos induced from anther tissue was published (Mozsar *et al.*, 1998). Likewise, the successful creation of transgenic plants in *V. vinifera* was done with somatic embryos developed from another culture and *Agrobacterium* (Harst *et al.*, 2000). After that, the protocol was tested successfully in seven major cultivars for wine production, namely: 'Cabernet Sauvignon', 'Shiraz', 'Chardonnay', 'Riesling', 'Sauvignon Blanc', 'Chenin Blanc' and 'Muscat Gordo Blanco' (Iocco *et al.*, 2001). However, some of the cultivars were resistant to transformation and experiments to optimize the protocol by changing media composition and *Agrobacterium* strains were carried out (Torregrosa, *et al.*, 2002).

The development of protocols to create transgenic plants in *V. vinifera* from different tissues such as embryos developed from leaf disks (Das *et al.*, 2002), cryopreserved cell suspension cultures (Wang *et al.*, 2005) and shoot apical meristems (Dutt *et al.*, 2007) was reported. An important advance was made with the successful transformation *in planta* using dormant buds. This protocol avoids the need to regenerate the plant which sometimes induces somatic mutations, and it also has the advantage of greatly reducing the time needed to obtain a fully developed plant because of the normal reproductive cycle of the grapevine (Fujita *et al.*, 2009). Besides the utilization of *Agrobacterium*, other protocols used particle bombardment of embryogenic cell cultures of the cultivar 'Chardonnay' (Vidal *et al.*, 2003; Vidal *et al.*, 2006a).

Other improvements made in transgenic plant protocols are the creation of marker-free transgenic plants to eliminate the presence of the antibiotic resistance gene in the plant genome (Dutt *et al.*, 2008), and the utilization of promoters with duplicated enhancers to increase the level of transcription of the gene placed downstream (Li *et al.*, 2004). Transgenic plants of several cultivars of table grape have been created, including 'Sugraone' and 'Crimson Seedless' (Lopez-Perez *et al.*, 2008), 'Thompson Seedless' (Dutt *et al.*, 2007) and 'Chardonnay' (Vidal *et al.*, 2003). There are still opportunities to improve the methodology for transgenic plants, creating an active area of research and studies to improve the utilization of *Agrobacterium* infection (Li *et al.*, 2006; Li *et al.*, 2008; Dhekney *et al.*, 2009a), as well as studies to improve the utilization of particle bombardment (Vidal *et al.*, 2003), and the development and maintenance of *Vitis* embryogenic cultures of several varieties and species (Dhekney *et al.*, 2008, 2009b).

The grapevine transgenic plants created by using a cold inducible transcription factor from *Arabidopsis* (*AtDREB1b*) have improved resistance to cold stress. Although the berries have not been tested, they could be resistant to cold stress and this is very important because grapes need to be stored at low temperatures after harvest (Jin *et al.*, 2009).

With regard to biotic stress, research has been done to improve grape resistance to phytopathogenic fungi. The induction of genes by the infection of both leaves and berries with *Uncinula necator* which causes the grapevine powdery mildew has been studied (Jacobs *et al.*, 1999). The induction of several pathogen related proteins encoding extracellular proteins such as acidic class III chitinase, a basic class I glucanase, a thaumatin-like protein and a basic class I chitinase was recorded.

Leaves of *V. vinifera* L. cv. 'Chardonnay' were infected with *Botrytis cinerea* to analyze the type of gene induced in the defense response, and a strong induction of the mRNA levels of a gene encoding a β-1,3-glucanase enzyme after five days of infection was found (Renault *et al.*, 2000). The induction of different chitinases in response to fungi infection was carried out, and it was found that the type of chitinase depends upon the infecting pathogen. Indeed, mRNA of class III chitinases accumulates in unripe berries infected with *Plasmopara viticola*, but it was not found in berries at later developmental stages infected with *Uncinula necator* or *B. cinerea*. In leaves, accumulation of the chitinase class III mRNA was found only with *B. cinerea* (Robert *et al.*, 2002). Another study was done infecting leaves and berries with *B. cinerea*. In leaves, the expression of genes encoding phenylalanine ammonia-lyase (PAL), stilbene synthase, an acidic chitinase, a basic chitinase and a polygalacturonase inhibitor protein was induced. On the other hand, in grape berries the expression of the same genes was observed with the exception of the acidic chitinase (Bezier *et al.*, 2002).

Studies to understand the defense response of grapevine against bacterial infection have also been carried out. Genes for chitinase class I putatively located in the vacuole and a chitinase class III were induced by the infection of grapevine leaves with *Pseudomonas syringae pv. pisi* (Robert *et al.*, 2002).

The polygalacturonase inhibitor protein (PGIP) is an important defense for the plant against the attack of fungi. Transgenic plants of the cultivars 'Chardonnay'

and 'Thompson Seedless' expressing pear PGIP were created (Aguero *et al.*, 2006). The development of *B. cinerea* infection rate in leaves of the transgenic plants was lower as compared with the control plants (Aguero *et al.*, 2005). Transgenic plants of tobacco overexpressing the cDNA encoding *VvPGIP1* (GenBank AF499451) were created, and showed up to 69% reduction in the susceptibility to the attack by *B. cinerea*. Furthermore, the *Vitis* enzyme PGIP1 isolated from the transgenic tobacco plants inhibited the *in vitro* activity of polygalacturonase from *Aspergillus niger* and *B. cinerea* (Joubert *et al.*, 2006).

Transgenic plants of grape expressing the class I chitinase gene from rice, *OsRCC2* (GenBank X56787), showed enhanced resistance against the attack of *Uncinula necator* which causes powdery mildew and a mild resistance to *Elisinoe ampelina* which causes anthracnose (Yamamoto *et al.*, 2000). In contrast, an experiment with transgenic grape plants expressing cDNAs encoding chitinase and a ribosome inactivating protein have not shown a higher resistance of the plants under field conditions against *U. necator* and *P. viticola*, a heterothallic oomycete which causes grapevine downy mildew (Bornhoff *et al.*, 2005).

Bacterial infection is also a potential biotic stress for grape berries. Because of this, transgenic plants have also been created with the goal to increase the resistance against the attack of bacteria. In this context, transgenic grape plants expressing a pear PGIP were found to delay the development of infection in leaves infected with *Xylella fastidiosa*, the causal agent of Pierce's disease. Furthermore, these plants were found to have lower bacterial titers and better recovery after pruning as compared with the control plants (Aguero *et al.*, 2005).

Magainin peptides were isolated from *Xenopus* skin and have been shown to have antimicrobial broad spectra of activity (Matsuzaki *et al.*, 1997). Thus, transgenic plants of *V. vinifera* 'Chardonnay' were obtained by particle bombardment of a construct expressing a natural magainin-2 or a synthetic derivative. Transgenic plants growing in the greenhouse were infected with either *A. tumefaciens* vitis strain or *U. necator*. The transgenic plants showed a great resistance to the infection by *Agrobacterium* but a rather mild resistance against *U. necator*. The authors concluded that magainin confer better resistance against bacteria than fungi (Vidal *et al.*, 2006b).

Not all the experiments mentioned above were on grape berries; however, the biotechnological potential of these genes to increase berry resistance to biotic stress imposed by fungi and bacteria challenge is clear.

The analysis of a transcription factor isolated from a *V. vinifera* L. cv. 'Cabernet Sauvignon' grape berry expression library, called *VvWRKY2* (GenBank AY596466) was reported. *VvWRKY2* was found to be active in berries and leaves and induced by signals playing a role in plant defense including salicylic acid and hydrogen peroxide (Marchive *et al.*, 2007). Although transgenic grape plants were not created, transgenic tobacco plants overexpressing the mentioned transcription factor were found to be more resistant to the attack of *B. cinerea* in leaves, *Alternaria tenuis* in seeds and *Phytium spp* in roots (Mzid *et al.*, 2007). Considering that this is a gene active in grape berries and that *B. cinerea* is an important pathogen for grapes, transgenic berries overexpressing this gene most likely will be more

resistant to the attack of *B. cinerea*. The partial sequence of a *V. vinifera* lipid transfer protein 1 called *VvLTP1* (GenBank DQ136029) was isolated. Analysis of the promoter sequence found several *cis*-acting regulatory elements involved in the defense response. Moreover, the promoter was induced by a non-specific fungal elicitor in transgenic cell suspension cultures of *V. vinifera* and *VvLTP1* mRNA in grape plantlets that also showed higher resistance to *B. cinerea* due to the gene induction (Laquitaine *et al.*, 2006). Although no transgenic grape plants were created, *VvLTP1* constitutes a good opportunity for future use with the aim of enhancing the resistance of grape berries to pathogen attack.

Besides biotic stress resistance, genes involved in fruit quality characteristics have also been studied. Although transgenic plants were not usually created to improve fruit quality characteristics, the genes to be described are good candidates for future use to manipulate grape berries' quality. The expression of genes *VvPME* (GenBank AY043232) encoding pectinmethyl esterase, *VvPG1* (GenBank AY043233) and *VvPG2* (GenBank EU078975) encoding polygalacturonases was evaluated in grape skin during ripening to define their function in skin ripening. High levels of *VvPME* mRNA were found throughout berry development until the end of color change. *VvPG1* mRNA levels increased during color change correlating with softening of the fruit, whereas expression of *VvPG2* began before veraison and was low during skin ripening. There was a clear relationship between the initiation of fruit ripening and fruit softening with the induction of the *VvPG2* and *VvPG1*, respectively (Deytieux-Belleau *et al.*, 2008).

Berry sugar content is an important quality component in grapes. An experiment was carried out to study the expression of *VvHT1* (GenBank AJ001061) encoding a hexose transport protein, which seems to be specific for sink tissues such as berries. Transgenic tobacco plants were created and there was an induction of the hexose transport by sugar, sucrose and the sucrose isomer palatinose. It was found that *VvHT1* helps in the intake of sugar into grape berries and importing leaves of grape, suggesting that perhaps the manipulation of its expression can help to increase the levels of sugars needed in grape berries to improve fruit quality (Atanassova *et al.*, 2003).

Ripening of *V. vinifera* berries does not depend on ethylene, because it is not a climacteric fruit. However, it has been found that brassinosteroids are hormones playing a role in the ripening phenomena of this fruit. A study was reported in which the expression of the cDNAs encoding brassinosteroid-6-oxidase and the brassinosteroid insensitive gene was studied during grape berry development (Symons *et al.*, 2006). The results clearly showed that there was an increase in the amount of brassinosteroids at the beginning of grape berry ripening. Furthermore, the application of external brassinosteroids induced ripening, whereas the use of biosynthetic inhibitors delayed the development of grape ripening. Therefore, there is a biotechnological potential of those genes in the manipulation of grape berry development.

Color is also an important quality characteristic of grape berries, and therefore several studies have been carried out to analyze the changes in the activity of genes related to the anthocyanin biosynthesis. Transgenic grape plants were

created with a construct containing the transcription factor *VvMYBA1* that control the gene encoding the enzyme UDP-glucose: flavonoid-3-O-glucosyltransferase, which is critical for anthocyanin biosynthesis and it was cloned from *V. vinifera* (Ford *et al.*, 1998). In grape, this gene has been found to be active specifically in red skin grapes (Kobayashi *et al.*, 2001; Castellarin and Di Gaspero, 2007). Although grape berries were not analyzed, all the transgenic tissues showed a very intense red coloration indicating the activation of the anthocyanin pathway. Moreover, the activation of a gene encoding an enzyme putatively playing a role in the vacuolar transport of anthocyanin was also recorded (Cutanda-Perez *et al.*, 2009).

The expression of a transcription factor *MYB12* was shown to activate several genes encoding proteins playing a role in the anthocyanins biosynthetic pathway in response to different environmental stimuli like light or shading (Matus *et al.*, 2009). A cDNA was isolated from a 'Cabernet Sauvignon' berry library and called *VvMYB5b*, and was found to encode a protein belonging to the R2R3-MYB family of transcription factors (Deluc *et al.*, 2008). Based on its similarity to other transcription factor regulating anthocyanin biosynthesis, it was tested whether this gene is able to play a similar role. Transient expression in cells found that this gene product could activate genes encoding enzymes of the flavonoid pathway. Furthermore, tobacco transgenic plants overexpressing the *VvMYB5b* in tobacco were found to accumulate anthocyanin. It is quite likely that the manipulation of these transcription factors could lead to grapes with a better color.

Besides the studies of transcription factors, specific genes encoding enzymes of the anthocyanin biosynthetic pathway have also been analyzed. In this context, the expression of the dihydroflavonol reductase gene was studied by creating transgenic plants expressing a construct in which the *uidA* gene encoding *β*-glucuronidase was controlled by the promoter of the mentioned gene (Gollop *et al.*, 2002). Expression of the reporter gene was found in roots, leaves and stems. Although the expression was not tested directly in fruits, cell suspension from fruits also showed expression of the reporter gene. Furthermore, deletion of the promoter region showed that there is a specific responsive element in the promoter playing a function in the expression of the gene in the fruit (Gollop *et al.*, 2002). The synthesis of anthocyanins is derived from metabolites of the phenylpropanoid pathway. Genes encoding the enzymes flavonoid 3′-hydroxylase and flavonoid 3′, 5′-hydroxylase were isolated and studied from this pathway. These genes were found to be active in all tissues of grape that accumulate flavonoids and in the skin of ripening red berries that synthesize mostly anthocyanins (Castellarin *et al.*, 2006). Other studies also analyzed the activity of the mentioned genes, finding that flavonoid 3′,5′-hydroxylase gene was active in the berry skin at the harvest stages in which there is a large accumulation of delphinidin-based anthocyanins (Jeong *et al.*, 2006). Another study, also analyzing the genes involved in the flavonol biosynthesis in grape, found that two genes encoding flavonol synthases showed different expression. Moreover, one of them was expressed at very high levels during ripening of grape berries correlating with flavonol content increase in the berries (Downey *et al.*, 2003). The transcription factor *VvMYBF1* responsible for the activation of the flavonol synthase

gene was already cloned and shown to be active specifically in the skin of grape berries during ripening (Czemmel *et al.*, 2009).

Anthocyanins are stored in the vacuoles after synthesis, which is important for color development. Two genes encoding anthocyanin transporters were isolated and shown to be very active during the accumulation of anthocyanin in grape berry ripening. Constructs with green fluorescent protein (GFP) showed that these genes are located in the tonoplast, strongly suggesting their role in the transport of anthocyanin to the vacuole (Gomez *et al.*, 2009). In support of the study just mentioned, two glutathione-S-transferases shown to play a role in the formation of the ligand anthocyanin-glutathione before transport to the vacuole, were cloned and studied (Conn *et al.*, 2008).

Recently, phytochemicals have been the subject of an intense research mainly due to their positive effects on human health. Grapes have high concentrations of proanthocyanidins, which are known to have beneficial effects on human health (Yahia, 2010). The increase in these compounds can improve the quality of the grape berry because of the additional health benefit related to the fruit consumption. Some studies have been done with the genes involved in the biosynthesis of proanthocyanidins in grapes. The cloning of a transcription factor called *VvMYBPA1* was reported (Gagne *et al.*, 2009). *VvMYBPA1* was found to induce the activity of the genes encoding the enzymes leucoanthocyanidin reductase and anthocyanidin reductase. Both enzymes have been found to play an important role in the proanthocyanidins biosynthesis (total tannin, catechin and epicatechin) at the beginning of berry growth as well as during the change in color of grape berry skin (Gagne *et al.*, 2009). Furthermore, it was found that *VvMYBPA1* does not induce transcription of the gene *VvUFGT* (GenBank AB047099) encoding the enzyme UDP-glucose: flavonoid-3-O-glucosyltransferase which is critical for anthocyanin biosynthesis (Bogs *et al.*, 2007).

Some of the most helpful genetic components during the design of transgenic plants are the promoter sequences because they direct the gene expression of the gene downstream in specific tissues and during a specific stage of development. From here the importance of work in which the cloning and study of three genes with a very large activity during different stages of berry development was described. One of them, *VvAdh1*, was found to be active during the early stages of berry development whereas the other two, *VvAdh2* and *VvGrip4*, were active during grape berry ripening (Tesniere *et al.*, 2006).

An interesting work has recently been published in which a protein induced by a virus infection, VIGG, was found to correlate with changes in the composition of organic acids and phenols content. Transgenic plants were not created, and VIGG induction by the virus did not bring the same response in the composition of the fruit (Katoh *et al.*, 2009). It would be worthwhile to study the mode of action of this protein in order to be able to improve fruit quality of grape berries by DNA recombinant technology tools in the future.

Grape berries with reported transcriptomes are the result of the great interest in manipulating berry development and quality for both economic and health reasons. However, the knowledge of grape berry development and the regulation of ripening

are scarce. Before 2003 there were just over 400 sequences deposited in GenBank; however, 146 075 sequences were deposited in NCBI for several *Vitis* species in September 2003. A total of 135 541 ESTs from 58 cDNA libraries representing cv. 'Cabernet Sauvignon', 'Chardonnay', 'Pinot Noir', 'Shiraz', 'Regent', 'Ugni Blanc' and 'Chasselas' were taken from different plant organs including berry, leaf, flower, roots, compound bud, berry without seeds, stem, petiole, berry pedicle, shoot tip, seeds and berry skin. Of these ESTs, 48 806 (25.6%) are from berries.

Deluc *et al.* (2007) reported an mRNA expression profiling of grape berries to investigate the transcriptional network responsible for controlling berry development. The mRNA expression patterns of transcription factors, abscisic acid (ABA) biosynthesis and calcium signaling genes, identified candidate factors likely to participate in the progression of key developmental events such as veraison, as well as candidate genes associated with such processes as auxin partitioning within berry cells and aroma compound production.

The dbEST had 361 433 ESTs for *Vitis vinifera* at its release on May 21, 2010 and it represents the tropical specie with more EST deposited. Moreover, the grape genome was sequenced and its length is 487 Mb in 19 chromosomes (Jaillon *et al.*, 2007) (see Table 9.3).

9.12 Guava (*Psidium guajava* L.)

There is a recently published review of available protocols for organogenesis, somatic embryogenesis and the first attempts to create guava transgenic plants (Rai *et al.*, 2010). Protocols for guava organogenesis were developed more than 20 years ago where whole plants were induced from nodal explants excised from field grown trees and also from explants of *in vitro* growing seedlings (Amin and Jaiswal, 1987). These protocols were improved by including the establishment of nodal explants cultures before shoot induction (Amin and Jaiswal, 1988). Further research in this area gave rise to protocols in which organogenesis was induced from shoot tips of growth chamber growing seedlings (Papadatou *et al.*, 1990), from *in vitro* germinated seedlings hypocotyls (Singh *et al.*, 2002) and from nodes excised of plants growing in the greenhouse as well as axillary buds from *in vitro* growing seedlings (Ali, *et al.*, 2003). Besides organogenesis, somatic embryogenesis has been successfully carried out from ten-week-old zygotic embryos (Rai *et al.*, 2007) and from mesocarp (Chandra *et al.*, 2004).

Although regenerating protocols for guava have been available for some time, the first efforts to create transgenic plants have been carried out only very recently. Indeed, successful introduction of cDNAs encoding *β*-glucuronidase (*uidA*) and

Table 9.3 Tropical and subtropical plant genomes sequenced

Fruit	Latin name	References	Type of project
Grape	*Vitis vinifera* L.	Jaillon *et al*., 2007	Complete large-scale
Papaya	*Carica papaya* L.	Ming *et al*., 2008	Complete large-scale

neomycin phosphotransferase (*nptII*) under the control of the CaMV35S promoter was done by infection of guava plants with *Agrobacterium tumefaciens* strain LB4404 (Biswas *et al.*, 2007). There is also a report where it was attempted to improve the resistance of the guava fruit to low temperature injury by the introduction of the transcription factors *CB1, CB2* and *CB3* (which stands for C-repeats binding factors), known to be induced by a low temperatures gene (Gilmour *et al.*, 2004). However, the regeneration of the complete transgenic plants was not possible (Rai *et al.*, 2010).

The aroma of guava fruit is an important quality characteristic. The isolation and study of a cDNA encoding a fatty acid 13-hydroperoxide lyase from guava (*CYP74B5*) has been reported. Expression of the cDNA in *E. coli* showed that this enzyme is able to convert the 13(S)-hydroperoxylinolenic acid into 12-oxododec-9 (Z)-enoic acid and 3 (Z)-hexenal, clearly demonstrating the nature of the enzyme encoded by the isolated cDNA (Tijet *et al.*, 2000). This cDNA will be available to be used in the improvement of guava fruit quality once more efficient protocols for guava genetic transformation have been developed.

9.13 Kiwifruit (*Actinidia deliciosa*)

The creation of transgenic kiwifruit plants by *Agrobacterium* infection has been carried out successfully (Janssen and Gardner, 1993), although the first efforts were initiated a long time ago (Candy, 1987). The isolation of promoters with activity in fruit tissue is very important in the application of DNA recombinant technology to improve fruit quality. In this context, cysteine protease enzymatic activity was assayed and found to be present from the stage of development in which the fruit weighed about half of the maximum until harvest when the activity was very high. The promoter of the gene encoding this enzyme was identified and cloned fused to the *β*-glucuronidase reporter gene and found to be active (Ershen *et al.*, 1993).

Stilbene synthase is an enzyme involved in the synthesis of the phytoalexin trans-resveratrol that is proposed to play a role in plant protection against fungal pathogens (Dercks and Creasy, 1989) since it inhibited conidia germination of *B. cinerea* under *in vitro* conditions (Adrian *et al.*, 1997). In order to increase the resistance of kiwifruit to fungal pathogens, transgenic plants that overexpressed *pSV25* encoding the stilbene synthase from three Vitis species (*V. vinifera, V. labrusca* and *V. riparia*) were created with the CaMV35S promoter (Kobayashi *et al.*, 2000). Instead of resveratrol, the transgenic plants accumulated a glucosil derivative piceid that was inactive against fungi according to the results. When transgenic plants producing the highest amount of piceid in leaves were infected with spores of *B. cinerea*, no reduction in fungi infection was found. Although resistance was not found in these kiwifruit transgenic plants, they could be healthier for the consumer due to the presence of the piceid molecule that undoubtedly improves fruit quality.

Several cultivars of kiwifruit develop a physiological disorder known as low temperature breakdown that reduces the quality of the fruit (Lallu, 1997; Burdon *et al.*, 2007). Knowing the molecular fruit responses to low temperature stress

can allow the design of kiwifruit with better resistance to this physiological breakdown.

Several genes related to the ethylene pathway were isolated from an *Actinidia* EST database, including ethylene receptors *AdETR1* (GenBank EU170628), *AdETR2* (GenBank EU170629), *AdETR3* (GenBank EU170630) and *AdERS1a* (GenBank EU170626); *AdCTR1*-like (Raf-like protein kinase; GenBank EU170631) and *EIN3*-like (transcription factor) genes *AdEIL1* (GenBank EU170633), *AdEIL2* (GenBank EU887511), *AdEIL3* (GenBank EU887512) and *AdEIL4* (GenBank EU887513). The expression of four tested genes encoding ethylene receptors (*AdERS1a, AdETR2* and *AdETR3*), one of the Raf-like protein kinase (*AdCTR1*) and the four *EIN3*-like genes, were up-regulated in response to low temperatures (Yin *et al.*, 2009). It has been found in plants that *EIN* genes can control the activation of ethylene-responsive element binding factors in response to several abiotic stresses such as wounding, cold, high salinity, or drought (Fujimoto *et al.*, 2000). Therefore, the four *EIN3*-like genes found to be induced during cold stress could be playing a role in the kiwifruit molecular responses to low temperature, perhaps by activating low temperature induced genes. Therefore, they have a potential biotechnological application in improving the resistance of kiwifruit to low temperature breakdown.

Kiwifruit is a very important source of vitamin C and several studies have been made to analyze the expression of the enzymes involved in its biosynthesis pathway. The gene *AdGDH* (GenBank EU525847) encoding the L-galactose dehydrogenase (GDH) was cloned (Laing *et al.*, 2004). GDH catalyzes the last step in vitamin C biosynthesis (Giovannoni, 2007) and it has been useful to study the effects of the environment in its activity as well as in the amount of vitamin C in kiwifruit. The effects of growing kiwifruit at high temperature were evaluated; kiwifruit heated during either cell division or starch accumulation resulted in a reduction of 60% in vitamin C content. Moreover, *AdGDH* mRNA levels were reduced in the kiwifruit, suggesting the importance of this gene in the ascorbate biosynthesis (Richardson *et al.*, 2004).

Expression analysis of the genes *AdGME* (GenBank GU339037) encoding GTP-mannose-3′,5′-epimerase and *AdGGT* encoding GTP-L-galactose guanyltransferase was done in *A. eriantha, A. chinensis* and *A. deliciosa*. These *Actinidia* species accumulate different amounts of vitamin C. Both enzymes are located in earlier steps of the biosynthesis pathway of vitamin C (Giovannoni, 2007). A strong correlation was found between expression levels of *AdGME* and *AdGGT* and the amount of vitamin C in *A. eriantha*, which can accumulate an amount of vitamin C up to 16 times higher as compared with the other two species. These results suggest that GME and GGT enzymes are important control points in the biosynthetic pathway of vitamin C. Confirmation of this finding was carried out by creating *Arabidopsis* transgenic plants overexpressing either *AdGGT* or both *AdGME* and *AdGGT* genes. Transgenic plants expressing both genes accumulated a higher amount of vitamin C than control plants (Bulley *et al.*, 2009). The regulatory function uncovered for GME and GGT can be helpful to design protocols to increase the amount of vitamin C in kiwifruit or other fruits and in this way improve the overall fruit quality.

A transgenic kiwifruit was created expressing a construct in which a transcription factor of *V. vinifera, VvmybA1-2* was controlled by the CaMV35S promoter. MybA1-2 transcription factor regulates the expression of genes encoding enzymes for the biosynthesis of anthocyanins. The transgenic kiwifruit plantlets showed a reddish-purple color due to the accumulation of cyanidin. Although fruits were not analyzed, this is a clear example of the potential of the DNA recombinant technology to improve the color in fruits by the introduction of specific genes (Koshita *et al.*, 2008).

Lipoxygenase (LOX) is an important enzyme playing a role in the developing of the characteristic flavor (Chen *et al.*, 2004) and aroma (Perez *et al.*, 1999) in some fruits. Six genes *AdLOX1* (GenBank DQ497792), *AdLOX2* (GenBank DQ497797), *AdLOX3* (GenBank DQ497795), *AdLOX4* (GenBank DQ497793), *AdLOX5* (GenBank DQ497796) and *AdLOX6* (GenBank DQ497794) encoding lipoxygenases were cloned from the kiwifruit EST database (Zhang *et al.*, 2006). The expression of the *AdLOX* genes was evaluated during kiwifruit ripening. *AdLOX1* and *AdLOX5* were highly expressed during ripening and their expression was inhibited by 1-MCP treatment, whereas *AdLOX2, AdLOX3, AdLOX4*, and *AdLOX6* were down-regulated during fruit ripening. LOX1 and LOX5 are important for the development of the characteristic aroma in kiwifruit. Although no transgenic plants were created, there is a clear biotechnological potential of the *LOX* genes studied to improve the aroma in kiwifruit (Zhang *et al.*, 2009).

Fruit softening is very important in the final quality of the fruit. In order to understand this phenomenon in kiwifruit the isolation and expression of six cDNA clones *AdXET1, AdXET2, AdXET3, AdXET4, AdXET5 and AdXET6* encoding xyloglucan endotransglycosylases (XET) from ripe kiwifruit was reported (Schroder *et al.*, 1998). Northern analysis indicated that *AdXETs* expression was induced in ripening kiwifruit, when endogenous ethylene could be first detected and the highest expression of *AdXET* genes was in climateric samples when kiwifruit were soft. XET enzymes depolimerize xyloglucan from cell walls.

Moreover, genes *AeXTH1* (GenBank EU494946), *AsXTH2* (GenBank EU494947), *AeXTH3* (GenBank EU494948), *AdXTH4* (GenBank EU494949), *AdXTH5* (GenBank EU494950), *AdXTH6* (GenBank EU494951), *AdXTH7* (GenBank EU494952), *AdXTH8* (GenBank EU494953), *AhXTH9* (GenBank EU494954), *AdXTH10* (GenBank EU494955), *AcXTH11* (GenBank EU494956), *AeXTH12* (GenBank EU494957), *AdXTH13* (GenBank EU494958) *and AdXTH14* (GenBank EU494959) encoding xyloglucan endotransglucosylase/hydrolase (XTH) were isolated and characterized (Atkinson *et al.*, 2009). XTH enzymes disassemble xyloglucan loosening the plant cell walls. The XTHs contained the two conserved glutamic acid residues at the active site of XTH. XTH5, XTH7 and XTH14 were overexpressed in *E. coli* and recombinant proteins had XTH activity. Quantitative RT-PCR was done to identify XTH transcripts in different tissues and ripe fruit. *AdXTH4, AdXTH5* and *AdXTH7* were much more expressed in ripe kiwifruit; *AdXTH7* was highly expressed in outer pericarp and its levels decreased during the rapid softening phase of the fruit. This work is the first to investigate the role of XTH in fruit ripening.

Crowhurst *et al.* (2008) produced ETS from kiwifruit (*Actinidia* spp.) to discover genes related to fruit quality and health. The ESTs were obtained from *A. chinensis, A. deliciosa, A. arguta* and *A. eriantha*. From a total of 132 577 ESTs, 41 858 resulted in unigenes. Within the flavor-related gene family, acyltransferases were identified, and in the fragrance-related gene family, carboxylesterases that are involved in the terpenoid biosynthesis pathway were identified. Also ESTs were identified for genes of color pathways controlling chlorophyll degradation and carotenoid biosynthesis. ESTs related to health are from genes involved in ascorbic and quinic acid biosynthesis. These large collections of ESTs will allow the researchers to undertake the challenge of investigating the molecular basis of genetic diversity in this fruit.

9.14 Litchi (*Litchi chinensis* Sonn.)

In litchi, there are no protocols for the creation of transgenic plants. However, the introduction of a construct containing the green fluorescent protein (GFP) transcriptionally controlled by the CaMV35S promoter was attempted by *Agrobacterium* infection. The expression of GFP in regenerated leaves and callus after four weeks of infection was reported, although no transgenic plantlets were obtained (Puchooa, 2004). Somatic embryogenesis and plant regeneration of litchi from protoplasts (Yu *et al.*, 2000) and leaves (Raharjo and Litz, 2007) have been reported, since these are important steps in the developing of transgenic plants; indeed, in the near future litchi transgenic plants will be produced.

Fruit cracking is a problem in some cultivars of litchi, leading to a reduction in fruit quality. Advances in the understanding of the molecular basis of this phenomenon were made in a work in which the expression of two cDNAs *LcEXP* (GenBank EF446292) and *LcEXP2* (GenBank EF446293) encoding expansins (EXP) were studied at different stages of development in the pericarp and aril of the cracking susceptible cultivar 'Nuomici' and of the cracking resistant cultivar 'Huaizhi'. Differences in the expression of the two genes during the different stages of fruit development as well as in the different tissues of the fruit were reported. It was concluded that these genes play a role during fruit growth and perhaps in the fruit cracking phenomena (Yong *et al.*, 2006).

9.15 Longan (*Dimocarpus longan* Lour.)

For longan fruit there are no protocols available to create transgenic plants; however, somatic embryogenesis and establishment of plantlets have been carried out successfully from longan calli cryopreserved (Matsumoto *et al.*, 2004). Therefore, longan transgenic plants may be available in the near future.

Aril breakdown is a very important problem reducing fruit quality during postharvest shelf life of longan fruit. With the goal of understanding the molecular mechanism underlying this phenomena, the expression of three genes *DlEXP1*

(GenBank EU416313), *DlEXP2* (GenBank EU416314) and *DlEXP3* (GenBank EU416315) encoding expansins and three genes *DlXET1, DlXET2* and *DlXET3* encoding xyloglucan endotransglucosylases was studied during fruit storage at 25°C or 4°C (Zhong *et al.*, 2008). The expression of these genes correlated well with either early (*DlXET3*) or late aril breakdown (*DlEXP3* and *DlXET1* and *DlXET2*) during storage at the different temperatures. Furthermore, one of the expansin genes expression (*DlEXP2*) was found to be induced within 12 hours after the fruit transference from low temperature to room temperature (Zhong *et al.*, 2008).

Three endo-1, 4-beta-glucanase genes *DlEG1* (GenBank GQ261871), *DlEG2* (GenBank GQ261872) and *DlEG3* (GenBank GQ261873) were characterized during longan fruit growth and development. These *DlEGs* presented different expression patterns in different tissues and only *DlEG3* showed fruit-specific expression (Chen *et al.*, 2009).

9.16 Loquat (*Eriobotrya japonica* L.)

In loquat fruit there are no transgenic plants created; however, a protocol has recently been published to develop plantlets in three cultivars of this species from cultured anthers (Li *et al.*, 2008). Perhaps this new development will help in the near future in the development of transgenic plants.

Chilling injury (CI) can reduce the postharvest quality of some cultivars of loquat fruit (Cai *et al.*, 2006). With the aim of understanding the involvement of ethylene in this physiological disorder, expression of some genes involved in the ethylene signal transduction pathway was studied in two loquat cultivars differing in their susceptibility to develop CI. Changes in expression of three ethylene receptor genes *EjETR1* (GenBank FJ624867), *EjERS1a* (GenBank FJ624871), *EjERS1b* (GenBank FJ624870), one *CTR1*-like (GenBank FJ624869) gene, and one *EIN3*-like *EIL1* (GenBank FJ624868) were isolated and characterized in ripening fruit (Wang *et al.*, 2010). These genes were differentially expressed within and between fruit of these two cultivars during low temperature storage of the CI susceptible cultivar 'Luoyangqing' and of the CI resistant cultivar 'Baisha'. Although the main differences were in the transcript expression levels rather than in the type of pattern, an interesting finding was that the expression of *EjETR1* and *EjEIL1* was highly correlated during the CI development symptoms in the loquat cultivar 'Luoyangqing'.

The firmness of fruit is an important quality characteristic during postharvest shelf life. A study aiming to explain the genes involved in loquat fruit softening analyzed the expression of four genes *EjEXPA1* (GenBank EU123919), *EjEXPA2* (GenBank EU123920), *EjEXPA3* (GenBank EU123921) and *EjEXPA4* (GenBank EU123922) encoding expansins in the loquat cultivars 'Luoyangqing' and 'Baisha' and found a close correlation between the increase in expression of the *EjEXPA1* and lignification development in the 'Luoyangqing' cultivar during storage at 0°C. Softening of the 'Baisha' cultivar was correlated with the increased

expression of *EjEXPA1* and *EjEXPA4* isozymes. It was concluded that *EjEXPA1* may have a role in lignification development and *EjEXPA1* and *EjEXPA4* isozymes may be active in fruit softening during development (Yang *et al.*, 2008).

Another study also related to fruit firmness was conducted to identify genes playing a role in the lignification of some loquat cultivars, which is an important phenomena reducing the quality of the fruit. Six cDNAs encoding enzymes with a role in lignin biosynthesis were isolated from 'Luoyangqing', a cultivar with the tendency to develop lignin during development, namely: *EjPAL1, EjPAL2* encoding phenylalanine ammonia lyase (PAL), *Ej4CL* encoding 4-coumarate: coenzyme A ligase (4CL), *EjCAD1* and *EjCAD2* encoding isozymes of cinnamyl alcohol dehydrogenase (CAD), as well as *EjPOD* encoding a peroxidase enzyme (POD). The expression of the genes was compared between a cultivar with a tendency to develop lignin, 'Luoyangqing', and a cultivar not developing lignin, 'Baisha'. It was found that the gene expression of *EjCAD1* and *EjPOD* correlates more with lignification of loquat flesh tissue. Also, the *EjCAD1* was highly expressed in loquat fruit developing chilling injury at low temperatures, and so perhaps may play a role in lignification of flesh tissue, one of the symptoms of the chilling injury physiological disorder in loquat (Shan *et al.*, 2008).

9.17 Mango (*Mangifera indica* L.)

In mango fruit the induction of embryos from nucellar tissues has been carried out successfully from either polyembryonic cultivars (Litz *et al.*, 1982; Rivera-Domínguez *et al.*, 2004) or monembryonic ones (Litz, 1984). Now, it is possible to get a complete plant from nucellar tissue as follows: induction of embryogenic culture from nucellus, maintenance of embryogenic culture, development of morphologically normal embryos and germination of somatic embryos into well-developed plantlets (Krishna and Singh, 2007). Transgenic mango plants were developed using *Agrobacterium* infection in somatic embryos and introducing the gene encoding *β*-glucuronidase reporter gene. Stable integration of this construct was probed by Southern blot analysis (Mathews *et al.*, 1992).

Ethylene triggers the initiation of mango fruit ripening and because of that, any delay in the initiation of its synthesis could allow the manipulation of the postharvest shelf life of this fleshy fruit (Bapat *et al.*, 2010). An attempt to reduce the expression of two enzymes from the ethylene biosynthetic pathway was made by introducing in antisense orientation the genes encoding the ACC oxidase and the ACC synthase in mango somatic embryos (Cruz-Hernández *et al.*, 1997). The presence of the *nptII* gene encoding the neomycin phosphotransferase enzyme in the mango genome was confirmed by PCR and Southern blot. Furthermore, the researchers were able to create mature transformed embryos and it is expected that the transgenic mango will show the effects of either very low levels or the complete absence of ethylene in the plant and fruit.

In order to understand the genes playing a role in the ethylene mode of action, the expression of *MiETR1* encoding an ethylene receptor from mango was

evaluated (Martínez *et al.*, 2001). *MiETR1* (GenBank AF227742) is expressed in the mesocarp during ripening and in response to wounding. It was interesting to find that this gene was induced both by wounding and by ripening. Another study reported the isolation from ripe mango fruit of *MiPEL1* (GenBank AY987389), encoding the cell wall enzyme pectate lyase (Chourasia *et al.*, 2006). *MiPEL1* was highly expressed during fruit ripening and it was reduced in 1-MCP treated fruits, suggesting the role of ethylene in its induction. Furthermore, *MiPEL1* gene expression was correlated with the increase in pectin solubilization. The authors of the work suggested that this is a gene playing an important role in fruit softening.

A gene *MiCEL1* (GenBank EF608067) encoding an endo-β-1,4-glucanase (hydrolysis of cellulose) was also cloned and characterized (Chourasia *et al.*, 2008). Analysis of the gene product allowed the identification of a cellulose binding domain as well as a signal peptide characteristic of enzymes targeted to the cell wall. Furthermore, *MiCEL1* was expressed only in fruits and CEL1 enzyme activity during fruit ripening correlates with a reduction in the amount of the cellulose of the fruit cell wall suggesting a role for this enzyme in fruit softening.

An important characteristic of fruit quality is the texture of the flesh, and in mango the development of uneven flesh softening is a frequent physiological disorder (Chaplin *et al.*, 1990). PCR based subtractive hybridization was carried out from healthy and spongy tissue affected mango fruit to investigate the molecular basis of that disorder and the expression of selected genes was analyzed. Expression of two ESTs (GenBank CD002004 and CD002000) related to catalase, an EST (GenBank CD002005) related to alcohol dehydrogenase and one EST (GenBank CB933771) related to keratin-associated protein were induced in the spongy tissue of mango fruit. Expression of one EST (GenBank CB933774) related to a ribosomal protein, one EST (GenBank CB933772) related to fructose-bisphosphate aldolase and (GenBank CB933775) related to cysthathionine gamma synthase was reduced in spongy tissue. This study will help to design a strategy to control the spongy tissue physiological disorder (Vasanthaiah *et al.*, 2006).

Several studies dealing with the analysis of genes putatively playing a role in flesh softening in mango have been published. The cloning and characterization of *MiEXPA1* (GenBank AY600964) encoding an alpha expansin enzyme was reported. *MiEXPA1* was active during fruit softening and induced by ethylene. Moreover, treatment of the mango fruit with 1-MCP inhibited the transcription of *MiEXPA1* as well as fruit softening, strongly suggesting the role of this expansin in mango fruit softening (Sane *et al.*, 2005).

In a recent work (Pandit *et al.*, 2010), 18 cDNAs related to the physiology and biochemistry of mango fruit cv. 'Alphonso' were isolated and their expression was evaluated. The 18 identified were: *MiIPPI* (GenBank EU513264) encoding isopentenyl pyrophosphate isomerase, *MiGPPS* (GenBank EU513265) encoding geranyl pyrophosphate synthase, *MiMTPS* (GenBank EU513266) encoding monoterpene synthase, *MiGGPPS* (GenBank EU513267) encoding geranylgeranyl pyrophosphate synthase, *MiFPPS* (GenBank EU513268) encoding farnesyl pyrophosphate synthase, *MiSqTPS* (GenBank EU513269) encoding sesquiterpene synthase, *MiIsoCH* (GenBank EU513270) encoding isochorismate hydrolase, *MiGT*

(GenBank EU513271) encoding glucosyl transferase, *MiLOX* (GenBank EU513272) encoding lipoxygenase, MiMDHAR (GenBank EU513274) encoding monohydrogenase ascorbate reductase, *Mi14-3-3* (GenBank EU513275) encoding a 14-3-3 protein, *MiMT* (GenBank EU513276) encoding a metallothionein, *MiMeTr* (GenBank EU513277) encoding a methyltransferase, *MisHSP* (GenBank EU513278) encoding a small heat shock protein, *MiCHIT* (GenBank EU513279) encoding a chitinase, *MiCysPI* (GenBank EU513280) encoding a cysteine proteinase inhibitor, *MiERF* (GenBank EU513281) encoding an ethylene response factor, *MiUbqPL* (GenBank EU513282) encoding ubiquitin-protein ligase and *MiEF1* (GenBank EU513283) encoding elongation factor 1-alpha.

There are 68 mango ESTs deposited in the dbEST release 052110, as well as one ETR (GenBank AY685130) and one EST for PAL (GenBank GU266281). This is an area of opportunity to produce more ESTs since mango has economic value.

9.18 Mangosteen (*Garcinia mangostana* L.)

In the case of mangosteen, there are no protocols available to create transgenic plants; however, somatic embryogenesis has been carried out successfully from seeds (Van Minh, 2005) and organogenesis has been induced from seeds (Normah *et al.*, 1992), leaves (Goh *et al.*, 1990; Te-chato and Lim, 1999) and cotyledons (Goh *et al.*, 1988). Therefore, the protocols to utilize the tools from the DNA recombinant technology are already developed and perhaps in the near future we will see the first efforts in this direction.

The color development is an important quality characteristic in mangosteen fruits. In this regard, three type MYB transcription factors *GmMYB1* (GenBank FJ197135), *GmMYB7* (GenBank FJ197136) and *GmMYB10* (GenBank FJ197137) were cloned and found to regulate anthocyanin biosynthesis during mangosteen ripening. Genes *GmPAL* (GenBank FJ197127) encoding the enzyme PAL, *GmCHS* (GenBank FJ197128) encoding the enzyme chalcone synthase, *GmCHI* (GenBank FJ197129) encoding the enzyme chalcone isomerase, *GmF3H* (GenBank FJ197131) encoding the enzyme flavanone-3-hydroxylase, *GmF3'H* (GenBank FJ197132) encoding the enzyme flavonoid 3′-hydroxylase, *GmDFR* (GenBank FJ197130) encoding the enzyme dihydroflavonol-4-reductase, *GmLDOX* (GenBank FJ197133) encoding the enzyme leucoanthocyanidin dioxygenase and *GmUFGT* (GenBank FJ197134) encoding the enzyme UDP-glucose:Xavonoid 3-O-glucosyltransferase were also cloned. *GmMYB10* mRNA and *GmUFGT* mRNA levels were highly abundant with onset of pigmentation and during red coloration. The results suggest that *GmMYB10* is important for the regulation of anthocyanine biosynthesis and *GmUFGT* is a key biosynthesis gene in mangosteen pigmentation. In fruits treated with 1-MCP the color development was delayed, suggesting the role of ethylene in the induction of *GmMYB10* expression. The data generated in this work clearly suggest the importance of the transcription factor MYB and the enzyme UFGT in the color development of mangosteen fruit (Palapol *et al.*, 2009).

9.19 Melon (*Cucumis melo* L.)

In melon somatic embryogenesis from cell suspension cultures was reported long ago (Oridate and Oosawa, 1986). Since then, the successful induction of somatic embryos has been carried out from cotyledons of mature seeds, hypocotyls of seedlings as well as leaves and petioles of young plantlets (Tabei *et al.*, 1991). By then, the creation of transgenic plants expressing the enzymes *β*-glucuronidase, dihydrofolate reductase and neomycin phosphotransferase under the CaMV35S promoter had already been reported (Dong *et al.*, 1991). Later, other protocols have been developed to create transgenic melon (Guis *et al.*, 2000). Moreover, the finding of a genotype with optimal characteristics for manipulation and transformation was reported (Galperin *et al.*, 2003) and a more efficient *Agrobacterium* mediated creation of transgenic melon that includes embryogenesis from cotyledon and hypotocyls explants was also obtained (Akasaka-Kennedy *et al.*, 2004).

The development of protocols to create transgenic melon plants is still an active line of research and the utilization of cotyledon, hypocotyl and true-leaf explants from both female and male parental lines of Galia melon has been reported (Nunez-Palenius *et al.*, 2007a). However, the manipulation of melon *in vitro* is still not easy and, for unknown reasons, the organogenesis and somatic embryogenesis cannot be applied to all melon genotypes; thus, it has to be done for every genotype on an individual basis. Therefore, the utilization of DNA recombinant technology is considered a good choice to improve the melon fruit characteristics (Nunez-Palenius *et al.*, 2008). There are two reviews on the subject of manipulation *in vitro* of melon explants and protocols to create transgenic melon plants (Li *et al.*, 2006; Nunez-Palenius *et al.*, 2008).

Fruit firmness is an important quality characteristic; however, in melon fruit controversy exists as to whether the PG enzyme plays an important role in this parameter. In this context, three cDNAs with significant homology to other genes encoding polygalacturonases were cloned from melon fruit ripening, *MPG1* (GenBank AF062465), *MPG2* (GenBank AF062466) *and MPG3* (GenBank AF062467). The expression levels of three polygalacturonase genes were high during fruit ripening. *MPG1* mRNA was accumulated at higher levels and for a longer period of time during fruit ripening (Hadfield *et al.*, 1998).

Carbohydrate composition in fruits is important to define their final quality in the market. Although melon fruit has not been found to accumulate large amounts of starch (Hubbard *et al.*, 1989), expression analysis of three cDNAs encoding one small subunit and two large subunits of ADP-glucose pyrophosphorylase, an enzyme playing a role in starch biosynthesis, were found to be highly active at the beginning of the melon fruit ripening (Park *et al.*, 1998). This finding must be related to the small amount of starch present at the beginning of ripening in some cultivars of melon fruit (Hubard *et al.*, 1989). Melon fruit during ripening accumulate stachyose and raffinose that are galactosyl-sucrose containing oligosaccharides (Gao *et al.*, 2002). The enzyme *α*-galactosidase initiates the degradation of the oligosaccharides mentioned by removing the terminal *α*-galactosyl residue. Two cDNAs *CmAGA1* and *CmAGA2* were cloned from

ripening melon fruit with high homology with genes encoding α-galactosidase enzymes. Expression of the proteins encoded in *CmAGA1* and *CmAGA2* was carried out in *E. coli* and allowed confirmation of their identity using several substrates in which various monosaccharides are covalently joined to the p-nitrophenyl (Carmi *et al.*, 2003).

Fruit softening is an important quality characteristic for melon fruits; however, there are some cultivars that soften quickly, reducing the postharvest shelf life of the fruit. It has been found that softening in melon fruits is ethylene dependent (Nishiyama *et al.*, 2007). Because of that, attempts have been made to reduce the rate of softening in melon by reducing ethylene biosynthesis. Melon cultivar 'Galia' was transformed with the gene *CmACO1* encoding 1-aminocyclopropane-1-carboxylate oxidase (ACC) in antisense orientation. The transgenic melons showed firmness levels about twice those of the wild type and a delay in the softening advance (Nunez-Palenius *et al.*, 2007b). Besides the reduction in the ethylene biosynthesis, the researchers also found a reduction in the general advance of fruit ripening and an increase in the polyamine putrescine and abscisic acid in a transgenic melon cultivar 'Vedrantais' expressing *CmACO1* in antisense direction. The increase in polyamines was eliminated by ethylene treatments (Martinez-Madrid *et al.*, 2002).

Promoter analysis of the melon *CmACO1* gene in transgenic tobacco has revealed the presence of an ethylene responsive element (Bouquin *et al.*, 1997). Analysis of gene expression during ripening of transgenic melon fruit expressing *CmACO1* in antisense has identified genes responsive and genes not responsive to ethylene, and also genes regulated by other developmental factors suggesting that ethylene is not the only factor controlling melon fruit ripening (Hadfield *et al.*, 2000).

Two ethylene receptor genes were isolated and are similar to the *Arabidopsis* ethylene receptors ETR1 and ERS1; they were named *CmETR1* (GenBank AB052228) and *CmERS1* (GenBank AB049128). Expression analysis of both transcripts during fruit development was carried out and *CmERS1* was slightly active in the pericarp before the large expression of *CmETR1* in response to ethylene production, suggesting that the two receptors play a different role in two different stages of fruit development (Sato-Nara *et al.*, 1999).

The aroma in fruits is a very important quality characteristic and in melon some of the genes playing a role in aroma have been cloned and studied. Four genes encoding enzymes with activity of alcohol acyl transferase, important in aroma development, were cloned from melon. Although no transgenic plants were created, the four genes *CmAAT1* (GenBank AB075227), *CmAAT2* (GenBank AB104414), *CmAAT3* (GenBank AB109053) and *CmAAT4* (GenBank AY859054) were expressed in yeast and studied. The gene products with the exception of AAT2 did not show activity. Furthermore, AAT1 generated a range of short- and long-chain acyl esters although with a tendency to produce E-2-hexenyl acetate and hexyl hexanoate. Moreover, AAT3 notably produced benzyl acetate whereas AAT4 showed a strong tendency to generate cinnamoyl acetate (El-Sharkawy *et al.*, 2005). The products of carotenoids hydrolysis have been found to be part of

the melon fruit aroma. In this regard, the gene encoding a dioxygenase able to hydrolyze the *β*-carotene in the position 9,10 producing *β*-ionone was cloned, expressed in *E. coli* and analyzed. The recombinant dioxygenase produced geranylacetone from phytoene, alpha-ionone and pseudoionone from delta-carotene, pseudoionone from lycopene and beta-ionone from beta-carotene. The gene was expressed during fruit development (Ibdah *et al.*, 2006).

The aroma of melon fruit also includes products derived from the catabolism of amino acids and transamination reactions. Two clones *CmARAT* and *CmBCAT1* were identified in the melon EST database (http://www.icugi.org/) and the encoded proteins were produced in *E. coli*. Analysis of the enzymatic activity of the recombinant proteins showed that they were able to convert L-isoleucine, L-leucine, L-valine, L-methionine or L-phenylalanine into their respective *α*-keto acids, using the alpha-ketoglutarate as the amine acceptor. Both genes were highly expressed in ripening fruit showing their role in melon fruit aroma development (Gonda *et al.*, 2010).

Besides the above mentioned, alcohol dehydrogenases also play a role in aroma development by synthesizing substrates to be used in the synthesis of ester molecules. Two cDNAs *CmADH1* (GenBank DQ288986) and *CmADH2* (GenBank DQ288987) encoding alcohol dehydrogenases from melon fruit var. 'Cantalupensis' were cloned, and expression analysis showed that both genes are up-regulated during melon fruit ripening. ADH1 and ADH2 were overexpressed in yeast and recombinant proteins were shown to have alcohol dehydrogenase activity (Manriquez *et al.*, 2006). In agreement with this, it has been demonstrated that ethylene plays an important role in the biosynthesis of some of the aroma volatiles in melon fruit (Flores *et al.*, 2002). In the future, the knowledge of the genes playing a role in melon aroma development will allow the improvement of this important quality characteristic. The dbEST has deposited 6921 ESTs from *C. melo* subsp. Agrestis and 5943 ESTs from *C. melo* muskmelon by release 052110.

9.20 Nuts

9.20.1 Brazil nut (*Bertholletia excelsa* HBK, Lecythidaceae)

In the case of the brazil nut, to our knowledge neither transgenic plants nor successful protocols for somatic embryogenesis have been reported until now. However, several efforts have been made to improve the nutritional quality of legume proteins by the introduction of cDNAs encoding rich methionine proteins of brazil nut. Indeed, there have been reports of the introduction of the cDNA *pBN2S1* encoding the 2S albumin protein in *Brassica napus* (Guerche *et al.*, 1990), canola (Altenbach *et al.*, 1992), the tropical forage legume *Stylosanthes guianensis* (Quecini *et al.*, 2006), in the bean *Phaseolus vulgaris* (Aragão *et al.*, 1992; Aragão *et al.*, 1996; Aragão *et al.*, 1999), potato (Tu *et al.*, 1998), in the grain legume *Vicia narbonensis* (Pickardt *et al.*, 1995; Saalbach *et al.*, 1995), and in *Nicotiana tabacum* (Saalbach *et al.*, 1994). Also, in an effort to increase the amount of methionine in legumes, a chimeric gene was designed by ligation of part of the sequence of the *Arabidopsis* 2S albumin gene 1 (*At2S1*) at different places into a brazil nut 2S

albumin cDNA clone sequence *pBN2S1*. The chimeric gene was introduced in *Arabidopsis*, tobacco (*Nicotiana tabacum*) and *Brassica napus* (De Clercq *et al.*, 1990). Although it was possible to increase the amount of the amino acid methionine in legumes and to increase the quality of the legume protein as a consequence, unfortunately, the 2S albumin protein was shown to have the potential to be allergenic (Teuber *et al.*, 1998). Therefore, this approach created a food with probable negative consequences for human health and that suggested the necessity to test the possible allergenic effects as one of the important side effects of the consumption of genetically modified foods (Lack, 2002).

9.20.2 Cashew apple and nut (*Anacardim occidentale* L., syn. *Anacardium curatellifolium* A. st. Hil)

In the case of cashew apple and nut there are no available protocols to create transgenic plants; however, organogenesis and embryogenesis protocols have been developed in this specie. In the case of organogenesis, shoot induction was carried out using as explants, axillary bud sections of plants with an age between six months and one year. It was found that the combination of fructose and maltose was the best treatment recording the largest percentage of shoots with the greatest shoot length (Gemas and Bessa, 2006). Shoot induction was also carried out from cotyledons explants excised from *Anacardim occidentale* L. mature embryos (Ananthakrishnan *et al.*, 2002). On the other hand, somatic embryogenesis has been successfully carried out from callus induced from nucellar tissue (Ananthakrishnan *et al.*, 1999; Gogte and Nadgauda, 2000; Cardoza and D'Souza, 2002). Furthermore, this protocol has been tested successfully in several cultivars, including 'Goa 11/6', 'Kanaka' and 'Ulla1-3' (Anil and Thimmappaiah, 2005). Besides nucellar tissue, somatic embryogenesis has also been induced from seed coat explants (Martin, 2003) and immature zygotic embryos (Gogate and Nadgauda, 2003). From the above, it seems that the stage is set for the first attempt to introduce specific genes in this specie to improve the postharvest fruit quality.

9.20.3 Macadamia (*Macadamia integrifolia*, *macadamia tetraphylla*)

In macadamia there are no reports of the creation of transgenic plants or somatic embryogenesis. However, protocols for organogenesis have been developed in both species. In the case of *M. tetraphylla*, the induction of shoot regeneration from immature cotyledon explants has been reported (Mulwa and Bhalla, 2006), as well as from node segments excised from plants two years old and from four month old seedlings (Mulwa and Bhalla, 2000). However, the induction of roots from macadamia shoots has been difficult and a protocol to obtain a whole plant is not yet available (Gitonga *et al.*, 2008). Macadamia contains an unusually large amount of monounsaturated fatty acids of relatively low molecular weight (Kaijser *et al.*, 2000) and the consumption of this nut has been found to have positive effects on the plasma lipid profile in persons with high levels of cholesterol in the blood (Garg *et al.*, 2003) and to reduce the risk of coronary artery disease (Garg

et al., 2007) which is an important quality characteristic of this fruit. Because of this, a study was undertaken to isolate the cDNAs encoding the acyl–acyl carrier protein desaturase playing a putative role in the biosynthesis of monounsaturated fatty acids (Gummeson *et al.*, 2000). Several cDNAs were isolated from developing nut seeds and one of them was expressed in *E. coli*. Unfortunately, analysis of the desaturase showed a preferential activity for fatty acids with 18 carbons and not 16 carbons, suggesting that this enzyme is not the one responsible for the large amount of monounsaturated fatty acids with 16 carbons characteristics of macadamia oil. It will be interesting to isolate and study the desaturases playing a role in the biosynthesis of the low molecular monounsaturated fatty acids in macadamia to use in the quality improvement of other tropical fruits.

A low molecular weight antimicrobial peptide AMP1, encoded in *MiAMP1*, was originally isolated from the kernel of *M. integrifolia* and was also found to be present in other plant species (Manners, 2009). *MiAMP1* was cloned and expressed in *E. coli* (Harrison *et al.*, 1999) and the recombinant AMP1 inhibited 14 phytopathogenic fungi such as *A. helianthi*, *B. cinerea*, *C. paradoxa*, *C. falcatum*, *C. gloeosporioides*, *F. oxysporum*, *L. maculans*, *M. phaseolina*, *P. cryptogea*, *P. graminicola*, *S. sclerotiorum*, *S. rolfsii*, *V. dahliae* and *A. fumigatus in vitro*. AMP1 also inhibited the growth of phytopathogenic bacteria *C. michiganensis* and *P. rubrilineans* (Marcus *et al.*, 1997). Because of this, the potential utilization of AMP1 to increase the resistance of many transgenic crops has already been proposed (Kazan *et al.*, 2002a). The construct including the *MiAMP1* cDNA under the control of the CaMV35S was introduced in tobacco and canola (*Brassica napus* L.). The AMP1 protein obtained from the leaves of the transgenic plants was able to inhibit several phytopathogenic fungi *in vitro*. Furthermore, cotyledons from seven independently transformed canola plants inoculated with *Leptosphaeria maculans*, the causal agent of blackleg disease, showed a lower lesion development as compared with the isogenic lines and an azygous plant, from the segregating progeny of a transgenic canola plant (Kazan, *et al.*, 2002b). Clearly, the antimicrobial macadamia peptide deserves further research to explore the possibility of increasing the biotic stress resistance of many highly susceptible tropical fruits.

9.20.4 Pistachio (*Pistacia vera* L.)

In pistachio the improvement of the existing cultivars has been very difficult because of the large degree of heterozygosity and low amount of regenerated good quality plant material (Onay, 2005), as well as the genetic erosion of the *Pistacia* germplasm (Ozden-Tokatli *et al.*, 2010). In this context, plant biotechnology has to be utilized and in fact it seems to be the only option. Although there are no protocols to create pistachio transgenic plants, successful organogenesis and somatic embryogenesis have been reported for this specie. Indeed, somatic embryogenic has been carried out from kernels derived from immature fruit (Onay *et al.*, 1995) and a protocol to store these embryos for a short time using calcium alginate gel has been reported (Onay *et al.*, 1996). In this work, it was found that embryos could be recovered after 60 days of storage at 4°C. Furthermore, the same protocol

has been tested successfully in several pistachio cultivars already (Onay *et al.*, 2007a). Besides that, somatic embryogenesis has been also successfully done with callus tissue derived from mature zygotic embryos of pistachio (Onay *et al.*, 2007b) and from female flower buds with a size between 5 and 8 mm harvested two weeks before pollination (Onay *et al.*, 2004). Besides somatic embryogenesis, an efficient protocol for *Pistachio* organogenesis using *in vitro* regenerated leaves explants developed from mature shoot tips obtained from a 30 year old tree was also reported (Tilkat and Onay, 2009). As can be seen, the first step toward the utilization of tools derived from the DNA recombinant technology has been taken in this specie. Therefore, we will see in the near future efforts to create transgenic plants harboring specific genes for quality improvement in this specie; up to now there are 1299 ESTs deposited at the dbEST release 052110.

9.21 Olive (*Olea europaea* L.)

In olive protocols for organogenesis, somatic embryogenesis and creation of transgenic plants have already been developed. In fact, there are several published works reviewing these biotechnological tools (Rugini *et al.*, 2005; Rugini and Pesce, 2006; Fabbri *et al.*, 2009). Olive organogenesis has been carried out from petioles (Mencuccini and Rugini, 1993), from shoot apical bud excised of an olive tree growing in the field (Zacchini and De Agazio, 2004), from nodes located at different places in olive plants growing in the greenhouse (Otero and Docampo, 1998) or in the field (Cozza *et al.*, 1997). On the other hand, somatic embryogenesis has been carried out from several explants, such as immature zygotic embryos at different stages of development (Rugini, 1988), petiole tissue or callus induced from petiole tissue (Rugini and Caricato, 1995), callus tissue induced from cotyledon fragments (Trabelsi *et al.*, 2003; Brhadda *et al.*, 2008) and leaf tissue explants (Lopes *et al.*, 2009). It is important to mention that in olive, secondary embryogenesis is not yet completely developed to obtain healthy plants (Rugini *et al.*, 2005).

The first attempts to create transgenic plants in olive were made in 1984 using *A. tumefaciens* mediated transformation. There is a reported attempt to introduce the genes *rolA, rolB* and *rolC* of *A. rhizogenes* with the aim of increasing the capacity of the explants to produce adventitious roots. Although most of the roots produced were not transformed, an increase in root number was observed (Fabbri *et al.,* 2009). Utilization of microprojectile bombardment in somatic embryos was able to get transient expression of the *β*-glucuronidase gene under the control of the CaMV35S promoter (Lambardi *et al.,* 1999). An improvement in the microprojectile bombardment protocol was made by the utilization of a construct in which the *β*-glucuronidase gene was under the control of the ubiquitin promoter from sunflower. In this case, a much higher frequency of *gus* expression sites per bombarded tissue was recorded (Perez-Barranco *et al.*, 2009). On the other hand, *Agrobacterium* infection of olive somatic tissues was able to introduce in the olive genome the genes *rolA, rolB* and *rolC* from *Agrobacterium rhizogenes* as shown by Southern blot (Mencuccini *et al.*, 1999). Later, a protocol for developing transgenic plants using

Agrobacterium infection of somatic embryos was able to develop transgenic olive plants expressing the *β*-glucuronidase gene (Torreblanca *et al.*, 2009).

A transgenic olive plant was created expressing the osmotin gene from tobacco (*Nicotiana tabacum*) under the control of the CaMV35S promoter. Although it was not the objective of the experiment, it is quite possible that overexpression of osmotin would increase the resistance of the fruit to attack by fungi (D'Angeli and Altamura, 2007). Although analysis was not made in fruit, an increased resistance of the transgenic olive plant to the olive leaf spot caused by *Phytophthora crown* was observed (Fabbri *et al.*, 2009). Moreover, in order to facilitate the harvest of olive fruit, it has been suggested that it may be possible to create a transgenic olive plant expressing a construct in which the ACC synthase gene is under the control of the promoter from chalcone synthase, anthocyanidin synthase and expansin genes from olive. This is because these genes showed the highest activity during the latest stage of olive fruit development (Ferrante *et al.*, 2004). However, still more experimental work is needed in this direction.

Olive oil is an important quality characteristic of olive fruit and its consumption has been shown to bring positive benefits to human health (La Lastra *et al.*, 2001). Several genes related to the biosynthesis of the different components of the olive oil have been cloned and studied. Two cDNAs, *OeFAD2* (GenBank AY733076) and *OeFAD6* (GenBank AY733075), that encode omega-6 fatty acid desaturases, responsible for synthesizing linoleic from oleic acid were isolated. Analysis of gene expression showed that *OeFAD2* presents the maximum expression in mesocarp of the olive fruit, suggesting an important role in lipid storage (Banilas *et al.*, 2005). Further research in those genes showed that most likely FAD2 is the enzyme responsible for the linoleic acid content in olive fruit mesocarp tissue (Hernandez *et al.*, 2009). In a related work, two oleate desaturases encoded in *OeFAD2-1* and *OepFAD2-2* (GenBank AY733076) were isolated and studied. Analysis of their expression showed that *OeFAD2-2* was present during the development of seeds and during the ripening of the mesocarp, suggesting an important role for the encoded enzyme in the storage of oil in the mesocarp of the mature fruit (Hernandez *et al.*, 2005). Desaturases need the presence of an electron donor to carry out their activity. In this context, two cDNAs, *OeCYTB5-1* (GenBank AJ001369) and *OeCYTB5-2* (GenBank AJ001370), encoding cytochromes b5 were cloned and studied. These cytochromes seem to be involved, among other things, in providing redox potential to desaturases enzymes (Martsinkovskaya *et al.*, 1999).

The aroma of olive oil is an important quality characteristic and it is known that lipoxygenase is an enzyme involved in aroma development. Two cDNAs, *OeLOX2-1* (GenBank EU513352) and *OepLOX2-2* (GenBank EU513353), encoding lipoxygenases were cloned and overexpressed (Padilla *et al.*, 2009). The recombinant enzymes LOX2-1 and LOX2-2 were able to synthesize 13-hydroperoxides from linoleic and linolenic acids. Furthermore, both enzymes were expressed in mesocarp and seed during development and ripening of olive fruit. Altogether, biochemical and gene expression data suggested that both enzymes are involved in the development of the characteristic aroma of olive oil. In a related work, a cDNA *OeLOX1* (GenBank EU678670) encoding a

lipoxygenase able to synthesize 9 and 13-hydroperoxides from linoleic acid was isolated and studied. Gene *OeLOX1* was expressed mainly during the late stages of olive fruit development. Although it was suggested that this enzyme plays a role in ripening and senescence of olive fruit, its putative role in aroma development deserves further research (Palmieri-Thiers *et al.*, 2009).

Olive transcriptomes have been reported in the past years in an effort to gain insights into the genetic and molecular aspects controlling fruit development and ripening. A recent study reported the construction of suppression substractive hybridization libraries from three developmental stages of olive fruit: initial fruit set (30 days after flowering, DAF), complete pir hardening (90 DAF) and veraison (130 DAF) (Galla *et al.*, 2009). The objective was to identify differentially expressed genes during the ontogeny of olive cv. 'Leccino', a widespread Italian variety showing a short fruit developmental cycle and a high degree of synchronization of processes defining ripening. A total of 1132 differentially expressed genes were identified and classified according to cellular compartment, molecular function and biological process. The most represented genes were for nucleotide binding proteins, followed by transport proteins, kinases and other enzymatic activities. The most represented genes according to cellular compartments were from plastids and mitochondria, followed by cytosol, plasma membrane, endoplasmic reticulum and nucleoplasm. With regard to biological processes, carbohydrate metabolism, response to biotic and environmental stresses, generation of precursors, metabolites and energy were the most represented genes. The less represented were genes related to the secondary metabolites, metabolism of lipids, synthesis of amino acids and derivatives, metabolites and their precursors, and protein modification process.

The deposited sequences contributed to the public olive EST repertory. From the 1132 ESTs, 642 are unique sequences. Among these, 89 (14%) corresponded to 61 different key genes, and their expression was investigated. Quantitative real-time PCR experiments were done to evaluate the expression patterns of a subset of 61 different genes from the libraries (Galla *et al.*, 2009). Genes involved in carbohydrate metabolism were modulated in their expression, genes related to starch metabolism were coherent with a temporary role of starch as a storage compound during early fruit development. Sequences of enzymes related to pentose pathway, glycolysis and gluconeogenesis as well as starch and sucrose metabolism were up-regulated. Genes involved in fatty acid biosynthesis are up-regulated throughout development; however, specific enzymes accumulate at a different extent depending on the developmental stage. Genes related to the phenylpropanoid and alkaloid biosynthesis and caffeine, limonene and pinene metabolism appeared to be differentially expressed throughout fruit development. Four enzymes controlling flavone and flavonol as well as anthocyanin biosynthesis were up-regulated from 90 to 130 DAF, therefore suggesting an increased accumulation of the related metabolites during late development. Genes for chalcone synthase, flavanone 3-hydroxylase, dihydroflavonol reductase and anthocyanidin synthase were up-regulated at veraison stage (Galla *et al.*, 2009).

The first large scale information about structural and putative function of gene transcripts during olive development was also reported in 2009 (Alagna *et al.*,

2009). The objective of that work was to identify EST involved in phenolic and lipid metabolism during fruit development. The technique 454 pyrosequencing was used to sequence four cDNA libraries from olive (*Olea europea*) cv. 'Coratina' (a high phenolic content) and 'Tendellone' (a natural variant lacking oleurepein), and obtained a set of 261 485 ESTs to identify genes involved in expression of fruit quality traits, fruit metabolism and phenolic content during ripening. A comparative EST analysis was performed and provided more than 102 000 unigenes, after clustering consisting in 26 576 consensus sequences from clusters (TCs) or contigs and 75 570 singletons from the four libraries (Alagna *et al.*, 2009). The authors discussed a possible overestimate of the unigene set of *Olea* since short reads are obtained by the 454 pyrosequencing technology. The short reads result in unassembled segments of TCs and EST pertaining to the same transcript unit.

Expression differences between the fruit developmental stages were found. For example, TCs predicted to be related to photosynthesis (photoreception, Calvin cycle and oxidative phosporylation) were more represented at 45 DAF. On the other hand, transcripts associated with carbohydrate metabolism (glycolisis/gluconeogenesis, citrate cycle, fructose, mannose and galactose metabolism) were more represented at 135 DAF (Alagna *et al.*, 2009).

The EST database was also compared between the C and T genotypes. There were TCs specific for cv. 'Coratina' and some specific for cv. 'Tendellone'. These TCs encode proteins involved in hormone catabolism and regulation, abiotic and biotic stress, cell wall metabolism, lipid metabolism, steroid metabolism and phenylpropanoid metabolism. Several transcripts involved in the biosynthesis of steroids with nutritional and health benefits were reported exclusively in 'Coratina'. Two TCs specific to 'Coratina', encode R-limonene synthase 1 and 1,8-cineole synthase, which are related to the biosynthesis of important flavor compounds such as (+)-R-limonene, a monocyclic monoterpene and 1,8-cineole or eucalyptol, a monoterpenic oxide present in many essential oils (Alagna *et al.*, 2009).

9.22 Papaya (*Carica papaya* L.)

Transgenic papaya plants were first produced by microprojectile bombardment transformation of three different tissues: immature zygotic embryos, hypocotyl sections immediately after being dissected and mature somatic embryos. The introduced genes were *nptII* (conferring resistance to kanamycin) and *uidA* (encoding β-glucuronidase) (Fitch *et al.*, 1990). It seems that the event in 1992, when the papaya ringspot virus nearly destroyed all of the papaya fields in the Puna district of Hawaii (Ferreira *et al.*, 2002) and the lack of a natural resistance trait in the commercial papaya cultivars, induced an intensive research to develop efficient techniques to create transgenic plants. Also, efficient protocols for papaya somatic embryogenesis from immature zygotic embryos were already available (Fitch and Manshardt, 1990). Indeed, in 1992 the creation was reported of transgenic papaya plants by microprojectile bombardment expressing the papaya ringspot virus coat protein as well as the *nptII* and *uidA* genes. It was reported that

at least one transgenic line created was completely resistant to virus infection (Fitch *et al.*, 1992). After that, the successful creation of transgenic plants expressing the same construct just mentioned by *Agrobacterium* mediated transformation of somatic embryos was reported (Fitch *et al.*, 1993). Innovation of the *Agrobacterium* protocol was made by using as explants petioles from shoots micropropagated *in vitro* (Yang *et al.*, 1996), and wounding of somatic embryos with carborundum prior to *Agrobacterium* infection (Cheng *et al.*, 1996). On the other hand, several innovations in the microprojectile bombardment protocol were made by the utilization of a thin layer of somatic tissue developed from immature somatic embryos (Cai *et al.*, 1999). Utilization of the gene *PMI* encoding the enzyme phosphomannose isomerase (Zhu *et al.*, 2005) and the green fluorescent protein (Zhu *et al.*, 2004a) instead of antibiotics to select for transgenic tissues was a good effort to avoid the concerns related to the presence of antibiotic resistance genes in the genome of transgenic plants (Kuiper *et al.*, 2001).

The strategy of introducing the gene encoding a coat protein of the ring spot virus mentioned above could induce complete resistance to papaya according to evaluations carried out in Hawaii (Tripathi *et al.*, 2008), Jamaica (Tennant *et al.*, 2002; Tennant *et al.*, 2005), Taiwan (Bau *et al.*, 2003), Australia (Lines *et al.*, 2002) and Venezuela (Fermin *et al.*, 2004). Analysis of the molecular basis of the resistance has found that virus suppression takes place by post-transcriptional gene silencing and mRNA degradation (Lines *et al.*, 2002), although the virus is able to overcome the defense mechanism after a few generations (Ruanjan *et al.*, 2007). The phenomenon of post-transcriptional gene silencing requires homology between the transgene RNA and viral RNA (Baulcombe, 1996). Furthermore, it has been found that the differences between the sequence of the transgene being expressed by the papaya fruit and the sequence of the infecting virus can affect the level of resistance of the transgenic papaya fruit (Souza and Gonsalves, 2005). As a consequence of this, the creation of transgenic papaya expressing local varieties of ring spot virus showed higher levels of resistance as compared to the utilization of genes expressing the coat protein from virus isolated in other parts of the world (Fermin *et al.*, 2004).

Besides the utilization of the gene encoding the coat protein of the virus, another approach was made by introducing a 3 'truncated and 5' fragment of the gene *RP* encoding the enzyme replicase from the papaya ringspot virus under the control of the CaMV35S. A good level of resistance was found in several lines of transgenic papaya plants (Chen *et al.*, 2001).

The ripening phenomena of fruits initiated by ethylene affects the postharvest shelf life in optimal conditions. In papaya, the cDNAs for key enzymes of the biosynthetic pathway of ethylene, ACC synthase (Hidalgo *et al.*, 2005) and ACC oxidase were cloned (Chen *et al.*, 2003). The nucleotide sequence of one of these cDNAs was used to design transgenic papaya fruits that produce reduced levels of ethylene. A chimeric construct containing a truncated piece of the gene encoding the ACC oxidase enzyme under the control of the CaMV35S promoter was introduced into papaya by particle bombardment. Analysis of the transgenic fruits revealed a large reduction in ethylene production and respiration rate, and a delay in the ripening rate of papaya fruits was

also observed. It seems that the utilization of this approach has a good potential to increase the postharvest shelf life of papaya fruit (Lopez-Gomez *et al.*, 2009).

Papaya is a tropical fruit and therefore it is susceptible to chilling injury which causes postharvest papaya losses (Chen and Paull, 1986). To increase the resistance of papaya fruit to low temperatures, two genes (*CBF1* and *CBF3*) encoding one of the C-repeat binding factors, known to induce genes for cold acclimatizing, were introduced into papaya. Transgenic plants were shown to have the transgene by Southern blot. Although the response of the fruit to low temperatures was not tested, it was a good approach to reduce the postharvest papaya losses by low temperature stress (Dhekney *et al.*, 2007).

Attack by fungi is one of the most important biotic stresses of postharvest fruit losses in the world. In the case of papaya, *Phytophthora palmivora* can infect both plant and fruit tissues. In order to increase the resistance of papaya to *P. palmivora* attack, a construct containing the gene *DmAMP1* (GenBank A26963) encoding a defensin from *Dahlia merckii* was introduced into the papaya genome. A leaf extract of the transgenic papaya was able to inhibit the growth of *P. palmivora in vitro*. Also, plants infected by *P. palmivora* were better able to suppress the hyphae growth and to reduce the infection advance (Zhu *et al.*, 2007). Another approach to increase the resistance of papaya to *P. palmivora* was to introduce into the papaya genome a construct designed to express the cDNA *VvVST1* (GenBank XM002263926) encoding the enzyme stilbene synthase, which catalyzes the synthesis of the phytoalexin resveratrol that has been found to inhibit the fungi growth *in vitro*. The expression of the transgene was found in response to the fungi infection as expected. Also, the resistance of the fruit was not tested; however, an increased resistance to the fungi infection in several transgenic lines was reported (Zhou *et al.*, 2004b).

Color is an important quality characteristic in fruits. In papaya, there are some cultivars with red and yellow pulp. Two genes encoding the lycopene β-cyclase enzymes that catalyze the transformation of lycopene into β-carotene from the red cultivar 'Tainung' and the yellow cultivar 'Hybrid 1B' were cloned and designated *lcy-beta1* and *lcy-beta2*. Analysis of the nucleotide sequences of *lcy-beta1* and *lcy-beta2* revealed a mutation consisting of a TT insertion at position 881 that induces a premature stop codon in the *lcy-beta2* gene. Therefore, there is no conversion of lycopene into β-carotene, and the color of the papaya flesh is red. The analysis of gene *lcy-beta2* in several cultivars with red flesh also showed the presence of the same mutation (Devitt *et al.*, 2010). Due to the fact that there is one gene related to the phenotype of flesh color in papaya fruit, it will be relatively easy to design a strategy to create transgenic papaya fruit with a specific color.

The potential utilization of papaya to develop fruit based vaccine was shown by the creation of a transgenic papaya expressing synthetic epitopes designed to induce an immunogenic response against the porcine *Taenia solium* cysticercosis. The soluble extracts of clones were shown to induce immunity against cysticercosis in 90% of the fed mice tested (Hernández *et al.*, 2007). This is a good approach to improve the quality of the fruit by having a positive effect on the consumer.

The first commercial virus-resistant transgenic fruit tree to be sequenced is the 'SunUp' papaya. With a size of 372 Mb, it is three times the size of the *Arabidopsis*

genome; however, it contains fewer genes including significantly fewer disease-resistance gene analogues. This genome sequence gives an opportunity to reveal the basis of Carica's distinguishing morpho-physiological, medicinal and nutritional properties (Ming *et al.*, 2008).

A study of papaya ESTs associated with fruit ripening was reported in 2006 (Devitt *et al.*, 2006). A total of 1171 ESTs were generated from two cDNA libraries made from yellow- and red-flesh fruit varieties. The ESTs produced 117 contigs and 710 singletons. The EST sequences encode genes involved in fruit softening such as cell wall hydrolases, pectinmethyl esterase, beta-1, 3-glucanase, polygalacturonase-like protein, beta-glucosidase, polygalacturonase and beta-galactosidase precursor that had not been reported from papaya (Devitt *et al.*, 2006). Softening in papaya is a major issue because it is very rapid and affects shelf life, and therefore the availability of sequences of enzymes related to this parameter will be useful for future work aiming to maintain firm fruits. Sequences showing similarity to enzymes associated with fruit volatile biosynthesis were uncovered also. Between them the genes of the isoprenoid biosynthesis, shikimic acid pathway such as the cinnamate-4-hydrolase (C4H) were identified. Fatty acid derived volatiles are contributors to aroma and enzymes related to acyl lipid catabolism pathway. Two ESTs related to putative enzymes of peroxisomal fatty acid beta-oxidation were identified and encode the final two steps of fatty acid degradation to acetyl-coenzyme A, AIM1 and 3-ketoacyl-CoA thiolase (Devitt *et al.*, 2006).

9.23 Passion fruit (*Passiflora edulis* Sim)

There are no protocols available for the creation of transgenic passion fruit plants. Although no protocols for somatic embryogenesis have been reported, protocols for organogenesis have been published (Vaz *et al.*, 1993; Pinto *et al.*, 2010). Also, there is a review of biotechnological aspects of several species belonging to the genus *Passiflora* (Vieira and Carneiro, 2005).

Analysis of the expression of three cDNAs, *PeETR1* (GenBank AB015496), *PeERS1* (GenBank AB015497) and *PeERS2* (GenBank AB070652) encoding ethylene receptors in passion fruit showed differential expression. Only *PeERS2* was induced by ethylene during fruit ripening. Treatment with norboradiene, a reversible inhibitor of ethylene action, induced a reduction in *PeERS2* mRNA levels in the aril of the fruit; however, ethylene treatment eliminates this effect, suggesting the importance of *PeERS2* in controlling fruit ripening (Mita *et al.*, 2002). In a related work (Mita *et al.*, 1998), the expression of two ACC synthase encoding genes, *PeACS1* (GenBank AB015494) and *PeACS2* (GenBank AB015495), one ACC oxidase encoding gene, *PeACO1* (GenBank AB015493) and two ethylene receptors *PeETR1* and *PeERS1* were studied. Higher levels of *PeACS1* mRNA and *PeACO1* mRNA were detected in arils compared to the levels accumulated in seeds and these results agree with the larger production of ethylene that has been observed in the plant. *PeETR1* and *PeERS1* mRNA levels did not change during the course of ripening. This data strongly suggests the importance of

the genes *PeACS1* and *PeACO1* to carry out the biosynthesis of ethylene during passion fruit ripening. In addition, these results clearly suggest the procedure to follow in order to create transgenic passion fruit with low levels of ethylene by inhibition of the enzymes playing a role in the ethylene biosynthetic pathway.

9.24 Pineapple (*Ananas comosus* L. Merr)

In pineapple, creation of plantlets by organogenesis has been available for quite some time (Mathews and Rangan, 1979). Also, protocols for pineapple embryogenesis have been published (Sripaoraya *et al.*, 2003; Firoozabady and Moy, 2004). Efficient protocols for the creation of pineapple transgenic plants by microprojectile bombardment (Sripaoraya *et al.*, 2001; Sripaoraya *et al.*, 2006) and *Agrobacterium* infection using different explants are also available (Firoozabady *et al.*, 2006; Wang *et al.*, 2009).

Pineapple fruit is also susceptible to fungi infection (Rohrbach and Pfeiffer, 1976); therefore, transgenic plants resistant to fungi are desirable. On the other hand, magainin peptides have been shown to have broad spectra of antimicrobial activity in fruits (Matsuzaki *et al.*, 1997). Transgenic pineapple plants were created by introducing the magainin analog, MSI99, in order to increase the resistance of pineapple fruit to fungi attack (Mhatre *et al.*, 2009). Although pineapple fruits from the transgenic plants were not analyzed, the possibility that these fruits will have higher resistance to fungi attack is not discarded.

In a related work (Yabor *et al.*, 2006), transgenic plants expressing the *Phaseolus vulgaris* chitinase class-I gene under the control of the chimeric promoter OCS-35S CaMV-rice actin I and the tobacco (*Nicotiana tabacum*) *AP24* gene, encoding a wide spectrum antifungal protein, were created to increase the resistance of pineapple to *Phytophthora nicotianae* var. parasitica attack. A construct in which the *bar* gene is under the control of the maize ubiquitin 1 promoter was also introduced. The *bar* gene encodes the protein phosphinotricin acetyltransferase conferring resistance to the herbicide phosphinotricin. In the transgenic plants analysis of the biochemical changes due to the transformation was carried out (Yabor *et al.*, 2006). Furthermore, biochemical effects of the herbicide treatment (Yabor *et al.*, 2008) as well as changes in phenotype and enzymatic activity in the herbicide treated transgenic plants growing in the field were analyzed (Yabor *et al.*, 2010). Although the resistance to the fungi attack during the postharvest shelf life of the transgenic pineapple fruit was not evaluated, the experimental approach clearly showed the current state of the art of the biotechnological tools to improve the resistance to biotic stress in pineapple fruit.

Blackheart is a physiological disorder that reduces the quality of pineapple fruit and increases the postharvest fruit losses (Smith and Glennie, 1987). This disorder is thought to be induced by low temperature storage (Nanayakkara *et al.*, 2005) and polyphenol oxidase activity (Stewart *et al.*, 2001; Zhou *et al.*, 2003a). Two genes *AcPPO1* (GenBank AY149880) and *AcPPO2* (GenBank AY149881) encoding polyphenol oxidase enzymes were isolated from pineapple (Zhou *et al.*, 2003b).

Analysis of the expression of a construct in which the promoter from *AcPPO1* was driving the expression of *β*-glucuronidase showed that this promoter is wound and cold inducible, in agreement with the conditions known to induce the blackheart physiological disorder. Furthermore, giberellin responsive elements were found in the promoter and indeed, the mutation of the elements induced a delay in the promoter response to the giberellic treatment. Treatment with giberellic acid also induces the blackheart disorder in the absence of low temperatures stress, further suggesting the role of polyphenol oxidase enzyme in blackheart disorder development. In a related work (Ko *et al.*, 2006), a transgenic pineapple was created in which *AcPPO1* was introduced in sense and antisense direction under the CaMV35S and maize ubiquitin-1 promoter. The integration of the transgene was probed by Southern blot and it is possible that the pineapple transgenic fruit will show a low incidence of the blackheart, even though the effects of the transgene in either *AcPPO1* mRNA levels or polyphenol oxidase activity were not analyzed.

The postharvest shelf life of fruits in optimal conditions is important during their exportation. The potential has already been shown of the alterations in the genes encoding the two key enzymes of ethylene biosynthesis: ACC synthase and ACC oxidase. Although pineapple is a non-climacteric fruit, the genes encoding the ACC synthase (*AcACS1*) and ACC oxidase (*AcACO1*, GenBank AY049052) were cloned. *AcACS1* mRNA levels increased 16-fold in ripe tissue, compared with green tissue, while low levels of *AcACO1* mRNA accumulated in ripening fruit tissue (Cazzonelli *et al.*, 1998). The data generated in this work suggest that manipulation of the ethylene biosynthetic genes can be a good strategy to control the pineapple ripening phenomena. To our knowledge, there is only one report about reduced levels of *AcACS*2 (GenBank CS326585) in pineapple by co-suppression (Trusov and Botella, 2006). A delay in the flowering time of the transgenic pineapple plants was reported, but no analysis of the effect on pineapple fruit ripening was reported. However, careful analysis of the pineapple fruit ripening phenomena suggested that there were no effects on pineapple fruit ripening due to the gene silencing (José Ramón Botella, personal communication).

A pineapple EST project was carried out during fruit ripening to develop a valuable molecular resource for pineapple technology (Moyle *et al.*, 2005). Differential expression was determined between green unripe tissue and yellow ripe fruit tissue. A total of 480 unripe green pineapple and 1536 ripe yellow pineapple clones were sequenced. After raw sequences were edited and sequences of less than 150 bp were discarded, 408 green unripe and 1140 yellow ripe pineapple EST sequences with an average length of 785 bp were deposited in the GenBank dbEST. All edited sequences were clustered into 634 contigs. A metallothionein clone was the most abundant transcript in the amplified fruit cDNA pools and its expression was assessed by QRT-PCR; the expression of the metallothionein increased during fruit development and was highest in mature fruit. A second metallothionein gene was also discovered in the yellow fruit library and it is 80% identical to the other metallothionenin gene discovered in both libraries. Northern analysis was performed to validate the expression patterns of the most abundant EST species isolated in fruit

tissue at different developmental stages. Both metallothionenin genes exhibited similar expression patterns across the range of pineapple fruits sampled and are similarly up-regulated in older leaves. Metallothionenins are small, cystein-rich proteins required for heavy metal tolerance in animals, fungi and plants. It is not yet clear why metallothioneins are up-regulated during fruit development, or how they function in plants (Moyle *et al.*, 2005).

Functional classification analysis of the rest of the clones was performed and ten per cent of unripe green and six per cent of ripe yellow clones encode proteins falling within cell rescue, defense and virulence major class. Unripe green and yellow pineapple fruits expressed a high proportion of oxidative stress-related proteins, supporting the view that ripening is an oxidative phenomenon requiring a balance between the production of ROS and their removal by antioxidant systems. Major differences between the genes expressed in unripe green and ripe yellow pineapple fruits and Northern analysis revealed that fruit bromelain and bromelain inhibitor gene found to be abundant in the green fruit library are down-regulated during fruit ripening. Cinnamyl alcohol dehydrogenase (*CAD*) clones were isolated from both green and yellow fruit libraries, although they were more abundant in the green than in the yellow fruit. It has been suggested that *CAD* up-regulation plays a role in the interconversion of aldehydes and alcohols implicated in flavor as well as the lignification of vascular elements. A MADS box transcription factor homologue was the most abundant clone type in the yellow fruit library, while in the green fruit library putative PHD transcription factor clones were isolated. Northern analysis of the *MADS* box gene in developing pineapple fruit confirmed its increase during ripening and its abundance in yellow fruit. Many candidate genes for future analysis are available to build microarrays for the large-scale examination of gene expression over a range of fruit development (Moyle *et al.*, 2005). The ESTs reported here are a valuable tool for future studies of ripening pineapple. Up to now there are 5659 ESTs deposited at the dbEST.

9.25 Sapodilla (*Manilkara zapota*)

In the case of the fruit sapodilla, no protocols for organogenesis, somatic embryogenesis or transgenic plants have been published. However, due to the fact that this fruit softens very quickly after harvesting (Qiuping *et al.*, 2006), the possible role of expansin genes in the softening has been studied. The cDNAs for *MzEXP1* (GenBank EU139436) and *MzEXP2* (GenBank EU251387) encoding expansins were cloned and their expression was studied in the cultivars 'Makok-Yai' and 'Kra-Suay' (Kunyamee *et al.*, 2008). The gene *MzEXP1* was expressed during the early stages of fruit development, whereas *MzEXP2* was present by the end of fruit development and a few days after harvesting in both cultivars. Treatment with ethylene reduced *MzEXP2* mRNA levels, whereas treatment with 1-MCP induced its expression. This data suggest that ethylene negatively regulates *MzEXP2* mRNA levels suggesting that both genes act at different stages of development inducing fruit softening.

Other work reported the cloning of cDNAs for *MzEG* (EU819555) encoding endo-beta-1,4-glucanase, *MzPL* (EU819554) encoding pectate lyase and *MzPG* (EU139437) encoding polygalacturonase from sapodilla (Kunyamee *et al.*, 2010). Expression levels of *MzEG* correlated with fruit growth and not with loss of fruit firmness after harvest. *MzPL* and *MzPG* accumulated during postharvest ripening. Levels of *MzPG* mRNA correlated with PG activity and decrease of fruit firmness; thus, PG has an important role in the rapid softening of sapodilla fruits.

9.26 Conclusions

Overall, the knowledge of the genetic and molecular basis of ripening, senescence, biosynthesis of bioactive compounds and the biochemical processes that occur in the fruit will result in benefits for the consumer and the producer. Consumer perceptions of modified fruits as (genetically modified organism) GMO may change upon the commercialization of fruits expressing vaccines or antigens. Those are the benefits that plant biotechnology must emphasize to interest groups. Also, the understanding of the recombination process in plants may lead to better and more precise transformation methods, that do not depend on illegitimate recombination, where it is possible that the plant has unknown effects, such as those claimed in transgenic soy with Bt toxin.

Other basic biology processes such as DNA repair in plants (Liu *et al.*, 2000) are less well known but are no less important, for example, if irradiation techniques are used to sterilize foods, or if UV-C is used to induce physiological responses. In all cases, investment in basic science will lead to advances that in the medium to long term, will benefit market and consumers.

9.27 References

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10

Fresh-cut tropical and subtropical fruit products

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Abstract: Fresh-cut (FC) produce is one of the major growing segments in food retail establishments. However, FC tropical and subtropical fruits are still under study because of the difficulties in preserving their fresh-like quality for longer periods. This chapter covers different aspects of the FC industry which includes biochemical, physiological, microbiological, nutritional and quality changes in FC processing and storage and distinct equipment design, packaging requirements, production economics, marketing considerations and new trends for FC products. Aspects concerning specific conditions suggested for FC tropical and subtropical fruits in each one of the processing steps such as washing, sanitation, cutting, and dipping treatments, use of edible coatings alone or in conjunction with antioxidants and antimicrobials, and/or preservation under modified atmospheres, are thoroughly discussed. The chapter covers the most frequent causes of quality loss of FC tropical and subtropical products such as browning and other discolourations, softening, surface dehydration, water loss, translucency, off-flavour and off-odour development, as well as microbial spoilage and the techniques used to prevent or reduce these problems. Special attention is devoted to the new development of edible coatings that can be used as a complement or alternative to modified atmosphere packaging (MAP) or as carriers of controlled release systems that contain natural antioxidants and antimicrobials.

Key words: fresh-cut produce, tropical fruit, subtropical fruit, quality, preservation, safety.

10.1 Fresh-cut produce industry worldwide

Fresh-cut (FC) fruit products are those that have been cleaned, peeled, sliced, cubed or otherwise prepared for convenient consumption, but which remain in a

living, respiring physiological condition (Brecht *et al.*, 2004). This is as opposed to minimally processed or lightly processed products, which are usually products that are retained in a 'fresh-like' (non-living) state by mild processing techniques that may include blanching, ultra-high-pressure treatment, or osmotic dehydration. The attractiveness and convenience of FC fruits and vegetables has led to increased sales and consumption of the available products, which in turn has led to attempts to develop and optimize the production of new FC products such as those made from subtropical and tropical fruits. Commercial FC vegetable products are more commonly produced and consumed than FC fruits due to seasonal and inconsistent supplies, shorter post cutting life, and higher cost of the latter. FC subtropical and tropical fruits make up a small (probably less than 10%) share of the total FC produce market.

Nevertheless, wherever subtropical and tropical fruit crops are grown throughout Asia, Africa, Pacifica and the Americas, vendors at roadside stands, produce markets, grocery stores and supermarkets prepare local fruits for immediate sale as FC fruit products such as green coconuts ready to drink, sliced pineapples, mango and papaya slices and chunks, jackfruit and durian arils, de-spined and peeled Opuntia cactus fruit, and citrus fruit segments. A few countries in subtropical and tropical fruit producing regions, such as Thailand, also have specialized commercial FC processing companies that sell FC fruit products domestically and internationally.

In the US and EU, subtropical and tropical fruits imported from the producing countries as well as some domestically produced subtropical fruits are processed into FC products by specialized FC companies as well as on site at supermarkets. It is not possible to estimate the amount of subtropical and tropical FC fruit products sold compared to temperate FC fruit products, but according to the United Fresh Research & Education Foundation and the Perishables Group (UFREF, 2009) annual retail sales of all FC and other value-added fruit products in the US were approximately $3.33 billion, or about 15% of total fresh fruit sales. The most important subtropical and tropical FC fruit products in the US and EU are pineapple, mango and kiwifruit slices and chunks, watermelon and muskmelon chunks, loose grape berries, pomegranate arils, and citrus fruit segments.

10.2 Handling and conditioning of raw materials for processing

Non-climacteric fruits, such as citrus fruits and pineapples, must be harvested when fully ripe to assure good flavour quality. Climacteric fruits can continue to ripen after harvest and may be picked mature-green or partially ripe. In such cases, these fruits should be ripened to the optimal ripeness stage for cutting and to assure consumer satisfaction with their flavour. Since FC products of climacteric fruits continue to ripen, it is possible to cut the fruit before full ripeness when there is enough time between cutting and sale to allow for completion of the ripening processes. Optimal ripening temperatures range between 15 and 25°C

(the higher the temperature within this range, the faster the ripening). Relative humidity should be kept between 85 and 95% to reduce water loss and associated fruit shrivelling. Addition of ethylene gas at 100 to 150 ppm for one to two days to the ripening room promotes faster and more uniform ripening. Carbon dioxide concentration within the ripening rooms should be kept below one per cent by introduction of fresh air since higher concentrations reduce the efficacy of ethylene. Application of 1-methylcyclopropene (1-MCP) can be used to delay ripening of partially ripe fruit (see section 10.5).

Heat treatments of intact fruit prior to cutting have been evaluated for their effects on post-cutting rates of browning and softening, but there is currently no commercial application except in the use of warm water for cleaning and sanitizing some intact fruit before processing. Fruit destined for FC processing should be cleaned with chlorinated (100–150 ppm) or otherwise disinfected water to remove contaminants and reduce microbial load before moving the fruit to the processing area.

10.3 Sanitation of whole and fresh-cut fruits

In order to achieve FC fruits with fresh-like quality, safety, and high nutritional value, the industry needs to implement improved strategies by introducing or combining sustainable techniques, especially standard procedures for sanitation. The key elements for the production of safe FC fruits include screening materials entering the processing chain, suppressing microbial growth, reducing the microbial load during processing, and preventing post-processing contamination (Artés and Allende, 2005).

Over the past two decades, the FC industry has grown tremendously. However, the growth of FC tropical and subtropical fruit sales, with the exception of pineapple and mango, is much lower than other fruits such as cantaloupes, watermelon and apples. Sanitary practices have also changed and are now more complex. Food processing and preparation depend on more mechanized and large-volume processes. The foods produced by these processors and retailers are eaten by millions of people each day. Therefore, it is more and more important for workers to understand sanitary food-handling principles and food hygiene. Workers who understand why food sanitation is so important are more likely to use safe practices. Therefore, proper personal hygiene and proper sanitation of equipment and whole produce are the most important keys in the handling chain of fruit processing, especially when tropical and subtropical fruits are transformed into FC products, because no mechanical equipment or atomizing systems have been developed yet.

The FC fruit industry needs appropriate sanitation procedures and new strategies for reducing microbial load of whole and FC produce to assure high quality and safe commodities. The removal of soil and microorganisms is the most important target for keeping overall quality of FC fruits. In order to avoid fruit contamination that affects the final quality of the product, every step in the

production chain must to be considered. The sanitation of the raw material and the FC fruit is the principal step that reduces microbial load throughout the production chain. This, in combination with proper postharvest handling and optimal sanitation techniques, enhances maintenance of fruit quality.

Among the different techniques used for fruit sanitation, chlorine treatment (applied as sodium or calcium hypochlorite) is the most widely used worldwide. However, chlorine presents a major disadvantage: the risk of the formation of undesirable by-products upon reaction with organic matter, which may lead to new regulatory restrictions in the future. Moreover, its efficacy has been demonstrated to be low for some products. Consequently the FC processing industry demands safer and natural alternatives. Artés-Calero *et al.* (2009) reported that antimicrobial washing solutions, O_3, UV-C radiation, intense light pulses, super high O_2, N_2O and noble gases, alone or in combination, are the most promising sanitization treatments. However, it is well known that the use of conventional or new sanitizers, especially those of natural origin, requires knowledge of the benefits, and the possible negative effects of the treatments on the quality attributes of the treated produce that limit consumer acceptance. González *et al.* (2009a) described the different emerging technologies such as ultraviolet irradiation (UV-C), edible coatings, active packaging and natural additives, to preserve the quality of FC fruits; highlighting the areas in which information is still lacking, and commenting on future trends.

10.3.1 Sanitation in the processing plant

An effective sanitation programme for FC fruit and vegetable processing facilities requires the same basic components needed in other food operations: appropriate cleaning compounds and sanitizers, effective cleaning procedures, and effective administration of the sanitation programme. The ultimate goal is to provide a finished safe product with a proper shelf life for marketing. The sanitation programme for fruit and vegetable processing facilities requires hygienic design of facilities and equipment, training of sanitation personnel, use of appropriate cleaning compounds and sanitizers, adoption of effective cleaning procedures, and effective administration of the sanitation programme – including evaluation of it through visual inspection and laboratory tests. Effective sanitation starts with reduced contamination of raw materials, water, air and supplies. If the facility and equipment are hygienically designed, cleaning is easier and risk of fruit contamination is reduced.

Effective preservation of fruits and vegetables depends on the prevention of contamination by spoilage-causing and pathogenic microorganisms during production, processing, storage and distribution. Raw materials are potential sources for food spoilage microorganisms and contribute to bacterial pools within a processing plant.

During harvest and handling, fruit are exposed to many unclean environments and can provide additional contamination in the receiving, raw material storage, and processing areas. The incoming materials may contain hazardous chemicals

such as pesticide residues, and water could be contaminated with heavy metals and chemical residues. A good sanitation programme must be established in order to minimize the risk of contamination. Furthermore, the intermediate products may become contaminated in the processing steps from cleaning compound residues due to improper rinsing or inappropriate handling of produce. Also, raw material and packages may be contaminated with hazardous extraneous material such as metal, plastic, glass fragments and wood slivers.

Different sanitizers are being used in the FC industry and new sanitizers are in the process of approval. Proper concentration and contact time differ depending upon the treated produce. Table 10.1 provides information about chemicals appropriate to use for sanitizing fresh produce and handling facilities.

10.3.2 Equipment sanitation

Equipment needs to be cleaned and sanitized after FC fruit processing. Small fruit pieces and juice remain in the different parts of the machinery (cutting, peeling,

Table 10.1 Chemicals used for sanitation in food handling facilities

Class	Other name/ Example	Optimum pH	Optimum use temperature	Advantage	Disadvantage
Chlorine	Sodium hypochlorite	Neutral to slightly acidic	Room temperature	Kills broad spectrum of microorganism	Corrosive if not used correctly, short shelf life, organic matter reduces activity
Iodine Based	Iodophor	Acidic	Room temperature (<35°C)	Stable, long, shelf life, less corrosive	Can stain, less effective against bacterial spores
Quaternary Ammonium Compounds	Quat	Alkaline	Room temperature	Stable, long, shelf life, non-corrosive	Leaves residues, high foaming
Peroxyacetic acid	Peracetic acid	Acidic	Chilled to room temperature	Kills broad spectrum of microorganism, biodegradable	Hazardous in concentrated form
Acidic-anionic		Acidic	Room temperature to hot	Less corrosive	Narrower spectrum of antimicrobial activity
Alcohols	70% Isopropyl	Neutral to slightly acidic	Room temperature	Fast acting	Not effective against spores, limited effectiveness against viruses, flammable

Fig. 10.1 Sanitation and disinfection of workers before entering the processing area and cleaning the different areas of the plant (courtesy of Leonali Puebla, Mexico).

washing tanks, packing and labelling). These are nutrients that are substrates of microorganisms and, if they are not removed or eliminated, create good conditions for proliferation of microbes. Sanitizing equipment and surfaces requires two steps: cleaning and sanitizing. Cleaning removes the juice and small fruit pieces, and sanitizing destroys microbes that are left on the clean surface. Surfaces must be thoroughly clean for sanitizers to work properly as shown in Fig. 10.1.

Food-processing and foodservice operations use various chemical sanitizers for different areas and types of equipment. Most chemical sanitizers are liquids, but some chlorine compounds and ozone are gases. It is important not to expose workers to a toxic chemical if a gaseous sanitizer is used. It is also very important to make sure that the chemicals are safe to mix together to avoid dangerous reactions and not contaminate the produce. The effectiveness of chemical sanitizers depends on: exposure time, temperature, concentration, cleanliness, water hardness, bacterial attachment.

10.4 Mechanical and manual processing of fruits

The most important purposes of fruit and vegetable processing are: (1) to offer consumers more convenient products while maintaining fruit freshness, without losing its nutritional value; and (2) to create FC products with a shelf life adequate to make distribution feasible. However, the perishable nature of tropical and subtropical fruits makes achieving these goals very difficult, and great efforts are needed in order to accomplish them.

Different attempts have been made in order to establish a mechanical and continuous process for producing FC subtropical and tropical fruits, but the intrinsic characteristics of these fruits make it difficult to develop mechanical equipment to process them. Pineapple is the most successful tropical fruit marketed as a FC product. The characteristics of pineapple facilitate the design of different equipment for peeling and cutting. Therefore, FC pineapple can be found in different presentations (halves, spears, cubes and slices), as one of the top five FC fruits on the market.

For subtropical and tropical fruits, hand-peeling with sharp knives is used. The sharper the knife used for peeling and cutting, the less the tissue damage to the fruit. On the other hand, removing the skin of some tropical fruits (e.g., jackfruit) is very difficult and the flesh can be damaged during peeling. Another important factor that affects mechanical processing is the imperfections of the fruit surface and geometry. In general, subtropical and tropical fruits are not spherical, making it more difficult to design the equipment for fruit peeling and cutting. Therefore, most subtropical and tropical fruits are processed manually and this factor affects the total cost of production, since more personnel are needed for the process (e.g., mangoes, Fig. 10.2).

Other important factors to be considered in order to produce FC fruits with high quality are:

1. The use of good quality raw material (proper cultivar/variety, good agricultural practices, and optimal storage conditions before peeling and cutting).
2. The use of strict hygiene and good manufacturing practices, HACCP (section 10.10).

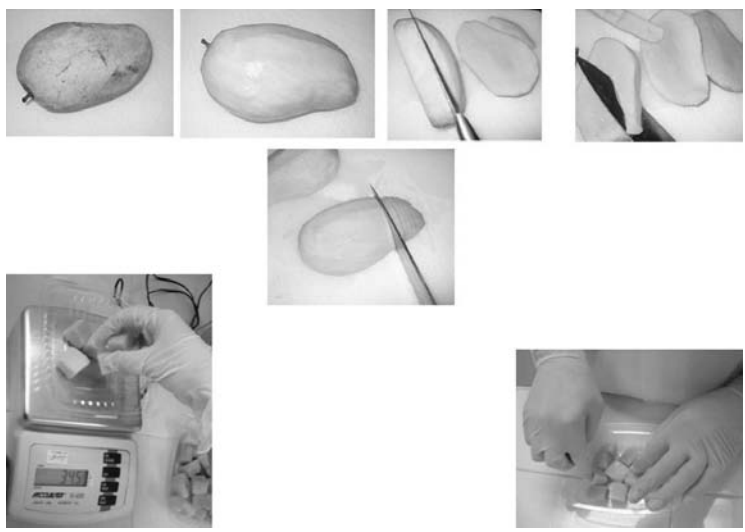

Fig. 10.2 Manual processing of FC mangoes (courtesy of Fresh-cut Lab CIAD, Mexico).

3. The use of low temperature (5–10°C) for processing the fruit.
4. The use of adequate methods for cleaning and/or washing before and after peeling and cutting.
5. The use of good quality water (sensory, microbiology, pH).
6. The proper use of sanitizers, and additives such as anti-browning and antimicrobial compounds.
7. Gentle spin drying after sanitation (if the product is not damaged by this).
8. Gentle peeling and cutting.
9. The use of correct and proper packaging materials and packaging methods (MAP, CA, low O_2).
10. The use of correct temperature and humidity during distribution and retailing.

Considering these factors, the quality demanded by consumers and the shelf life for marketing may be met. Other important factors for assessing the suitability of the cultivars to be processed as FC fruit are: (1) high processing yield; (2) low sensitivity to physiological disorders and microbial diseases; (3) high mechanical resistance of the tissue; (4) resistance to high CO_2 and/or low O_2 concentrations; (5) high sugar content (since sugar depletion may be responsible for energy stress); (6) low respiration rate (most tropical fruits have high respiration rates).

We have to work on the development of practical and efficient methods to preserve the quality of FC fruits. Some researchers in tropical countries have been working for a long time in conjunction with the food industry; and they have established some processing methods for tropical fruits. However, not many FC tropical fruits have reached international markets. Usually, tropical fruits are exported as intact fruit and then processed near the place of consumption. Further efforts have to be made in order to improve the actual processing practices used for tropical fruits.

Depending on the individual characteristics of the subtropical or tropical fruit, the FC presentation can vary significantly. Figure 10.3 shows different presentations used by processors for marketing FC subtropical and tropical fruits. The treatments applied to maintain the nutritional quality and prevent tissue browning, firmness and weight loss of FC products are discussed in subsequent sections (10.5, 10.6 and 10.7).

10.5 Physiological and biochemical aspects of fresh-cut produce

10.5.1 Effects of wounding on respiration and ethylene production rates

Figure 10.4 shows the effect of peeling and cutting on tissue damage. In general, wounding stimulates rates of respiration and ethylene production of subtropical and tropical fruits, including kiwifruit (Agar *et al.*, 1999), mango (Chantanawarangoon, 2000; Allong *et al.*, 2001; Gonzalez-Aguilar *et al.*, 2008),

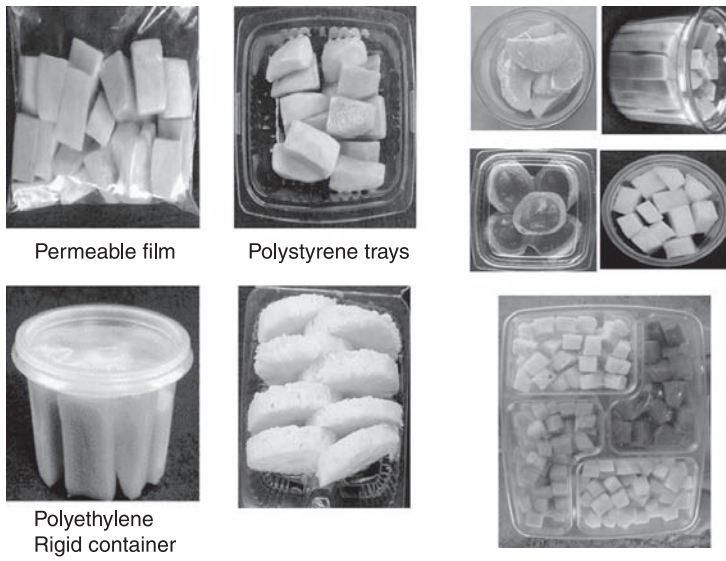

Fig. 10.3 Fresh cut presentation of subtropical and tropical fruits.

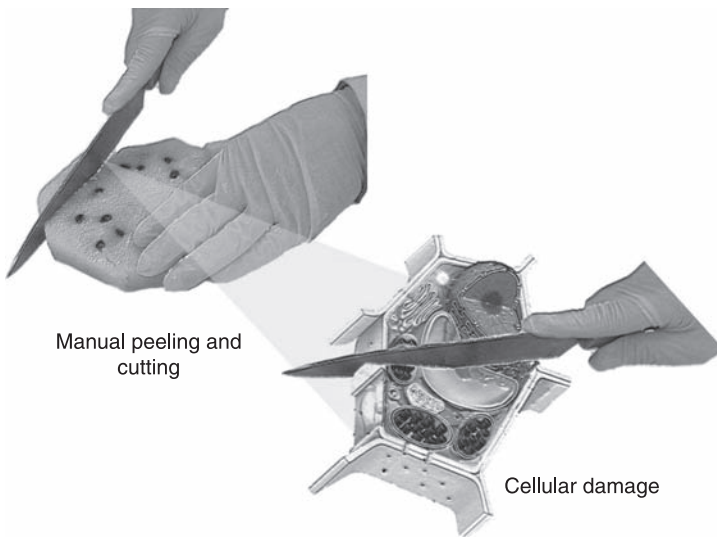

Fig. 10.4 Effect of cutting on tissue integrity (courtesy of Fresh-cut Lab CIAD, Mexico).

papaya (Paull and Chen, 1997), and pineapple (Budu *et al.*, 2001; Marrero and Kader, 2006).

Mango peels had the highest respiration and ethylene production rates, followed by whole mangoes and mango cubes, respectively (Chantanawarangoon, 2000).

Peeled whole mangoes had lower respiration and similar ethylene production rates compared to mango cubes. The C_2H_4 and CO_2 production rates of whole mangoes were about 1.5 to 2 times higher than peeled whole mangoes. The results indicate that mango peels are major contributors to C_2H_4 and CO_2 production by mango fruit. The CO_2 production rates of mango cubes was about 1.5 times higher than peeled whole mangoes, which indicated that cutting increased respiration rates of mangoes. However, the CO_2 and C_2H_4 production rates of whole mangoes were about 1.5 times higher than those of mango cubes. This means that the preparation steps of FC mango cubes, including peeling and cutting, resulted in the reduction of the CO_2 and C_2H_4 production rates. Therefore, wounding had a minor effect on physiology of FC mangoes, which is helpful in extending post-cutting life. Similar results have been shown in kiwifruit (Agar *et al.*, 1999).

After an initial increase due to wounding of pineapples, respiration rates one and two days after cutting at 10°C were almost twice as high in peel pieces as in pulp and core pieces (Marrero and Kader, 2001). Whole fruit showed an intermediate respiration rate between those of peel and pulp pieces. Five days after cutting, all pieces showed a much elevated CO_2 production rate and signs of microbial spoilage. Ethylene production was always higher in tissue pieces than in whole fruit, especially on day five after cutting. A significant increase in ethylene production by pieces was apparent two days after cutting, when respiration rates were still at their basal levels (Marrero and Kader, 2001).

10.5.2 Effects of 1-methylcyclopropene (1-MCP) treatments

Ethylene production is enhanced by wounding during processing, and the accumulation of this gas within the packages of FC fruits can be detrimental to their quality and shelf life. These effects can be reduced by exclusion and/or removal of ethylene from packages and treatment with 1-MCP to block ethylene action (Ergun *et al.*, 2006; Vilas-Boas and Kader, 2007; Toivonen, 2008).

Exposing partially ripe fruit to 1-MCP before cutting or after cutting may be a useful supplement to proper temperature and relative humidity management and chemical dips for maintaining quality of FC fruit products. Vilas-Boas and Kader (2007) evaluated the effect of 1-MCP on softening of kiwifruit, mango, and persimmon slices. Softening of FC kiwifruit slices was delayed and ethylene production decreased by 1-MCP whether it was applied before or after processing. 1-MCP applied directly on mango slices delayed their softening and darkening (decrease in L* value). Respiration rate of mango slices was not influenced by 1-MCP whereas the ethylene production rate was affected only during the latter part of their shelf life. Softening and darkening (decrease in L* value) were retarded whereas ethylene production was enhanced when FC persimmons were treated with 1-MCP before processing. 1-MCP did not affect the respiration rate of FC persimmons.

The most promising applications of 1-MCP seem to involve co-application with other treatments or appropriate atmospheres to achieve treatment response

synergies leading to consistent quality (sensory and microbial) and shelf life improvement (Toivonen, 2008).

10.5.3 Tissue browning

Polyphenol oxidase (PPO) enzymes catalyze the *o*-hydroxylation of monophenols (phenol molecules in which the benzene ring contains a single hydroxyl substituent) to *o*-diphenols (phenol molecules containing two hydroxyl substituents). They can also further catalyze the oxidation of *o*-diphenols to produce *o*-quinones. It is the rapid polymerization of *o*-quinones to produce black, brown or red pigments (polyphenols) that is the cause of fruit browning. The amino acid tyrosine contains a single phenolic ring that may be oxidized by the action of PPOs to form *o*-quinone. Hence, PPOs may also be referred to as tyrosinases.

PPOs exhibit either mono- or di-phenol oxidase activity, or both types of activities. Figure 10.5 shows the reactions involved in tissue browning. Peroxidase (POD) and phenylalanine ammonia lyase (PAL) are also found to be closely associated with the browning of FC fruits (He and Luo, 2007). Browning inhibition treatments include dipping in anti-browning solutions; modified atmosphere packaging (MAP); and heat shock and refrigerated storage (Beuchat, 1996; Artés *et al.*, 1998; Brecht *et al.*, 2004; Massantini and Mencarelli, 2007; Nicola *et al.*, 2009).

Enzymatic browning is not unique to FC fruits. PPO, a mixture of monophenol oxidase and catechol oxidase enzymes, is present in nearly all plant tissues, and

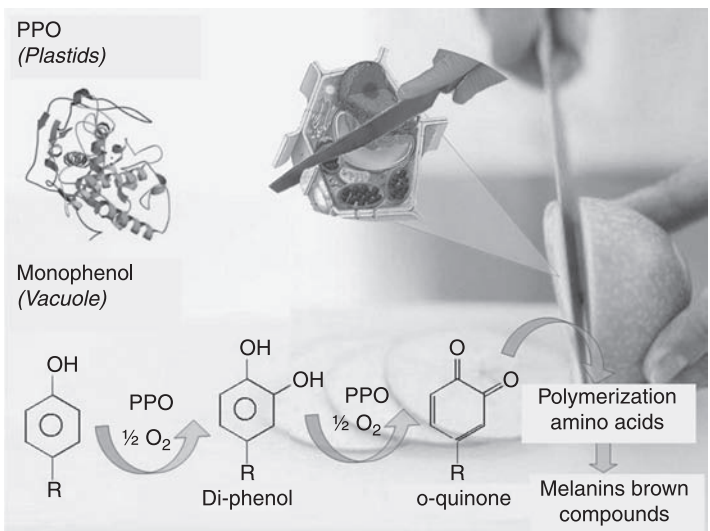

Fig. 10.5 Reactions catalyzed by PPO that deal with tissue browning (courtesy of Fresh-cut Lab CIAD, Mexico).

can also be found in bacteria, animals and fungi. In fact, browning by PPO is not always an undesirable reaction; the familiar brown colour of tea, coffee and cocoa is developed by PPO enzymatic browning during product processing.

10.6 Effects of peeling and cutting on overall quality: texture, flavour and colour

The quality of FC subtropical and tropical fruit products is dependent on the quality of the whole fruit prior to processing. The FC products must be sufficiently ripe to be 'ready-to-eat', while also possessing the potential to maintain satisfactory quality between cutting and sale, whether this period is a few hours at ambient outdoor temperatures or a week or more in refrigerated conditions of 0 to 5°C. Peeling and cutting can directly lead to many different changes in quality (Fig. 10.6). Peeling fruit removes the natural protection of the epidermis against loss of moisture. Water loss from FC fruit pieces may lead to undesirable tissue flaccidity and surface drying. The negative appearance related to cut surface drying of fruit tissues can be minimized by using the sharpest cutting instruments possible in order to minimize tissue damage at the cellular level. Clean cuts also reduce the development of cut edge softening, browning and tissue degradation as shown for FC mango (Dea *et al.*, 2010b).

Textural changes related to ripening and senescence of fruit tissues may be accelerated in FC tissues (Karakurt and Huber, 2003; 2007). Increased activities of both membrane and cell-wall hydrolases can contribute to textural changes in

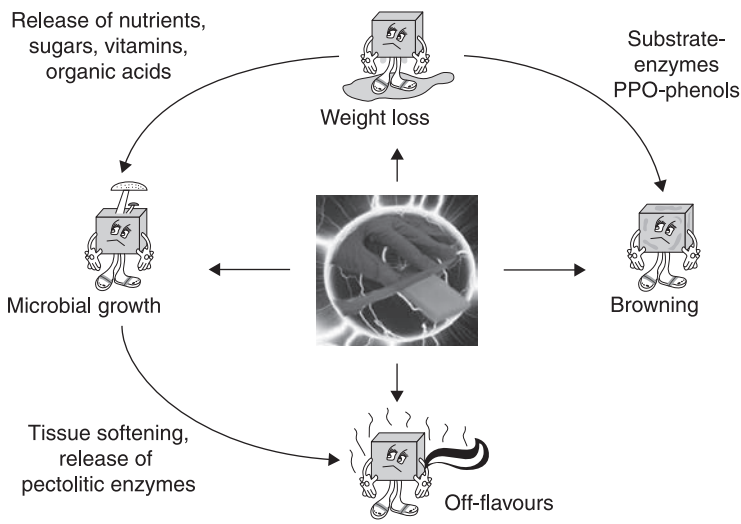

Fig. 10.6 Effect of peeling and cutting on overall quality of fresh-cut fruits (courtesy of Fresh-cut Lab CIAD, Mexico).

FC fruits. Membrane degradation due to wounding or to senescence causes leakage of juice from the cells into the intercellular spaces, resulting in water-soaked appearance and tissue flaccidity. Hydrolysis of cell wall polysaccharides weakens the cell wall structure and softens the tissue.

Accelerated wound-induced respiratory activity causes more rapid depletion of carbohydrates and organic acids used as respiratory substrates in FC products compared with whole fruit. These changes can cause loss of sweetness or loss of the sweet–tart flavour balance (SSC:TA ratio) of FC fruits such as mango and papaya.

Climacteric fruits that are immature when they are processed into FC products may maintain an acceptable appearance during marketing, but will likely not possess the sugar or aroma volatile content necessary for good flavour development. Likewise, unripe non-climacteric fruits that are processed into FC products may not complete the ripening process and develop good eating quality. While it is possible for unripe climacteric fruits to continue ripening after they have been processed into FC products, this will only happen if they are held at ripening conducive temperatures (15 – 25°C), which severely limit the potential postharvest life of FC products; ripening will not occur and desirable taste and aroma will not develop further if FC fruits are handled at 0–5°C in order to allow extended marketing.

It is well known that FC fruit flavour becomes bland during the course of extended handling even if the initial fruit ripeness was ideal (Beaulieu and Gorny, 2002). This is thought to be caused by premature synthesis (and loss) of volatile aroma compounds in response to wounding. The intensity of the wound response in terms of wound respiration and ethylene production has been shown to increase as fruit ripen (Brecht *et al.*, 2004). Beaulieu and Lea (2003) showed that FC 'Keitt' and 'Palmer' mango pieces prepared from firm-ripe fruit had longer appearance-based shelf life than pieces prepared from soft-ripe fruit (11 versus 7 days at 4°C), but the soft-ripe fruit pieces possessed better overall quality characteristics and volatile retention during their seven-day shelf life at 4°C based on subjective appraisals of colour, aroma, desiccation and edge or tissue damage. Dea *et al.* (2010b) found comparable behaviour for FC 'Kent' mango prepared from fruit of different ripeness stages.

For subtropical and tropical fruits handled at 0–5°C, flavour loss may be a consequence of chilling injury. Karakurt and Huber (2003) for papaya and Dea *et al.* (2010a) for mango found little evidence that the FC fruit are affected by chilling injury during exposure at 5°C. Dea *et al.* (2010a) noted that even though aroma intensity declined more at 5°C, the FC mango slices had longer shelf-life when stored at 5°C than at 12°C, because the negative appearance and aroma changes that occurred at the higher temperature were more objectionable than the relatively minor negative changes possibly caused by chilling injury that occurred at the lower temperature.

Removal of the epidermis also exposes the underlying tissues to atmospheric oxygen levels that may result in oxidative browning at the cut surface of many types of FC fruits (O'Connor-Shaw *et al.*, 1994; Matthews and Myers, 1995;

Weller *et al.*, 1997; Wright and Kader, 1997b; Dorantes-Alvarez *et al.*, 1998; Moline *et al.*, 1999; Gonzalez-Aguila *et al.*, 2000; Martinez-Ferrer *et al.*, 2002; Yoruk *et al.*, 2004; Chaisakdanugull *et al.*, 2007; Ding *et al.*, 2007). Cultivar differences in FC browning potential have been demonstrated for several fruit species such as carambola (Weller *et al.*, 1995) and mango (Rattanapanone *et al.*, 2001; Gonzalez-Aguilar *et al.*, 2008). Variability in browning potential among fruit species and cultivars and even within different parts of individual fruit (Dea *et al.*, 2007) can be related to the concentrations of pre-existing substrates for the browning reactions (phenolics, including anthocyanins) as well as relative induction of phenolic synthesis via PAL, which is induced by both ethylene and wounding, and the oxidases such as PPO that are responsible for brown pigment formation.

Carotenoids are responsible for yellow, orange and red colours in fruits, which increase in intensity during ripening. Increases in carotenoid synthesis are slowed at typical FC handling temperatures, so little increase in colour intensity is usually observed. Since carotenoids could conceivably be degraded as a consequence of wounding – due to exposure to acidic pH, oxygen or light or by co-oxidation during oxidation of fatty acids by lipoxygenase – all of which may occur when fruit tissues are disrupted by cutting, it has been speculated that losses of carotenoids could occur in FC products (Klein, 1987; Thompson *et al.*, 1987). Carotenoid loss would have negative consequences both for appearance and for nutritional quality.

Wright and Kader (1997a; 1997b) measured losses of almost 30% for β-carotene and about 40% for β-cryptoxanthin in FC persimmon held for eight days in air at 5°C, but carotene and lycopene contents did not change significantly and there were no apparent changes in the colour of the slices other than those changes that were due to oxidative browning. Wright and Kader concluded that, for most carotenoids in FC persimmon, the shelf life limit was reached prior to occurrence of significant losses in carotenoid content. Similar results indicating minor changes in flesh colour or carotenoid content during FC product storage have been reported for pineapple (Antoniolli *et al.*, 2007) and papaya (Rivera-Lopez *et al.*, 2005), while Gil *et al.* (2006) measured significant carotenoid losses and paler colour in FC mango and pineapple compared with the corresponding whole fruit, but only after extended storage times at 5°C that exceeded the product shelf life.

10.7 Preservative treatments for fresh-cut fruits

Preserving FC subtropical and tropical fruit products is a particularly challenging goal since this category includes fruits of quite diverse physiologies and physical structures. While most of these fruits have climacteric ripening patterns, some do not, and this can pose a challenge in regard to atmosphere and treatment effectiveness in controlling quality loss. Nevertheless, there have been many advances made in preservation of FC subtropical and tropical fruit products and the most interesting will be highlighted in this section.

10.7.1 Antioxidants and antimicrobial compounds

Post-cutting treatments to control microbial growth and maintain the quality of the fruit pieces are probably the most active area of research for FC fruits (Beaulieu and Gorny, 2002; Allende *et al.*, 2006). While antioxidant compounds are helpful in preventing or reducing discoloration of FC surfaces, some fruits, such as citrus, have very high antioxidant content (Artés -Hernandez *et al.*, 2007) and so may not show reduced browning in response to antioxidant application.

Antimicrobials are an important consideration for handling FC products since many cases of foodborne illness have been linked to this category (Beuchat, 1996). In some FC citrus fruits, the cell pH is generally below four (Pao *et al.*, 2009) and in such cases many microorganisms are expected to have a difficult time to survive and grow (Banwart, 1989). However, the pH of the surface of FC citrus is much higher and so microorganisms can readily survive and grow on the surface (Pao *et al.*, 2009). As a consequence, acidulating compounds have been used in the past to help reduce microbial spoilage and human pathogens on FC tropical and subtropical fruits (Pao and Petracek, 1997). However, that approach is becoming less attractive since it does not preserve the fresh flavour and texture that most consumers are coming to expect in FC products. Therefore, other approaches are being explored, including the assurance of low temperatures (<5°C) and good processing plant sanitation for handling FC tropical and subtropical fruits (Pao *et al.*, 2009).

Ascorbic acid, or an ascorbate salt, is the most commonly used antioxidant in FC fruits and vegetables (Toivonen and Brummell, 2008). The mode of action of this antioxidant is through reduction of quinones (the first products in the browning reaction) to their native phenolic form (Toivonen and Brummell, 2008) and this process is very effective in preventing the final reactions that result in brown or black pigment formation. Quite often the antioxidant formulation can be supplemented with other antioxidants (L-cysteine) and/or other agents that modulate tissue texture (e.g., calcium salts) and/or mild acidulants (Beaulieu and Gorny, 2002).

In FC subtropical and tropical fruits, there are demonstrated shelf life and quality benefits for some products with the use of antioxidant formulations. Application of 1–2.5% citric acid and 0.25% ascorbic acid was shown to reduce browning in carambola slices (Weller *et al.*, 1997). A mixture of 1 mM 4-hexylresorcinol, 50 mM potassium sorbate, and 500 mM ascorbic acid applied to MAP stored cut mangoes maintained good colour and flavour, while also controlling microbial growth (Gonzalez-Aguilar *et al.*, 2000). A mixture of 50 mM N-acetylcysteine and 500 mM citric acid was found to control browning in sliced bananas held at 5°C (Moline *et al.*, 1999). Alternately, oxalic acid has also been shown to be very effective to control browning in FC banana (Yoruk *et al.*, 2004). Manurakchinakorn *et al.* (2005) found that a mixture containing sodium erythorbate (a synthetic ascorbate analog) and calcium chloride were best for maintaining firmness and preventing browning of FC mangosteen stored at 4°C in MAP. A combination of 0.5% ascorbic acid and 0.5% citric acid was found to be effective in enhancing the shelf life of pomegranate arils (Gil *et al.*, 1996b).

The most commonly used antimicrobial, citric acid, is often also classified as an anti-browning compound. Citric acid provides antimicrobial activity via pH reduction at the cut surface (Pao and Petracek, 1997). Application of citric acid has been found beneficial in extending shelf life, primarily in FC citrus (Pao and Petracek, 1997; Artés-Hernandez *et al.*, 2007). A drawback to the use of citric acid is exemplified by de Souza *et al.* (2006) who found that citric acid provided no benefit in FC mango and actually led to accelerated softening in that product.

Ngarmsak *et al.* (2006) found that vanillin could be used as an effective antimicrobial to control decay microorganisms and human pathogens in FC mango. Other antimicrobials showing promise for FC subtropical and tropical fruits include sodium benzoate, potassium sorbate, and ethanol (Chauhan *et al.*, 2006; Plotto *et al.*, 2006; Sirichote *et al.*, 2008). A compound that works indirectly to increase fruit tissue resistance to microbial growth, methyl jasmonate, is effective at controlling microbial growth on FC pineapple (Martínez-Ferrer and Harper, 2005). Clearly, more work should be devoted to use of antimicrobials in FC fruit products, particularly since shelf life is most often limited by microbial growth (Allende *et al.*, 2006).

10.7.2 Edible coatings

A wide range of edible coatings have been tested to improve shelf life and quality retention in FC fruit products (Rojas-Graü *et al.*, 2009). Many coatings not only provide protection against water loss, they are also used as carriers for other functional additives, such as antioxidants (Rojas-Graü *et al.*, 2009). Pérez-Gago *et al.* (2005) found that incorporating ascorbic acid into a whey protein concentrate/beeswax coating enhanced the antioxidant functionality as compared with a simple dip of ascorbic acid in sliced persimmons. However, they also found that when MAP was also used, the water loss control benefits of the coating were no longer significant. Single component coatings have also been found to be effective in some instances. When whey protein concentrate was used alone in rose apple, browning and titratable acidity losses were controlled quite well (Worakeeratikul *et al.*, 2007) and higher CO_2 levels were maintained in the tissues. Likewise, when a sucrose fatty ester formulation was used to coat FC guava, weight loss and discoloration were somewhat controlled, and slightly higher CO_2 levels were maintained in the tissues (Thommohaway *et al.*, 2007b). Montero-Calderón *et al.* (2008) found that an alginate coating reduced the leakage of juice from FC pineapple pieces, but could not quantify any other benefit.

Chitosan coating, a compound extracted from shell fish exoskeletons, has been tested for use on several FC subtropical and tropical fruits. A chitosan coating was found to be the best option in FC mangoes (Ducamp-Collin *et al.*, 2009). Worakeeratikul *et al.* (2007) found that a chitosan coating reduced weight loss and browning, enhanced titratable acidity and soluble solids retention, and maintained higher CO_2 concentrations in FC rose apple. Similar results were found with chitosan coating in guava (Thommohaway *et al.*, 2007a).

The work to date shows that coatings can be very useful alone and as matrices to carry other functional compounds. Examples were cited in which antioxidants were incorporated into the coating, providing a more effective delivery of the antioxidant. However, no examples of incorporating antimicrobial compounds were found in the literature for FC subtropical and tropical fruits (Rojas-Graü *et al.*, 2009) and this is an area that has not received much attention in the food microbial safety research (Allende *et al.*, 2006). The gap in this area suggests that there is opportunity to explore the use of coatings for delivering antimicrobial compounds to the surfaces of FC subtropical and tropical fruits to control both decay and human pathogen growth.

10.7.3 Active packaging

Active packaging has been defined as MAP that has interactive features incorporated (e.g., evolution of antimicrobials from the film), is flushed with a gas mixture at the time of sealing, or has sachets enclosed that either absorb or emit gases or volatiles (Forney *et al.*, 2009) and also as a system in which the package interacts with the product or the headspace, in order to maintain the nutritional and sensory quality, fresh-like appearance, and safety of products (Ayala-Zavala *et al.*, 2008a). There is limited information available on the use of active packaging techniques in FC subtropical and tropical fruits.

Flushing MAP of FC pineapple with high or low O_2 did not influence the quality, microbiology or shelf life (Montero-Calderón *et al.*, 2008). In contrast, flushing with 2.5 or 5% CO_2 was beneficial for maintaining quality of shredded, packaged green papaya (Srilaong and Chansamrankul, 2007). Microbial quality was also improved with active packaging containing 10% CO_2 for mango cubes (Poubol and Izumi, 2005). A reason for the discrepancy between studies may relate to the packaging materials used, since Gil *et al.* (1996b) observed that despite distinct initial atmospheres in active package treatments, the steady state atmosphere compositions in all packages were soon similar. They attributed this to the fact that their film was quite permeable and therefore allowed the O_2 and CO_2 partial pressures to quickly adjust to levels determined primarily by product respiration and package permeability characteristics. Therefore, success with active packaging of subtropical and tropical FC fruits requires the selection of packaging films that will preserve the intended atmosphere over the expected shelf life as seen in section 10.7.4.

One of the observations for many FC subtropical and tropical fruit products has been that CO_2 and ethylene levels build up over time in the package systems used (Agar *et al.*, 1999; Chonhenchob *et al.*, 2007; Montero-Calderón *et al.*, 2008). This observation emphasizes the need to consider absorbents for control of CO_2 and ethylene in packaged FC subtropical and tropical fruit products, but currently no work is reported in the literature on potential benefits of such an approach.

The release of antimicrobial compounds into the packaging system could provide significant improvements for shelf life and safety of the FC subtropical

and tropical fruits. Plotto *et al.* (2006) found that ethanol was a good antimicrobial for FC mango. Toivonen and Lu (2007) developed a sachet technology that releases ethanol over time in the package. The use of such approaches might be helpful for application in FC subtropical and tropical fruits, particularly since that sachet also releases a ripening control agent (1-MCP) that controls softening of tissues. Another approach to applying antimicrobials is incorporation into packaging films (López *et al.*, 2007a; 2007b), but there are currently no reports on this approach for FC subtropical and tropical fruits. Certainly, the incorporation of antimicrobial release agents in packaging systems is an area that needs much more investigation in regard to FC subtropical and tropical fruit products.

10.7.4 Modified atmosphere packaging (MAP) and controlled atmospheres (CA)

The use of modified atmospheres (MA) in packaging FC produce, including subtropical and tropical fruit products, is essential for successful marketing of these products (Beaulieu and Gorny, 2002). The mechanisms for MAP and CA action have been well discussed previously (Beaulieu and Gorny, 2002) and therefore will not be discussed in this chapter. Specific atmosphere target recommendations are highly dependent on the fruit in question and limited, in many cases, to the research that has been conducted. Table 10.2 is a summary of current recommendations or best knowledge for optimal MA conditions for many subtropical and tropical fruits. Level of benefit varies, suggesting that in many cases a second technology needs to be applied to optimize quality. The application of antioxidants, antimicrobial compounds, coatings, and active packing have been found to improve response to MA for some subtropical and tropical fruit FC products (Beaulieu and Gorny, 2002; Vilas-Boas and Kader, 2006).

An important aspect of MAP is that it must always be applied in conjunction with appropriate temperature control. Quality retention achieved by MA for FC papaya and pineapple was directly correlated to the temperature at which the FC products were stored in MA (Rivera-Lopez *et al.*, 2005; Marrero and Kader, 2006).

While there are often recommendations for low O_2 and high CO_2, some products, such as pomegranate arils, do not show good quality improvement in response to such atmospheres (Gil *et al.*, 1996a). However, pomegranate arils when packaged in air supplemented with high CO_2 atmospheres (15–20%) show dramatic reductions in fungal decay, leading to significant improvement in shelf life (Hess-Pierce and Kader, 1997).

There are a number of FC subtropical and tropical fruit products for which no specific MA recommendations exist (Table 10.2). In many cases, those products have been shown to benefit from MAP, but no optimal atmosphere recommendations were derived from the work (Durigan *et al.*, 2005; Goldman *et al.*, 2005; Chauhan *et al.*, 2006; Voon *et al.*, 2006). This gap in information is an area of research that requires more effort, since understanding optimization of atmosphere composition is important to successful and reliable commercial application. To emphasize this

Table 10.2 Suggested holding temperatures and modified atmosphere targets for fresh-cut tropical fruit products

FC fruit and format	Temperature (°C)	Atmosphere recommendation		Relative benefit
		$\%O_2$	$\%CO_2$ achieved	
Banana, slices	5	2–4	5–10	Slight
Carambola, slices	4.4–10	16–18	1–2	Moderate
Coconut, slices	4	8–16	0–4	Good
Dragon fruit, slices	4	–	–	–
Durian, arils	4	–	–	–
Guava, slices	5	–	–	–
Jackfruit, slices	6	3	5	Good
Kiwifruit, slices	0–5	2–4	5–10	Good
Mango, cubes	5	2–4	5–10	Moderate
Mangosteen, peeled	4	5	9	Moderate
Orange, slices	0–5	14–21	7–10	Moderate
Papaya, cubes	3–6	–	–	–
Green papaya, shreds	6	3	6	Good
Persimmon, slices	0–5	2	12	Poor
Pineapple, cubes	0–5	2–8	10	Good
Pomegranate, arils	0–5	–	15–20	Good
Rambutan	4	8	20	Moderate

Sources: Beaulieu and Gorny (2004); Chauhan *et al.* (2006); Hess-Pierce and Kader (1997); Manurakchinakorn *et al.* (2005); Marrero and Kader (2001;2006); Saxena *et al.* (2008); Sinigaglia *et al.* (2003); Sirichote *et al*. (2008); Vilas-Boas and Kader (2006); Voon *et al*. (2006)

point, Budu and Joyce (2005) demonstrated the importance of proper modelling and package design for optimizing FC pineapple shelf life. Another emerging issue is that many subtropical and tropical fruits are preferred in fruit salad mixtures, which will also require more research to optimize, since different fruits have differing atmosphere responses (Chonhenchob *et al.*, 2007).

10.7.5 Natural compounds

Food safety and quality have always been important to consumers and continue to be a basic requirement of a modern food system. Chemical control of FC fruit decay (synthetic additives) has been used since the initiation of this industry as reliable preservative factors to control the amount of deteriorative factors in FC fruits and vegetables. However, most of these compounds do not satisfy the concepts of 'natural' and 'healthy' that consumers prefer and that the food industry, consequently, needs to provide. This necessity is underlined by many in agro-industries, legislatures and consumer organizations around the world.

Natural active compounds are a re-emerging alternative to FC produce preservation. The antimicrobial power of plant and herb extracts has been recognized for centuries, and mainly used as natural medicine. Plants produce a

wide range of volatile compounds, some of which are important flavour quality factors in fruits, vegetables, spices and herbs (Lanciotti *et al.*, 2004). A number of volatile compounds inhibit the growth of microorganisms (Burt, 2004). The ability of plant volatiles to inhibit microbial growth is one of the reasons why there is an increased interest in using them to control postharvest and post-processing decay of fruits and vegetables (Yao and Tian, 2005). Plant volatiles have been widely used as food flavouring agents, and many are generally recognized as safe (GRAS).

Essential oils (EOs) represent the most important aromatic fraction of plants and plant produce, constituted by a complex mixture of terpenes, alcohols, ketones, aldehydes, esters, and sulphur compounds, depending on the source of plants. Their antibacterial mode of action has been related to their individual active compounds. Tripathi and Dubey (2003) reported that the exact modes of action of antimicrobial compounds, such as thymol, eugenol and carvacrol, have not been well determined, although it seems that they may inactivate essential enzymes, react with the cell membrane or disturb genetic material functionality.

Plant EOs have shown a wide range of antimicrobial action against several bacteria and their toxins produced in foods, yeasts and moulds (Tripathi and Dubey, 2003; Burt, 2004). Therefore, EOs present a huge potential as food preservatives, especially because most of them are classified as GRAS. Citrus EO preserved the quality of FC fruit salads without affecting consumer acceptance of the products (Lanciotti *et al.*, 2004). Garlic oil preserved overall quality and antioxidant capacity of FC tomatoes (Ayala-Zavala *et al.*, 2008c). Cinnamon leaf and garlic oils showed antifungal activity against *Alternaria alternata* (Ayala-Zavala *et al.*, 2008d).

The antimicrobial activities of a variety of naturally occurring phenolics from different plant sources have been studied in detail (Burt, 2004). Phenolics from spices, such as gingeron, zingerone and capsaicin, have been found to inhibit germination of bacterial spores. Natural plant phenolic compounds are important food preservative factors and have, as a group, a remarkable antimicrobial range (Burt, 2004).

Methyl jasmonate (MJ) is a natural compound widely distributed in plants. It was first detected as a sweet fragrant compound in Jasminum spp. EO and other plant species (González-Aguilar *et al.*, 2006). Methyl jasmonate is known to regulate plant development and response to environmental stress (Demo *et al.*, 2005; Yao and Tian, 2005), affecting many biochemical and physiological reactions in the tissue of whole and FC fruits and vegetables and extending shelf life of whole and FC tomatoes, mangoes, guavas and strawberries (González-Aguilar *et al.*, 2006).

Ethanol, a GRAS compound, has been shown to be effective for controlling decay of whole fruits and vegetables, inhibiting microbial growth (Karabulut *et al.*, 2004). The mode of action of ethanol is by interaction with the membrane of microorganisms. Several devices have been designed to control ethanol release in the headspace of packaged fruits (Kalathenos and Russell, 2003). Ayala-Zavala

et al. (2007) reported that ethanol treatment in conjunction with MJ increased antioxidant capacity, volatile compounds, and postharvest life of strawberry fruit, as well as extending the shelf life of FC tomatoes (Ayala-Zavala *et al.*, 2005; Ayala-Zavala *et al.*, 2008c). Plotto *et al.* (2006) concluded that ethanol vapour applied for 20 hours, prior to processing whole mangoes, did not delay ripening; however, shorter time of exposure (10 h) suppressed fruit ripening.

Appropriate or compatible use of natural antimicrobial agents would involve using these compounds to add positive sensory characteristics in addition to improve food safety and/or extend shelf life of fresh fruits and vegetables (Tripathi and Dubey, 2003; Ayala-Zavala *et al.*, 2008a). Essential oils are effective antimicrobials; however, their aromatic volatile constituents can be absorbed by the food product. By choosing the right combination of aromas between the antimicrobial EO and the FC product, safety and flavour quality can be improved (Ayala-Zavala *et al.*, 2008a).

Organic acids are commonly used as antimicrobial acidulants and anti-browning agents in FC produce (Table 10.2) (Ruiz-Cruz *et al.*, 2007). Since many pathogens cannot grow at pH values much below 4.5, acidification may act to prevent microbial proliferation. Organic acids may also possess bactericidal capabilities. The antimicrobial action of organic acids is due to the pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid. Many types of produce, especially fruits, naturally possess significant concentrations of organic acids, such as acetic, benzoic, citric, malic, sorbic, succinic and tartaric acids, which negatively affect the viability of contaminating bacteria.

Organic acids, including lactic acid, acetic acid, citric acid, ascorbic acid and others are approved or listed in FDA regulations for various technical purposes, e.g., as acidulants, antioxidants, flavouring agents, pH adjusters, nutrients and preservatives. The Agency's decision about this use of organic acids was based on industry requests that were supported by data that showed that this application of organic acids meets FDA's definition (FDA, 2010). Therefore, products made from organic acid-treated produce do not have to declare the organic acids in the ingredients statement on the product label.

Lozano de Gonzales *et al.* (1993) used pineapple juice for anti-browning, considering that pineapple contains the enzyme bromelain, which is also capable of inhibiting enzymatic browning just as ascorbic acid. Pineapple juice was an effective browning inhibitor in both fresh and dried apples. All fractions of pineapple juice separated by different extraction methods inhibited enzymatic browning by at least 26%, as measured colourimetrically and by visual examination. Fractionation identified that the inhibitor is a neutral compound of low molecular weight.

Song *et al.* (2007) used rhubarb juice as a natural anti-browning agent for FC apple slices. They found that juices at 20% concentration containing 67 mg/100 g of oxalic acid inhibited browning. Yoruk and Marshall (2003) investigated the mode of inhibition of oxalic acid on PPO and determined that, by binding with copper to form an inactive complex, it reduces catechol-quinone product

formation. Oxalic acid was a more potent inhibitor of PPO compared with other structurally related acids. Other compounds, such as benzoic and cinnamic acids, are PPO inhibitors but have been found not to give prolonged protection during storage (Lamikanra, 2002).

10.7.6 Controlled release of antimicrobial compounds

Controlled release of antimicrobial compounds can be used to generate active packaging systems (see subsection 10.7.3). Since microbiological contamination of fruits and vegetables occurs primarily on their surfaces due to processing and post-processing procedures, in recent years special attention has been paid to the development of antimicrobial active packaging systems that deliver antimicrobial compounds to the packaged product in order to increase its shelf life and microbiological quality. These antimicrobial active packaging systems create an environment inside the package that delays microbial growth (Ayala-Zavala *et al.*, 2008b). Antimicrobial active packaging systems can be divided into four groups according to the mechanism of action of the antimicrobial compound: (1) the antimicrobial is released to the headspace of the package in order to interact with the product surface; (2) the antimicrobial compound is incorporated into the packaging material and is released to the product by a migration process; (3) the antimicrobial compound is immobilized on the surface of the package; and (4) the package material has inherent antimicrobial activity. In groups 3 and 4, direct contact between the package and the product is needed in order to obtain the desired antimicrobial effect (de Oliveira *et al.*, 2008). The retention/release mechanism of the antimicrobial compound will depend on the type of active packaging used. In the same way, the mode of action of the antimicrobial compound will be determined by the type of active packaging system used. For example, if the antimicrobial has to enter the cytoplasm, an immobilized antimicrobial or an intrinsic antimicrobial polymer would not inhibit the growth of the microorganism (Appendini and Hotchkiss, 2002; Rico *et al.*, 2007). In the next section each of the four groups of antimicrobial active packaging systems and its application to fresh fruits and vegetables will be analysed.

Headspace artefacts were the first antimicrobial active packaging commercialized in the market, in the form of sachets that are enclosed in the interior of the package or attached to it. They can be divided into two groups: indirect and direct antimicrobial activity. Headspace artefacts with indirect antimicrobial activity include O_2 and moisture scavengers and CO_2 absorbers/emitters. They are considered to be indirect antimicrobials because, even though their primary activity is to decrease spoilage due to enzymatic deteriorative reactions and prevent foggy film formation, the modification of the internal atmosphere (decrease of O_2 and moisture) inhibits the growth of aerobic bacteria. Headspace artefacts with direct antimicrobial activity include antimicrobial volatile compounds such as sulphur dioxide, ethanol, organic acids and EOs (Rico *et al.*, 2007; Park and Lee, 2008; Ayala-Zavala *et al.*, 2008b; Ayala-Zavala *et al.*, 2008c; Ayala-Zavala *et al.*, 2008d).

The effectiveness of headspace artefacts depends on the permeability of the sachet material to water vapour, the release of the volatile compound absorbed or encapsulated, the diffusion through the polymer, their vapour partial pressure, and the way in which the antimicrobial interacts with the microorganism. Recently, Ayala-Zavala *et al.* (2009) described a cyclodextrin EO microcapsule that was used as a headspace artefact to increase the shelf-life of FC produce. In this study, it was hypothesized that internal moisture can be the driving force that releases the antimicrobial compound from the complex. Reports by Almenar *et al.* (2006; 2007) showed that the inclusion of complexes of *β*-cyclodextrin-hexanal (1.1 μL hexanal L^{-1}) and *β*-cyclodextrin-acetaldehyde (0.12 μL acetaldehyde/L) were effective against *A. alternata, Colletotrichum acutatum* and *Botrytis cinerea*. A sachet containing different amounts of 2-nonanone (2.5, 5 and 10 μL), a volatile compound that is naturally found in strawberry fruit, impregnated in alumina as an adsorbent solid, was developed, studied, and finally incorporated as a part of an active packaging system.

Antimicrobials included in the package polymer as another type of antimicrobial active packaging artefact are those in which the antimicrobial compound is embedded in the bulk polymer and it has to migrate to the surface in order to interact with the microorganism. Different natural and synthetic polymers have been used as carriers; a review on this subject has recently been published (Rojas-Grau *et al.*, 2009). Mono and multilayer active packages using this technique have been developed (Ayala-Zavala *et al.*, 2008b). Typical multilayer films consist of four layers: outer layer, barrier layer, matrix layer (in which the antimicrobial is embedded) and control layer. Several organic and inorganic compounds have been used as antimicrobials; the most commonly used are silver zeolites, organic acids and their derivatives, peptides, enzymes, EOs, parabens, bacterocins, and volatile compounds, among others (Burt, 2004; Rico *et al.*, 2007; Ayala-Zavala *et al.*, 2009). In order for these active packaging systems to be effective, a minimum antimicrobial release has to be maintained (Ayala-Zavala *et al.*, 2008a; Del Toro-Sánchez *et al.*, 2010).

One of the main disadvantages of packages with antimicrobials included in the package polymer is that heat-sensitive compounds cannot be used, because they are inactivated during the processing of the package. An interesting option is the use of microencapsulation of active compounds in the case of heat-sensitive antimicrobials before incorporation into the polymer extrusion process (Ayala-Zavala *et al.*, 2008a). Figure 10.7 shows a package system that includes a microencapsulated active compound, which is released passively in the atmosphere and reaches the fruit surface, where it acts by suppressing deteriorative reactions.

10.8 Preservation of bioactive compounds and antioxidant capacity

Bioactive compounds (phytonutrients) are health-promoting compounds that can lower the risk of heart disease, cancer and other diseases; they include carotenoids

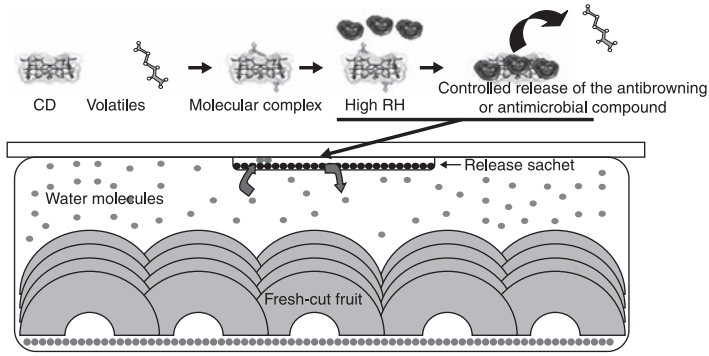

Fig. 10.7 Controlled release of antimicrobial compounds into the package containing the fresh-cut produce (adapted from Ayala-Zavala *et al.,* 2008).

and flavonoids (anthocyanins, phenolic acids, polyphenols). The antioxidant capacity of fruits is related to their contents of anthocyanins, phenolic compounds, carotenoids, ascorbic acid and vitamin E. In general, exposure of intact or FC fruits to stress increases biosynthesis of phenolic compounds and antioxidant capacity (Gonzalez-Aguilar *et al.*, 2007; Gil and Kader, 2008).

Alothman *et al.* (2009) reported that UV-C radiation induced an increase in antioxidants, including polyphenols and flavonoids, in FC pineapple, banana and guava products. Artés-Hernandez *et al.* (2010) concluded that UV-C illumination, if effectively applied over the whole product surface, could be a satisfactory sanitizing treatment for FC watermelon, and possibly for other FC fruits with delicate texture. All UV-C doses tested retarded microbial development compared to a non-treated control, but only the lower doses (1.6 and 2.8 kJ m^{-2}) preserved sensory attributes for up to 11 days at 5°C, with maintenance of lycopene and ascorbic acid contents, and increased total antioxidant capacity. The evaluation of the influence of FC processing is still a key factor in finding those technological conditions necessary to preserve the content, activity and bioavailability of naturally occurring antioxidants and other health-promoting constituents of fruits and vegetables. Research into the health benefits of fruits and vegetables needs to identify optimum conditions for maintaining these compounds after harvest and after FC processing operations (Gil and Kader, 2008).

10.9 Nutritional aspects of fresh-cut vs whole fruits

Wright and Kader (1997a) studied changes in vitamin C content of FC persimmons for eight days in CA of 2% O_2 in N_2, air + 12% CO_2, or 2% O_2 + 12% CO_2 in N_2 at 0°C and found that the post-cutting life based on visual quality ended before significant losses of vitamin C occurred. Storage of persimmon slices in 2% O_2 or air + 12% CO_2 tended to result in lower retinol equivalents after eight days, but

the loss was not significant for fruit stored in 2% O_2 + 12% CO_2. For persimmons, the limit of shelf life was reached before major losses of carotenoids occurred (Wright and Kader, 1997b).

Kiwifruit slices stored at 5 or 10°C exhibited a gradual decrease in reduced ascorbic acid (RAA) and an increase in dehydroascorbic acid (DHAA) content. The total vitamin C was 8, 13 or 21% lower than initial values in slices kept for six days at 0, 5 or 10°C, respectively (Agar *et al.*, 1999). Kiwifruit slices stored in ethylene-free air contained three times more RAA than controls. When dipped in 1% $CaCl_2$ after cutting and kept in an ethylene-free atmosphere, slices had a slightly higher RAA content than those treated with 1% $CaCl_2$ only (Agar *et al.*, 1999). Vitamin C content of FC kiwifruit slices kept in 0.5, 2 or 4% O_2 (balance N_2) at 0°C decreased by 7, 12 or 18%, respectively, after 12 days storage. Vitamin C content in slices kept in air + 5, 10 or 20% CO_2 decreased by 14, 22 or 34%, respectively (Agar *et al.*, 1999).

During storage at 5°C for 12 days, there were no significant differences in the changes of RAA, DHAA and total ascorbic acid (TAA) concentrations among mango cubes stored in different atmospheres (Chantanawarangoon, 2000). The RAA content of mango cubes declined, while DHAA content increased during storage. However, the increase of DHAA was not equivalent to the decrease in RAA and, consequently, TAA decreased during storage. After 12 days at 5°C, the amounts of RAA and TAA decreased by about 50% and 40% of initial amounts, respectively. Del Caro *et al.* (2004) found that citrus segments retained their nutritional quality until the end of their post-cutting life, based on appearance. Recently, it was reported that dipping treatment with ascorbic acid + citric acid + $CaCl_2$ positively affected the quality by delaying deterioration of FC 'Ataulfo' mango as compared with whole fruit. However, the dipping treatment affected consumer preferences for the FC mangoes (Robles-Sánchez *et al.*, 2009a). The same treatment was used on the 'Kent' cultivar and a significant increase in vitamin C was observed compared with untreated mango cubes. *β*-carotene was not affected by dipping treatments, and vitamin E showed a significant decline over storage time for both treated and untreated mango cubes. Dipped cubes had higher antioxidant activity measured as TEAC and % RSA than controls. In general, addition of ascorbic acid is effective as an anti-browning treatment (Robles-Sánchez *et al.*, 2009b). Other treatments such as the application of chitosan at 0.02 g mL^{-1} reduced deterioration and increased the shelf life of FC 'Maradol' papaya compared with controls during storage at 5°C (González-Aguilar *et al.*, 2009b).

Gil *et al.* (2006) investigated the influences of processing and storage on the quality indices and nutritional content of FC fruits in comparison with whole fruits stored for the same duration but prepared on the day of sampling. Fresh-cut pineapples, mangoes and kiwifruits and whole fruits were stored for up to nine days in air at 5°C. The post-cutting life based on visual appearance was shorter than six days for FC kiwifruit and shorter than nine days for FC pineapple. On the other hand, FC mango pieces were still marketable after nine days at 5°C. Losses in vitamin C after six days at 5°C were 25% in mango pieces, 10% in pineapple

pieces and 12% in kiwifruit slices. No losses in carotenoids were found in kiwifruit slices, whereas losses in pineapples were the highest at 25%, and losses in mango were 10–15% after six days at 5°C. No significant losses in total phenolics were found for any of the FC fruit products tested after six days at 5°C. In general, FC fruits spoil visually before any significant nutrient loss occurs.

Artés-Hernandez *et al.* (2007) concluded that to reach a ten-day shelf-life period, wedges, slices, and half slices of FC 'Lisbon' lemons should be kept at 0 to 5°C and protected from water loss by proper packaging with high relative humidity during distribution. Under these conditions, sensory quality of these FC lemon products was preserved with good retention of vitamin C and antioxidant capacity. However, a decrease of the total phenolics compounds throughout the shelf life was observed.

10.10 Hazard Analysis Critical Control Point (HACCP) and hygiene considerations for the fresh-cut produce industry

The production of safe food products requires that the HACCP system be built upon a solid foundation of prerequisite programmes. Monitoring finished FC produce is no guarantee of safety because unsafe samples may escape detection. Each segment of the food industry must provide the conditions necessary to protect food while the food is under that segment's control. This protection has traditionally been accomplished through the application of Good Manufacturing Practices (GMP). These conditions and practices are now considered to be prerequisite to the development and implementation of effective HACCP plans. Figure 10.8 shows some of the good hygienic and manufacturing practices used in the FC industry.

HACCP is a structured approach to the identification, assessment of risk, and control of the hazards associated with a food production process or practice. This system prevents physical, chemical or microbial contamination of FC produce. HACCP addresses the root causes of food safety problems in production, storage, transportation, receiving and processing and is preventive (FDA, 1994). It aims to identify possible problems before they occur and establish control measures at stages in production that are critical to product safety. One of the purposes of HACCP is to design safety into the process, thereby reducing the need for extensive microbiological testing of in-line samples and finished FC products (Silliker, 1995).

Design and implementation of a HACCP system involves following seven basic principles or steps:

Step 1: Conduct a hazard analysis. Flow diagram the steps of a process to determine where significant hazards exist and what control measures should be instituted.

Step 2: Determine Critical Control Points (CCPs) required to control the identified hazard. CCPs are any steps where hazards can be prevented, eliminated, or reduced to acceptable levels.

Step 3: Establish Critical Limits (CLs). These are specifications (target values and tolerances) that must be met to insure that CCPs are under normal control.
Step 4: Establish procedures to monitor CCPs. These are used to adjust the process to maintain CCP control.
Step 5: Establish corrective actions to be taken when monitoring indicates a deviation from an established CL.
Step 6: Establish verification procedures for determining if the HACCP system is working correctly.
Step 7: Establish effective record-keeping procedures that document the HACCP system.

The establishment of HACCP for the FC industry must be built around a series of preservative factors (hurdles) to control pathogen growth, because there is no definitive kill step in the processing operation. Fresh-cut hurdles include purchasing fresh produce from certified growers, implementing comprehensive plant sanitation programmes, using sanitizers and antimicrobial agents in the wash water and on whole and FC produce, and using MAP techniques and low temperature management.

HACCP functions as the final stage of an integrated food safety programme that includes Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Sanitation Standard Operating Procedures (SSOPs). In fact, HACCP can only be effective if these other programmes are in place and functioning properly. HACCP is based on the identification of Critical Control Points (CCPs) and the prevention of identified hazards through controlling CCPs. But a CCP has certain characteristics that limit its use to certain well defined situations. First, a CCP is a place in a process that, when not controlled, could result in a significant hazard. Second, a CCP must be a point where a hazard could be controlled through some defined process. Thirdly, it must be possible to quantify the process so it is possible to know if it is within critical limits. And, finally, it must be possible to monitor and/or measure the process at that point to know if the process is within the critical limits.

Identification of potential hazards and CCPs is the foundation of HACCP. First, a hazard analysis is performed for the process. Then, a CCP is identified as a step in the process that, if not controlled, could result in a significant hazard. Next, it must be possible to measure and document the proper limits of performance of the CCP to verify that it is under control. Finally, it must be possible to audit the written records to verify that the process is under control through an effective HACCP plan.

Prerequisite programmes provide the basic environmental and operating conditions that are necessary for the production of safe, wholesome food. Common prerequisite programmes may include, but are not limited to:

Facilities. The establishment should be located, constructed and maintained according to sanitary design principles. There should be linear product flow and traffic control to minimize cross-contamination from raw to processed materials.

Supplier control. Each facility should assure that its suppliers have in place effective GMP and food-safety programmes. These may be the subject of continuing supplier guarantee and supplier HACCP system verification.

Specifications. There should be written specifications for all ingredients, products and packaging materials.

Production equipment. All equipment should be constructed and installed according to sanitary design principles. Preventive maintenance and calibration schedules should be established and documented.

Cleaning and sanitation. All procedures for cleaning and sanitizing the equipment and the facility should be written and followed. A master sanitation schedule should be in place.

Personal hygiene. All employees and other persons who enter the manufacturing plant should follow the requirements for personal hygiene.

Training. All employees should receive documented training in personal hygiene, GMP, cleaning and sanitation procedures, personal safety, and their role in the HACCP programme.

Chemical control. Documented procedures must be in place to assure the segregation and proper use of non-food chemicals in the plant. These include cleaning chemicals, fumigants, and pesticides or baits used in or around the plant.

Receiving, storage and shipping. All raw materials and products should be stored under sanitary conditions and the proper environmental conditions such as temperature and humidity to assure their safety and wholesomeness.

Traceability and recall. All raw materials and products should be lot-coded and a recall system should be in place so that rapid and complete traces and recalls can be carried out when product retrieval is necessary.

Pest control. Effective pest control programmes should be in place.

Other examples of prerequisite programmes might include quality assurance procedures; SOPs for sanitation, processes, product formulations and recipes; glass control; procedures for receiving, storage and shipping; labelling; and employee food and ingredient handling practices.

10.11 Facilities, process design and equipment requirements

All fruit and vegetable processing facilities should be hygienically designed and easily cleaned to prevent contamination during processing of fresh produce. Therefore, adequate sanitation methods must be used in order to assure a final FC product free of contamination. But if proper sanitary methods are not used, FC fruits can be prone to contamination by microorganisms. Food hygiene practices are at least as important as the design of the physical plant in producing safe food.

Fig. 10.8 Good hygienic and handling practices used in the fresh-cut industry (courtesy of Fresh-Foods Co., Mexico).

In general, buildings in rural areas may cost more to construct because of higher transport costs for building materials, but rents in rural areas are usually lower than urban centres. The investment in construction or the amount of rent paid should be appropriate to the size and expected profitability of the business. A marketing study must be performed in order to design the right size of each processing area of the new buildings. Engineering has to be sure that the building is big enough for the expected production, but do not purchase or build an extra space that you do not need.

Within the building, fresh produce should move between different stages in a process without the paths crossing. This flow of materials reduces the risk of contaminating finished products by incoming, often dirty, crops, as well as reducing the likelihood of accidents or of operators getting in each other's way. There should be enough space for separate storage of raw materials, away from ingredients, packaging materials and finished products.

The locations and physical structures that currently support fresh fruit and vegetable packing and packaging, including FC produce, are diverse and of many different scales. Well-designed food processing facilities for FC fruit and vegetable products must use easy to clean materials, avoiding wood, soft metal, porous polymers, and carpeting. The building should be designed so that product flow is linear – that is, 'in' one end and 'out' the other. During FC processing operations, incoming raw produce and outgoing packed product should, ideally, never cross paths. If the available space is not linear, the physical separation of zones achieves an equivalent unidirectional traffic and product flow. In addition, separate or segregated zones for chemical storage and mixing and maintenance or fabrication shops should be planned for the facility.

Pallets and bins coming directly from the field may also be a source of contaminated soil and plant debris and they should never have an opportunity to reach the clean areas. Proper facility design can reduce this potential hazard. In

keeping with a linear flow design, non-washed produce should never contact the same surfaces that will contact produce at any other step. When this is unavoidable, a thorough cleaning and sanitation procedure must precede the use of common spaces or contact surfaces. Finally, the freshly cut but unprotected product should not be stored in the same loading dock or cold room location with the raw produce. The facility should have sufficient cold room space to keep the washed and/or graded product (being held for later shipping, packing or packaging) separate from incoming and stored raw materials. The key element to safe facility design is ensuring that unwashed produce enters at one separated area, moves in a linear or segregated flow, and exits at a terminal segregated shipping area.

Facilities may include design and installation of an air-filtration system for central air distribution and air flow counter to product flow. Clean, filtered air should move with a positive pressure from the cleanest areas at packaging and packing back towards the receiving area. A positive pressure flow design helps reduce the chance of airborne contamination along the linear facility design. Additional airflow barriers, such as air-curtains, help to isolate receiving and shipping areas that may be open to the outside environment. Equipment and workers should not move between segregated areas. Colour-coding of boxes, bins, clothing, cleaning tools and other items can help achieve this separation of traffic. Following these recommendations and the good practices mentioned in previous sections will assure the sanitation and quality of the final FC products. Producers have to consider each step of the processing line, implementing a good hygiene and sanitation programme for personnel involved in the different operations.

10.12 New trends in the fresh-cut fruit industry

Many subtropical and tropical fruits possess very attractive sensory attributes that are underappreciated by large segments of the world population. Many of these fruits, including mango, pineapple, mangosteen, durian, jackfruit, litchi and others, are somewhat intimidating to attempt to eat for people who are unfamiliar with them. Fresh-cut products that provide a convenient, ready-to-eat introduction to these fruits for the uninitiated consumer can serve to expand the consumption and economic demand for these fruits to new market segments. Creation of innovative, new combinations of different FC fruit mixes is one approach that may be taken. Development of new FC fruit products and research to determine the optimum procedures for their preparation and handling is a trend that can serve to stimulate the international market for more subtropical and tropical fruits.

The goal of many FC fruit companies nowadays is to deliver to consumers products that possess not only good appearance, but more importantly, the attractive texture, taste and aroma of the freshly picked tree-ripe fruit. As reported in this chapter, a number of approaches and technologies are available or are being researched that have the potential to help realize this goal for FC subtropical and tropical fruits. The best approach, however, is also one of the most obvious: start by choosing whole products that have inherently high quality in terms of maturity,

colour, SSC or SSC:TA ratio, texture, taste, and aroma. One trend in the FC fruit industry is that plant breeders and FC companies are developing and selecting cultivars that are inherently suitable for FC processing. This may involve selection of cultivars with inherently high quality potential or firm texture as well as those with slow ripening behaviour that minimizes sensory changes during FC shelf life.

Another trend in the FC fruit industry is a movement away from extended shelf life claims or requirements of two weeks or more that are based on product appearance, toward more realistic shelf life periods based on retention of good texture, taste and aroma qualities. Modified atmosphere packaging has been a standard component of FC handling throughout the history of the industry. An expected trend is a re-examination of MAP design in conjunction with the development of FC fruit products made from more mature or riper fruit and the desire to maintain more of a fresh-picked flavour. This trend will most likely utilize new advances in edible film and active packaging development. Use of natural compounds such as EOs that preserve or enhance FC fruit texture, taste and aroma is also likely, given the trend toward natural food preservation.

10.13 Economic and market considerations

Fresh-cut products are considered by most people to be convenience and luxury products. The price of FC fruit products is much higher than that of corresponding whole fruits, but most consumers probably recognize the added costs related to preparing the FC products as well as the reduction in waste compared to whole fruits. Still, price is a major restraint to FC sales. Most fruits are eaten in the home, but according to a recent report by the Perishables Group, food service dominates in sales of FC fruit products by about a three-to-one margin in the US (Christie, 2008). This probably relates to price expectations in grocery store versus restaurant sales.

The top retail sellers among FC fruit products in the US are already mostly subtropical and tropical fruit products: watermelon, mixed fruits (mostly cantaloupe, honeydew and grape), and pineapple. Those three items account for 72% of all FC fruit sales (UFREF, 2009). Because of the way FC fruit products are viewed by many consumers, these products would seem to be natural vehicles to entice a first-time buyer to try an unfamiliar subtropical or tropical fruit. Since three-quarters of FC fruit sales are in food service and because it is possible that consumers may be more likely to try an unfamiliar fruit in a restaurant environment, food service may be the most logical market to pursue for the introduction of new FC subtropical and tropical fruit products.

10.14 Conclusions

The growing trend toward increased consumption of FC tropical and subtropical fruits, and the high expectations of consumers for healthier, safer and more

nutritious foods, represents a big challenge for food technologists; since, when fruits are cut, they undergo rapid deterioration, with an increase in fruit respiration, accelerating the ripening process and causing senescence and loss of quality. To accomplish good quality FC tropical and subtropical fruits, it is necessary to optimize the different processing steps to assure the overall quality of FC produce.

Several technologies can be applied in order to preserve the quality of FC fruits, such as the use of antioxidants, antimicrobials, edible coatings, active packaging and MA. Nowadays, the use of safe technologies that include natural products that do not affect the sensorial attributes but do maintain the nutritional value of the final product are the most accepted by consumers.

An increased number of FC produce presentations offers a wide variety of products from which consumers can select the fruit of their preference in a most convenient manner, increasing fruit consumption and providing health benefits to consumers with healthy food that is rich in phytochemicals. The presence of FC tropical and subtropical produce in the market is a good alternative for fruit consumption and can be considered as a new vision of a functional food.

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Plate XII (Chapter 10) A processing facility for fresh-cut fruits.

Plate XIII (Chapter 10) Packaged fresh-cut fruit.

11

Preservation and processing of tropical and subtropical fruits

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Abstract: This chapter discusses the preservation processes commonly or potentially applied to tropical and subtropical fruit products. The chapter first discusses the agents and factors responsible for the deterioration of fruit products and biological hazards to health. It then presents the principles behind conventional technologies and provides examples of their application to tropical and subtropical products. Finally, the chapter discusses the most promising emerging technologies for the preservation of tropical and subtropical fruit products.

Key words: deteriorating agents and factors, preservation processes, emerging technologies.

11.1 Factors responsible for the deterioration of tropical fruits and their products

The processes adopted for the preservation of fruits and their products aim at inactivating pathogenic microorganisms and preventing or delaying the action of those responsible for deterioration. They also seek to inactivate enzymes that originate from the fruit itself, as well as preventing other undesirable physical changes and chemical reactions. Such agents of deterioration depend not only on conditions or factors intrinsic to the fruit product itself, but also on conditions related to the environment in which it is stored or factors extrinsic to the product.

The intrinsic determinants of deteriorative processes include the composition of the fruit product, water activity, pH and oxidation reduction reactions, while the extrinsic factors include temperature, relative humidity, and composition of gases in the environment or packaging in which the product is stored. These

factors determine not only the occurrence of deteriorative processes, but also the speed (rate) at which these changes occur.

11.1.1 Intrinsic factors

Water activity

Water activity is one of the most important intrinsic factors in processing, preservation and storage of fruits. It quantifies the degree of 'free water' contained in the product and therefore its availability to act as a solvent and participate in chemical, biochemical and microbiological transformations. It can be expressed as follows (Labuza, 1977):

$$A_W = \frac{P}{P_0} = \frac{\text{Water vapor pressure in equilibrium with fruit product}}{\text{Vapor pressure of pure water}} \quad [11.1]$$

The maximum activity of water is 1 for pure water; for many fruits and vegetables the value varies from 0.970 up to 0.996 (Chirife and Ferro Fontan, 1982).

All organisms have a minimum water activity (A_w) required for development. In general, bacteria are more demanding than fungi and yeasts, and can usually grow in media with high water activity. The growth of most bacteria and fungi is concentrated on A_w values above 0.90, but certain microorganisms that are significant for fruit preservation can even grow in fruit products having low A_w values or high concentration of solutes.

Acidity and hydrogen potential (pH)

The pH values which allow microorganisms to grow vary from 1 to 11, with the optimum pH for many microorganisms being close to neutrality. However, the optimum pH for the bacteria that produce acetic acid is between 5.4 and 6.3 and for those producing lactic acid between 5.5 and 6.0. In general, fungi and yeasts can grow at lower pH than bacteria, while the maximum pH for growth is similar for all three types of microorganisms.

The main line of demarcation in relation to the pH of foods, including fruit products, is set at 4.5. It is well known that below this value it is difficult for *Clostridium botulinum*, generally acknowledged as the most resistant food pathogenic bacteria, to grow. This distinction determines the type of heat treatment that the product should undergo. For foods with pH above 4.5, processing under pressure is usually required, and for foods with a pH below 4.5 mild heat treatments are sufficient. Foods in general and fruit products in particular are divided into three main groups based on their pH: (i) low-acid foods with pH above 4.5, including açai and avocado; (ii) acidic foods, with pH between 4.0 and 4.5, including some varieties of papaya and mango; and (iii) highly acidic foods with pH below 4.0, such as pineapple and passion fruit. The low-acid fruits are more prone to proliferation of pathogenic microorganisms and consequent spoilage (Franco, 1996). Foods in the acidic category, where most tropical fruits belong, experience the highest incidence of growth of fungi, yeast and some species of

bacteria, principally those producing lactic acid and some species of *Bacillus*. This is discussed in a later section.

Redox potential (E_h)

The redox potential (E_h) is a physicochemical parameter that determines the oxidizing or reducing properties of the medium, and is dependent on the composition of the fruit (thiol-containing amino acids, peptides, proteins and reducing sugars), pH, temperature, and most importantly the concentration of dissolved oxygen. This parameter plays an important role in the cellular physiology of microorganisms, affecting growth capacity, enzyme action and thermal resistance (Alwazeer *et al.*, 2003).

Fungi play an important role in the deterioration of fruits and their products, and these are aerobic, Some species of pathogenic bacteria are also aerobic, such as *Bacillus cereus*. Some aerobic bacteria multiply better in environments with low oxygen concentration (e.g., *Lactobacilli* and *Streptococci*) and these are known as microaerophilic bacteria (Franco, 1996). The bacteria that multiply both under aerobic and anaerobic conditions are called facultative anaerobes (e.g., *Enterobacteriaceae*). Some important yeasts for fruit deterioration are also either aerobic or facultative anaerobic.

Nutrients

Microorganisms' demand for nutrients can vary according to environmental conditions such as temperature and water activity as well as other diverse factors such as water, energy source, nitrogen source, vitamins and minerals. Different foods have different chemical compositions and will thus favor the growth of certain microorganisms over others, depending on their nutrient content. The microorganisms can use sugars, alcohols and amino acids, as well as lipids in some cases, as energy sources. The most important sources of nitrogen are amino acids, but nucleotides, peptides and complex proteins can also be used. Vitamins, including the B complex, biotin and pantothenic acid are important nutrients for the growth of microorganisms, and these form part of several coenzymes involved in metabolic reactions. Although required in very small quantities, these minerals are essential for microbial multiplication, since they are involved in many enzymatic reactions (Franco, 1996).

11.1.2 Extrinsic factors

Extrinsic factors which affect microbial growth are temperature, relative humidity and the gaseous composition of the environment.

Ambient temperature

Microorganisms can multiply in a wide range of temperatures, usually expressed as minimum, maximum and optimum temperatures for growth. Similarly, the enzymes have optimum temperatures of activity, and their action is decisive in shaping the conservation process. Microorganisms are classified according to their ideal temperature for multiplication. Although there is controversy surrounding these divisions, Table 11.1 presents one of the most accepted classifications.

Table 11.1 Groups of spoilage microorganisms in foods according to their growth temperature

Group	Temperature (°C)		
	Minimum	Optimum	Maximum
Psychrophilic	−15	10–15	18–20
Psychrotrophic	−5	20–30	35–40
Mesophilic	5–10	30–37	45
Thermoduric	15	42–46	50
Thermophilic	25–42	50–80	60–85

Source: Massaguer (2005)

A large majority of thermophilic bacteria which cause spoilage in food and are pathogenic belong to the genera of *Bacillus* and *Clostridium*. Fungi are able to grow in a wider range of temperatures than bacteria and can multiply even in refrigerated (chilled) foods, while yeasts are generally mesophilic or psychrophilic (Franco, 1996; Massaguer, 2005).

Relative humidity

There is a close correlation between the A_w of a food and the equilibrium relative humidity (E_{RH}) of the environment. When the vapor pressure of water is in equilibrium with that of the environment, at a certain temperature, A_w can be expressed as $A_w = E_{RH}/100$. Thus, if a fruit product is stored in an environment with different RH it will either absorb moisture from the environment or lose moisture to the environment, thereby altering its A_w. These changes trigger consequent modifications in microorganisms' growth capacity.

Gas composition of the environment

The composition of the gaseous environment surrounding a food can determine the predominant types of microorganisms in this atmosphere. The presence of oxygen favors the multiplication of aerobic microorganisms while its absence favors anaerobes, which affects the oxidation reduction potential. Vacuum packing with modified atmosphere, where oxygen is totally or partially replaced by other gases, as well as active packaging, are both used as technological advances to extend the shelf life of fruit products.

11.1.3 Implicit factors

Implicit parameters are mutual influences, which may be synergistic or antagonistic, between the primary selection of organisms that result from the influence of the intrinsic and extrinsic parameters (Veld, 1996). Thus, implicit parameters are the result of the development of a microorganism which may have a synergistic or antagonistic effect on the microbial activity of other microorganisms present in the fruit product (Mossel *et al.*, 1995). This concept is applied in the development of alternatives for combined methods of preservation, based on hurdle technologies.

11.2 Microbiological aspects

Many organisms, particularly acid-loving or acid-tolerant bacteria and fungi (yeasts and molds), utilize fruit as their substrate, causing spoilage, product discoloration and consequently producing off-flavors and odors (Esteve and Frígola, 2007). If the contaminating microorganisms are pathogens, they could also cause human illness. Before pasteurization, fruit juices contain a microbial load which is representative of the organisms normally found in fruits during harvesting, along with additional postharvest contaminants associated with transport, storage and processing. Of all types of microorganisms, yeasts are known to be the predominant fungi involved in juice spoilage (Parish and Higgins, 1989).

Fruits which are damaged and rotten have a high level of contamination, and it is possible for only a small number of these to contaminate the whole processing line. Thus the implementation of Good Agricultural Practices (GAP), at agricultural level, as well as Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP), during processing, is essential in assuring a high level of safety in these products (Roever, 1998). Awareness of the close association between water- and food-borne diseases is growing; as a result, rational water use management plans must be developed within the food industry in order to maximize levels of protection against such diseases (Kirby *et al.*, 2003).

The microbiota responsible for contaminating fruits and their products may already be present in part in the pulp and juice; these may also be introduced if the hygienic conditions of the processors and industrial installations are unsatisfactory. Generally the high level of acidity in these products does not favor the proliferation of microorganisms, especially pathogens (Siqueira and Borges, 1997).

It is also important that the pH value should not be looked at in isolation but should be considered as one of several factors which can influence the microbial multiplication. Certain strains of microorganisms can develop resistance to lower pH levels and can therefore multiply under adverse conditions (Uboldi Eiroa, 1996).

While some microorganisms can grow at extremes of pH, many microorganisms, mainly bacteria, have an optimum pH close to neutrality and grow between pH 5.4 and 6.3. Filamentous fungi and yeasts are acid-tolerant microorganisms, which grow well in pH below 4. Some bacteria, including some species of *Lactobacillus*, can also cause deterioration in acidic fruits (pH < 4.5),

Kimball (1999) suggests that the major cause of juice rejection is the buttermilk-like off-odor, found especially in citrus juices. These non-desirable characteristics in juices are caused by a compound called diacetyl which in turn is produced by the growth of lactic acid bacteria (*Lactobacillus* and *Leuconostoc*) (Hendrix and Red, 1995), with *Lactobacillus brevis* among the most important of the flavor-altering microorganisms.

According to Warth (1986), the main yeasts associated with the deterioration of acidic products are *Zygosaccharomyces rouxii*, *Z. bailii*, *Z. bisporus,*

Torulopsis sp, *Pichia membranaefaciens*, *Candida krusei*, *Brettanomyces spp* and *Schizosaccharomyces pombe*. Tandon *et al.* (1983) reported that the majority of fungi and yeasts isolated from guava pulp prepared with the use of additives consisted of *Alternaria sp*, *Helminthosporium sp*, *Candida sp*, *Kloeckera sp* and *Saccharomyces*.

Uboldi Eiroa (1983) highlights the spoilage caused by *Z. baillii* yeast as the greatest risk to biological stability and quality of acidic foods or foods with high sugar content, because of its high tolerance to chemical preservatives at low pH and A_w and in the presence of ethanol, glucose or sodium chloride. Features of the deterioration caused by *Z. baillii* are the production of undesirable odors, film formation on the surface, gas production, changes in texture, color or formation of slime. A lack of good hygiene and sanitation practices can lead to microbial contamination in production line equipment.

Tchango *et al.* (1997) studied the resistance of two yeasts, *Candida pelliculosa* and *Kloeckera apis*, isolated from fermented pasteurized pineapple juice, guava and passion fruit nectars. The study evaluated the efficiency of the pasteurization process used for beverages such as pasteurized tropical fruit juices and nectars produced in Cameroon. The results showed that 22% of the pasteurized fruit juice samples contained fungi, which could be a result of either inadequate pasteurization or post-pasteurization contamination occurring during bottle cooling and bulk storage.

Although only a limited number of *Aspergillus* and *Penicillium* species can attack living plant tissues, they are frequently encountered as postharvest contaminants of fruit products. *Aspergilli* and *Penicillia* can contaminate fruits at different stages, including during harvest, processing and handling. Postharvest contamination can lead to changes in the quality and nutritional value of fruits. The most important aspect of food spoilage caused by these organisms is the formation of mycotoxins, which results in harmful effects on human and animal health (Varga *et al.*, 2008).

Drusch and Aumann (2005) reported that most fruits grown in tropical and subtropical regions seem to be contaminated with *Aspergillus* species because they are ideally adapted to the climatic conditions prevailing in these regions. The optimum temperature for mycotoxin formation by *Aspergillus* species is 25–28°C, whereas the optimum temperature for mold growth lies between 30 and 35°C. *Aspergillus species* were present in 70% of Argentinean grapes while *Alternaria* was present in 80% of grape samples (Drusch and Aumann, 2005).

Tropical fruits can be contaminated with heat resistant molds and bacterial spores, especially in fruits such as pineapples, which grow near the soil (Reyes *et al.*, 2004). Spores of heat resistant molds, especially *Byssochlamys fulva* and *Byssochlamis fulva*, are extremely resistant to heat treatment and other preservation methods, and are potential producers of mycotoxins such as patulin, which is of major concern for food processors (Ferreira *et al.*, 2009).

Since the outbreaks of pathogens, particularly *Eschericchia coli* O157:H7, which have been reported in some fresh juices (Cody *et al.*, 1999; Singh *et al.*, 1996; Sharp and Reilly, 1994), the image and safety of juices have been affected,

and the FDA recommends that juice manufacturers should increase safety measures by introducing the Hazard Analysis and Critical Control Points (HACCP) system in their processes and should apply a pasteurization treatment that will ensure five decimal reductions of the target pathogen (U.S. Food and Drug Administration, 2001).

11.3 Enzymes

The presence of enzymes in fruits is responsible for several processes such as ripening and flavor formation. As a rule most of these changes are undesirable, even if the enzymes do not provoke reactions which may be hazardous for consumption. The principal changes caused by enzymes in fruits are browning, loss of texture and modification of flavor. The enzymatic hydrolysis of proteins frequently leads to the formation of a bitter taste. Due to the presence of pectin in virtually all plant tissue, pectinolytic enzymes lead to loss of texture, causing excessive softening in many fruits, which promotes further microbial attack.

11.3.1 Enzymatic browning

Browning reactions are common in fruits, usually occurring when they are processed by cutting, crushing or pulping or when they are mechanically injured. Typical examples of tropical fruits which display enzymatic browning are banana, pineapple and avocado.

Enzymatic browning converts phenolic compounds into pigments such as melanins which are brown or dark in color. The peroxidase (POD) and polyphenoloxidase (PPO) enzymes are known to be responsible for causing loss of quality in many products. These enzymes can participate in a large number of reactions including oxidative degradation (such as chlorophyll degradation), oxidation of phenols, oxidation of indole acetic acid, lignin biosynthesis, etc. The occurrence of these reactions can lead to darkening of the product and changes in taste and can even change the product's nutritional value (Valderrama *et al.*, 2001).

The characteristics of these enzymes vary from fruit to fruit and even between cultivars of the same fruit. For example, a comparison of peroxidase between two pineapple cultivars showed an optimal temperature activity of between 45°C and 50°C for one, and between 50°C and 55°C for the other. Both peroxidases showed optimal activity at pH 4.5 and were stable at pH between 4.0 and 9.0, retaining more than 80% of activity after 24 hours of incubation at 50°C.

11.3.2 Pectic enzymes

Pectins are present in the cells of almost all plants, and are released when these are triturated. Pectin is a chain of α 1,4 glycosidic galacturonic acid. There are three types of pectic enzymes: pectin esterase, pectin lyase and polygalacturonase.

Pectin esterases hydrolyse the methoxyl group of pectin molecules to form the low-methoxyl pectin and polygalacturonic acid. Polygalacturonases hydrolyze the α-1.4 links of polygalacturonic acid to produce oligogalacturonic compounds and galacturonic acid. This enzyme exists in both exo and endo form: the former hydrolyzes the polymer at random while the latter hydrolyzes the polymer sequentially, from the far ends of the molecule. However, endopolygalacturonase causes more rapid depolymerization of the pectin molecules.

One of the biggest causes of turbidity in juices is attributed to the presence of pectins. As some of the carbohydrates in the pectin molecule are esterified, the colloidal stability of particles is ensured, preventing sedimentation in juices and pulps. However, in the presence of pectic enzymes, pectin molecules are hydrolyzed, losing their colloidal characteristics, which facilitates sedimentation. Pectic enzymes are thus often used for the clarification of juices, for which other processes such as filtration or centrifugation are also employed. However, in the production of higher viscosity juices, fruit which contains high levels of pectic enzymes undergoes thermal inactivation in the early stages of processing, preventing the juice having low viscosity.

Most of these processes control enzyme activity through the use of some form of heat application. Alternatively, chemical treatment, either alone or in combination with heat treatment, can be used aiming at reducing the negative effects of heat and ensuring that the fruit and fruit products have greater freshness. However, such chemical treatments sometimes involve chemicals that are increasingly restricted by consumer preferences and legislation worldwide.

11.4 Principles of conventional methods of preservation

The most common methods of food preservation in general, and of fruit preservation in particular, are based on the adequate control of intrinsic and extrinsic determinants as discussed above. Thus, any change in the conditions ideal for the occurrence of deteriorative processes – including growth of pathogenic and spoilage microorganisms, enzymatic reactions, chemical processes, lipid oxidation reactions, non-enzymatic browning and physical alterations such as clustering – can prevent or slow down deterioration, consequently allowing increased storage time, which in turn facilitates transportation, marketing, handling, distribution and consumption.

Other methods involve subjecting the food to high temperatures, thereby destroying or inactivating the agents that affect quality and food safety. These methods involve increasing the temperature of food to above the upper limit for growth of microorganisms or for activity of deteriorating enzymes. Depending on temperature, exposure time and heat resistance of the deteriorating agent, the effect may be a high or low level of inactivation or complete destruction. Several processes involve the application of heat to preserve food and these comprise mainly thermal pasteurization, sterilization and thermal blanching. The selection of the appropriate procedure to be used for preservation depends on the

characteristics of the fruit and on the expected results related to shelf life and storage conditions.

Another principle of fruit preservation processing involves the reduction of the temperature to a level below that required for the development of deteriorative processes. In this case processing operations include refrigeration and freezing, which act to delay or slow down the deteriorative processes that occur during storage. However, these operations do not destroy the agents responsible for deterioration. As a result, the processes that are terminated during low temperature storage can often restart at the onset of better conditions for their growth.

The decrease in water activity is one of the main intrinsic factors associated with the decrease of deteriorative processes. It is the basis of other conservation processes such as drying or dehydration, as well as being the motivation behind alternative strategies such as the addition of solutes, such as sugars, to the fruit. Thus the goal is also to change those conditions of the fruit which are favorable to decay processes such as growth of microorganisms and reactions catalyzed by enzymes.

The adjustment of pH, another intrinsic factor, is achieved through food acidification or by promoting fermentation, which results in the production of lactic and acetic acids. Since many tropical fruits are acidic, this treatment is less common, and is generally used in association with other processes, such as heat application, which can then be of lower intensity as a result. Tropical fruit chutneys, such as that made from mangoes, are one example of this, as the acidity provided by vinegar is combined with other preservation agents such as sugar and condiments, which also provide the appropriate taste.

Processes associated with the control of oxidation reduction potential consist mainly of removing or decreasing the availability of oxygen for deteriorative reactions such as oxidation of lipids, vitamins or pigments. Moreover, the growth of microorganisms and enzymes is delayed. This is the basis for vacuum application in products prior to the closure of packages as well as in processes such as controlled or modified atmospheres, in which oxygen concentration is altered or adjusted in order to prevent deterioration.

11.5 Fruit preparation for preservation purposes

11.5.1 Preliminary treatments

Preliminary treatments include operations which are also carried out for other processes such as drying, and are particularly important for cooling and freezing, where the main objective is to preserve the freshness characteristics of the fruit.

Some details regarding cleaning are necessary in view of its importance to the processes that involve cooling as a form of preservation. This step must occur separately from the other operations so as to avoid cross-contamination. Quality standards should be well established; however the higher and more stringent the standards applied, the greater the losses.

This stage covers cleaning, washing and disinfecting processes, which are carried out in order to separate, remove and reduce contaminants. The fruit then has a clean surface, limiting the chances of recontamination.

Cleaning involves the removal of foreign material, such as branches, twigs, stems, stalks, soil, insects and fertilizer waste from raw material. This process also prevents damage to equipment that may be caused by foreign objects. The separation of light materials from heavy materials can be facilitated by gravity, screening, etc. This step can also remove parts that are damaged or diseased, according to a pre-established quality standard. Correct preprocessing procedures should be ensured so as to remove the extraneous materials as soon as possible.

Washing can be preceded by a pre-wash which serves to remove the dirt attached to the plant. A washing system (water spray, shower, sink, bath with bubbling air, etc.) is appropriate for the removal of dirt that is retained particularly in small fruits. The presence of sand, for example, can dramatically decrease the life of equipment such as colloid mills and homogenizers, which are commonly used in the processing of fruit juices.

The temperature of the wash water, contact time, and use of surfactants (concentration, monitoring) are important factors in the efficiency of this operation. Detergents (liquid soaps) are commonly used by spraying or immersion with or without stirring, for 10 to 15 minutes. Rinsing should be carried out with drinking water with 2 to 5 ppm free residual chlorine.

To sanitize the equipment, 100 to 200 ppm of free residual chlorine is usually used (when allowed by the legislation) for 10 to 15 minutes of contact. Once the fruit is sanitized, it should be rinsed with water containing 2 to 5 ppm of available chlorine (again, if permitted by legislation). The most common substances permitted for sanitizing are dichloroisocyanurate or sodium hypochlorite. The concentration should follow the manufacturer's recommendations, taking into consideration the nature and quantity of soil material, the type of product or plant material, the shape of the surface, the water quality such as color, odor, taste, hardness, the pH and microbial contamination. Sanitation is generally performed at the washing stage, when it is important to determine which sanitizing agents should be used, how they should be prepared, added and controlled and the minimum contact times and temperatures required. Laboratory testing for confirmation of its suitability for use may be necessary, especially in the case of organic products.

Blanching is not only carried out before more intense heat processes, but also in the preparation of the raw materials for other preservation techniques. Blanching of fruit is much more complex than blanching of vegetables; however, its main objectives are relatively similar: partial reduction of the microbial load, mainly on the surface of the product, and inactivation of enzymes.

Blanching can be performed by using hot water, steam, pressurized steam, hot air or microwave. However, whichever method is used (application of steam is the most common), the time needed for inactivation of enzymes is variable, and

Table 11.2 Inactivation conditions of enzymes commonly present in fruits

Fruit	Temperature/Time conditions for thermal inactivation
Mango	80°C/5min
Guava	85°C/7.5min
Papaya	70°C/3min
Avocado	85°C/10min
Assai	90°C/5min

Table 11.2 presents some specific data for some fruits, noting that nutrients such as minerals, vitamins such as thiamine, riboflavin, niacin, ascorbic acid, and free amino acids are lost or reduced during blanching since thermal damage is almost inevitable.

Even for blanching, water consumption should be evaluated. In general, for each ton of product, the water consumption involved in each treatment is as follows: hot water – > 4.000L; vapor – 200L; microwave – 177L; hot air – 0.08L; and pressurized steam (~0). However, the effectiveness of each treatment should also be evaluated. Of all these treatments, the pressurized steam best meets the requirements and offers certain advantages: it is an automated, computerized control process taking less than two minutes, depending on the pressure and speed used, and has 80% thermal efficiency. Additionally, the same equipment can be used to facilitate peeling.

The efficiency of blanching is based on the evaluation of either peroxidase, measured with guaiacol solution of 0.05%, or of polyphenol oxidase, which is analyzed by using a solution of 0.2M catechol, and these evaluations should be performed regularly.

Besides the microorganisms which cause spoilage of vegetative cells, the main target of blanching are oxidative enzymes. Chemical blanching through sulfitation is still widely used for fruits, although several countries have restricted its use and there is a trend towards its removal, taking Codex standards into account. Chemical blanching shows positive results in fixing the color, reducing losses of carotenoids and vitamin C, inhibiting the Maillard reaction and controlling the growth of yeasts and molds, but can impart strange tastes, destroys vitamin B1, causes equipment corrosion in metal containers and packages, besides being relatively toxic and allergenic to people suffering from asthma. Sulfitation can be performed by exposing the fruit to an atmosphere of up to 2% of SO_2 or by dipping the fruit in water containing up to 1000 ppm SO_2, which is easy and widely carried out. Another alternative means of inhibiting the oxidative enzymes is to dip the fruit in a solution containing 1% citric acid and 0.1% ascorbic acid, followed by consequent draining of the fruit so as to facilitate its drying.

11.6 Refrigeration and freezing

11.6.1 Principles

The cooling process is usually employed for food storage at temperatures between −1°C and 8°C (Fellows, 2006). A large majority of fruit pulps do not freeze at −1°C, as the soluble solids dissolved in the food reduce the freezing point, and temperatures of 8°C or 10°C are already able to reduce the speed at which enzymatic reactions and microbial growth occur in food. Cooling alone is not sufficient to maintain the integrity of food, and other complementary forms of preservation, such as pasteurization, the addition of chemical preservatives or even fermentation, are required.

When fruit temperature is lowered below its freezing point, it forms ice crystals. Both the immobilization of water in ice crystals and the concentration of non-crystallized solutes will contribute to a decrease in the water activity of the fruit. The accelerated lowering of temperature and decreased water activity may ensure the preservation of fruit products for prolonged periods, both from the sensorial and the nutritional point of view.

For both refrigeration and freezing, prior cleaning of the fruit is essential. Neither refrigeration nor freezing alone will be able to reduce the number of microorganisms in the product to satisfactory levels. Even after freezing, thawed contaminated fruits still preserve much of their active microbiota. Even if the cold does cause microbial injury, this should not be considered sufficient microbial control. The basic rule is that fruit which is to be cooled or frozen must already have been sanitized.

11.6.2 Cooling

The main factors which influence the cooling conditions are the chemical composition of the food, its geometry (shape, thickness and volume), its thermal properties (conductivity, diffusivity and specific heat), the initial temperature and the temperature of the coolant, the cooling medium, the interference of biochemical processes and the cost of the process.

Some technological aspects which must be considered are the type of product to be obtained (whole fruit, fruit in pieces, fruit pulp), the fruit variety, the pre- and postharvest maturation stage, and preprocessing methods such as washing, cutting, enzymatic control by heating or chemical blanching procedures.

According to Siriphanich (2002) the only clear physiological difference between the postharvest physiology of tropical and temperate fruit is that the former are sensitive to chilling while the latter are generally not. Most of this knowledge was derived from work with banana, mango, pineapple and papaya. For other tropical fruit, the studies have focused mainly on handling and storage; the lack of physiological research has limited the expansion of tropical fruit in the world market. Individual tropical fruit species are unique and require detailed studies. For example, durian fruit suffer from uneven ripening and mangosteen are affected by husk hardening and a translucent pulp disorder.

Storage of guava at 0 to 10°C extends the postharvest life by about two weeks, while for mangoes the sensitivity decreases as fruit matures and ripens. Mature green mangoes can be stored at 9 to 10°C, but temperatures lower than recommended can cause injury at this stage of development. Mangoes that are almost ripe and have not yet softened can be stored at 7°C without chilling injury (Jagtiani *et al.*, 1988). Bender *et al.* (1995) recommended three weeks at 12°C for mature green mangoes and the same period at 8°C for tree ripe mangoes.

Papaya is sensitive to low temperature, and chilling injury is manifested as a lack of normal ripening, with non-appropriate development of color in flesh and skin. Further negative characteristics resulting from chilling injury include the development of soft and watery pitted tissues, increase of diseases and lower reducing sugar content at 7°C or below. The recommended storage temperature for papaya is at 10°C (Akamine and Goo, 1977).

In fresh non-blanched fruits cooling must be carried out quickly in order to reduce respiration. The most common forms of cooling are forced air circulation, cold water or application of ice. Forced air cooling is the most common and involves the placing of fruit in a cold room. The cold air leaving the evaporator enters the upper part of the roof and moves horizontally over the product, which may be packaged or unpackaged, returning to the evaporator through the product, a path that will cause the least possible resistance (Teruel *et al.*, 2002).

The product is usually cooled at the same place where it will be stored, thereby reducing the amount of handling required. However, when there is limited space, a relocation of the product inside the cooling chamber can be performed, since there is a greater need for cooling space than for storage space. Cooling may be performed alongside other useful techniques for maintaining fruit quality such as that of a controlled atmosphere.

Postharvest cooling of fruits can significantly decrease the biochemical changes and other alterations caused by fungi during storage. In general, for each increase of 10°C above the optimum temperature for storage the viability of fruits decreases two to three times.

Plant size and time required to cool a product are calculated based on methods of transient heat transfer. Several observations can be made to simplify the calculation. A uniform initial temperature which remains constant throughout the fruit or fruit product and a constant cooling temperature are usually assumed, with the respiration activity and all the thermal properties of the fruit to be stored taken into account.

Once the product is cooled, its temperature should be maintained during storage. In order to achieve this, cold rooms are usually equipped with a system of cold air circulation and the fruits or fruit products are distributed throughout the chamber on pallets or shelves so as to ensure excellent circulation of the cooling medium.

In the case of modified atmosphere storage of fruits as examined in previous chapters, the atmospheric conditions change according to the respiratory activity of the fruit as the oxygen is consumed and CO_2 is produced. However, in controlled atmosphere storage the concentration of O_2, CO_2 and sometimes ethylene is monitored and regulated (Fellows, 2006). In fruit storage, the concentration of O_2

should not be too low because it can promote anaerobic fermentation leading to the formation of unpleasant alcoholic off-odors.

11.6.3 Freezing

In freezing, the growth of microorganisms is completely inhibited at temperatures below −9.5°C. In general, chemical reactions become very slow and almost negligible at temperatures below −18°C. However, despite the sharp decrease in temperature some enzymes can act even at temperatures as low as −73°C. For practical purposes a temperature of about −18°C is used in the storage of frozen foods. However, it is necessary to control enzyme action and the most common way to achieve this control is through prior blanching of the fruits.

If fruits are not blanched or if freezing drastically changes its properties, the fruit should be frozen as soon as possible in small volumes, so that these can also be thawed quickly, without requiring any heat. In cases where blanching is applied, freezing must be carried out immediately afterwards, preferably with forced air circulation in a fluidized bed.

Freezing can occur in two basic forms: slow or fast. Slow freezing results in the formation of a lower number of larger crystals, while quick freezing causes the formation of several smaller nuclei of crystallization. Large ice crystals tend to break down the cell structure and cause irreversible damage to the texture and organoleptic properties of the fruit. As fast freezing forms smaller ice crystals, it causes less cell damage leading to lower leaking of intracellular material and salt diffusion, resulting in a thawed product with better organoleptic characteristics. Variation in freezing temperatures during storage may cause recrystallization, suggesting that storage temperature control is critical to the success of the process. It is worth noting that most of these sensory changes become visible only when the fruits are thawed.

In slow freezing, a temperature of about −18°C is normally used and carried out in smaller installations, while quick freezing is typically associated with larger installations and uses temperatures of about −40°C.

In addition to quick freezing, some other measures which could minimize the negative effects of the freezing process are slow thawing under cold conditions, addition of solutes such as sugar and anti-oxidants, effective blanching operations, the use of containers which protect the product from oxygen and moisture loss, and the maintenance of the cold storage and distribution chain at a constant temperature of about −18°C. Sugar syrups, especially of sucrose and glucose, are widely used in fruits because they provide a sweet taste, allow the retention of aroma and volatiles, decrease the quantity of frozen water and restrict enzymatic browning due to the barrier to O_2. Sodium chloride is also used as a cryoprotectant solute for frozen fruit. Solutions of between 1 and 3% are generally used. Immersion of very soft fruit in $CaCl_2$ solution varying from 0.05 to 0.1% minimizes loss in fruit texture.

The main antioxidants used in the freezing of fruits and fruit pulps are sulfur dioxide, organic acids and ascorbic acid. Sulfur dioxide acts chemically on enzymes and substrates. However, sulfur dioxide treatment is not recommended for fruits that will not undergo any further heating operations since the odor in the

final product could be unpleasant. Furthermore, the use of these salts may be restricted in accordance with the legislation of certain countries, as previously mentioned. The action of organic acids is related to the lowering of pH in regions where enzyme action is at its weakest. The acid keeps the phenolic substances in a reduced state and hence does not alter the original color. Table 11.3 presents some methods for preparing the fruit for preservation by freezing.

11.6.4 Freezing process

In fruit freezing, two procedures are used. The product can be packed and then frozen and vice versa. Packaged products are frozen by forced air circulation on plates. Freezing is very common for fruit pulps packaged in plastic films for the retail market. Forced air freezing can occur both in cabins and in freezing tunnels, in continuous processes. Unpackaged products can also be frozen by forced air in conveyors or in fluidized bed process, where the direct contact of the coolant with the product leads to relatively fast freezing. It is also possible to apply the principle of Individual Quick Freezing (IQF), where the air flow or the movement of the conveyor belt allows individual fruits or fruit slices to freeze without becoming attached to each other. In this case the frozen products can be packaged after freezing.

Another freezing method is cryogenic freezing, in which a refrigerant, normally liquid, is sprayed on the product and as the cryogenic agent evaporates quickly, freezing takes places extremely quickly. One of the most widely used cryogenic agents is liquid nitrogen at −196°C, which is able to remove 358kJ kg^{-1} of latent heat of vaporization, which when added to its sensitive heat equals about 690kJ kg^{-1}. The product is packaged in an isolated stainless steel chamber and sprayed with liquid nitrogen. Centrifugal fans then distribute the gas, causing evaporation and standardizing a uniform temperature in the container. This technique, although quite efficient, is usually more suitable for low volume production or processing of products with high added value. Table 11.4 presents

Table 11.3 Preparation methods for freezing

Fruit	Preparation	Packaging
Avocado	Peel and separate the core. Cut into halves and the pulp disintegrating.	Add 2.5 g of ascorbic acid per kg of puree. Packed in small size for a quick freezing.
Citrus fruits or slices	Select the firm, free of spots, wash and peel.	Wrapped in a syrup to 40% sugar or in the juice. Add 2.5 g of ascorbic acid for each kg of product/juice or syrup.
Melons and watermelon	Select firm, well colored and mature. Wash, cut into slices or cubes.	Wrapped in a syrup of 30% or dry. The pulp can also be crushed (except watermelon), adding 15 g of sugar for every 250 g of pulp. Packed in small size for a quick freezing.

Source: Adapted from Archuleta (2003)

Table 11.4 Estimated time for various types of freezing

Method	Approximate freezing time
Air blast	3 to 5 h
Plate	1/2 to 2 h
Bulk freezing – air blast:	
Belt	20 to 30 min
Fluidized belt or tray	5 to 10 min
Cryogenic freezing	1/2 to 1 min

Source: Fellows (2006)

the freezing times associated with different types of freezing processes, based on a package of 280g of food, according to Fellows (2006).

Some specific details regarding the freezing of tropical fruits were described by Reid and Barrett (2004), Kendall (2008) and Jagtiani *et al.* (1988), as follows:

- Avocado presents a challenge for commercial freezing due to its high oil content (which easily becomes rancid) and also because of its very active oxidative browning system. Avocado puree preservation is enhanced by lowering the pH to below 4.5 with citric acid, by avoiding oxidation through the addition of ascorbic acid and by adequate packaging, possibly under nitrogen. Vacuum packaging has also been employed together with rapid freezing in order to achieve a storage temperature around −18°C.
- Sliced mango can be frozen in syrup containing ascorbic acid to inhibit browning induction by polyphenol oxidase. Purees can be single or double strength according to concentration level and the storage temperature should also be at −18°C or below. Higher storage temperatures can result in a significant browning problem for mangoes as a result of non-enzymatic browning.
- Melon must be frozen when the texture is firm enough to allow cutting into cubes or balls that retain textural integrity. If it is too ripe, a very mushy product may result due to considerable texture losses commonly verified in fully thawed melon. Melon can be better frozen in 30% sucrose syrup.
- Papaya puree must be prepared from ripe fruit that can be sliced and crushed following the separation of the skin from the pulp. The pulp is acidified to a pH below 4.5 and is then passed through a heat exchanger to inactivate enzymes before cooling and freezing to −25°C.
- Pineapple for freezing can be prepared in the same way as pineapple for canning. Rectangular chunks are filled in syrup into cans or bulk containers and frozen. The cans are next frozen in a blast tunnel, the bulk containers in a blast freezer. The 'Smooth Cayenne' variety should preferably be used for freezing because the 'Red Spanish' variety has a tendency to develop off-flavors.
- Shredded coconut can be frozen without any particular preparation. The rate of freezing must be sufficiently rapid to minimize microbiological contamination and the storage, in large containers, should be at −18°C.

- Passion fruit pulp pasteurization inevitably leads to some losses of fresh fruit flavor and its high starch content causes accumulation of gelatinous deposits on the surfaces of heating exchangers. Freezing is commonly chosen as the method of preservation with no prior heat treatment, if this is allowed by country legislation.

The shelf life of frozen fruits or purees is generally long, reaching up to several months or even years. However, there is often moisture loss if the fruits or fruit slices are not protected by materials impermeable to water and water vapor, which then often results in weight loss.

The time to freeze solid foods can be estimated based on Plank's Law, described in Equation 11.2:

$$t_F = \frac{\rho \Delta H}{T_F - T_\infty}\left[\frac{L}{6}\left(\frac{1}{h_C} + \frac{x}{k_1}\right) + \frac{L^2}{24k_2}\right] \quad [11.2]$$

Where,

t_F = Freezing time (s)
L = Length of the cube (m)
h_C = Coefficient of convective heat transfer on the surface ($Wm^{-2}k^{-1}$)
T_F = Initial freezing point (°C)
T_∞ = Temperature of the cooling medium (°C)
ρ = Density of the unfrozen food (kgm^{-3})
ΔH = Latent heat of crystallization ($J\ kg^{-1}$)
x = Thickness of the package (m)
k_1 = Thermal conductivity of the packaging material ($Wm^{-1}k^{-1}$)
k_2 = Thermal conductivity of food ($Wm^{-1}k^{-1}$).

Numbers 6 and 24 in the equation are factors related to cube geometry. For a flat plate, the constants used are 2 and 8; for a cylindrical shape, the values are 4 and 16; and for sphere-shaped food materials constant values of 6 and 24 are used.

The engineering properties of foods are important parameters in the design of a freezing process, but very often the thermal diffusivity, thermal conductivity and specific heat of many fruits, juices and purees are very similar to those of water, which can therefore be used as approximate values. In the 12 fruit products (fruits, juices, concentrated juice and fruit preserve) listed by Hayes (1992) the thermal conductivity ranged from 0.415 to 0.571 W/mK; the specific heat from 3.01 to 3.98 kJ/kg°C and the thermal diffusivity from 1.17 to 1.39 10^{-7} m^2/s, while water showed respectively 0.594 W/mK (at 0°C); 4.2 kJ/kg°C and 1.48 10^{-7} m^2/s (at 30°C). Other approximate values for engineering properties of foods and useful equations for their prediction can be found in Alvarado and Aguilera (2001).

Regarding the ideal storage conditions for the avoidance of undesirable reactions, the classic Arrhenius model relates the reaction rate, k, to the temperature at which the reaction takes place, according to Equation 11.3.

$$k = k_0 \cdot e^{\left(-\frac{Ea}{R}\cdot\frac{1}{T}\right)} \quad [11.3]$$

Where,

E_A = Activation energy (kJ mol^{-1})
R = Constant of the gases = 8.31439 (J mol^{-1} K)
k = Reaction rate (time^{-1})
k_0 = Arrhenius constant (time^{-1})
T = Temperature in Kelvin.

This is the most universally accepted means of describing the variation in reaction rate as a function of temperature. Numerous reactions of chemical dynamics can be explained by this equation (Formosinho and Balconies, 1986).

The coefficient Q_{10} is the ratio of speed (k_T) of a reaction at a given temperature T in relation to the other reaction rate at a temperature 10°C above the first (k_{T+10}), as in Equation 11.4:

$$Q_{10} = \frac{k_{T+10}}{k_T} \quad [11.4]$$

As the temperature drops, the rate of most of the degradation reactions also falls and shelf life can be related to Q_{10} as follows, according to Equation 11.5.

$$Q_{10} = \frac{k_T}{k_{T-10}} = \frac{S.L._{(T-10)}}{S.L.} \quad [11.5]$$

where,

$S.L.$ is the shelf life at room temperature T and
$S.L._{T-10}$ is the shelf life-at a temperature below 10°C.

Based on such equations it is clear that the storage temperature should be as low as possible to avoid undesirable changes in quality. Storage temperatures of frozen products are commonly at −18°C, but may vary between −18°C and −23°C.

11.7 Drying

Drying is defined as the application of heat under controlled conditions to remove the water contained in food through vaporization. The main objective is to prolong the shelf life of the fruit by reducing water activity, which inhibits microbial growth and slows enzymatic activity. The slowing of enzyme activity is usually reversible and the enzymes contained in fruit can regain their activity if moisture is reintroduced, which leads to changes in color and texture as well as loss of vitamin C.

The main advantages of the dehydration of fruits are:

- Direct consumption of dried fruits which commonly have a sweet flavor and moderate acidity. In addition, the nutritional value is concentrated. Table 11.5 presents the nutritional values of some fresh and dried fruits.
- The utilization of dried fruits in the formulation of products such as yogurts, fruit juices, cakes and other ready-to-eat preparations.
- Reduction in weight that can reach levels as high as 80%, which means a substantial saving in freight, transport, packaging and physical space for the dehydrated product.

11.7.1 Dehydration process

The drying of fruits can be performed in commercial dryers or can be achieved naturally by the sun (solar drying). In the case of solar drying, the fruit is either sliced or used whole, provided that the geometry is appropriate. Fruits placed in trays exposed to the sun tend to achieve equilibrium with the relative humidity of the environment, so the final moisture content is generally around 25% w/w. In commercial dryers, drying conditions are better controlled and the drying process is more efficient, so processing time is reduced and the quality of the product is maintained. In this case, the final moisture content may be in the range of 25% to 5% w/w. Just as in sun drying, fruits may be sliced in order to increase the surface area, which results in a consequent increase in drying rate.

Fruit in the form of a puree or juice can be dried in a drum dryer, where the puree passes through rolls or drums, or in spray dryers where drying is performed by atomization. Lyophilizers are a very versatile piece of equipment where fruit presentation is concerned, as they can dehydrate food materials in particle form or

Table 11.5 Comparison of nutritional values of some fresh and dried fruits

Fruit	Water	Protein	Carbohydrates	Kcal 100 g^{-1}
Red guavas				
Fresh	85.0	1.1	13.0	54
Dry	5.0	7.0	82.3	357.2
Jabuticaba				
Fresh	83.6	0.6	15.3	58
Dry	5.0	3.5	88.6	368.4
Aden Mango				
Fresh	82.3	0.4	16.7	64
Dry	5.0	2.1	89.6	367.1
Pitanga				
Fresh	88.3	0.9	10.2	41
Dry	5.0	7.3	82.8	360.5
Umbu				
Fresh	89.3	0.8	9.4	37
Dry	5.0	7.1	83.5	362.2

Source: adapted from NEPA (2006)

small whole fruits, juices or purees. Finally there is an increasing interest in developing the technology of preserving fruit by osmotic dehydration, as this technique improves the overall quality of fruit whether whole or in slices, with a saving in energy compared to traditional processes of drying. The use of this technique also allows products to be obtained that maintain a high degree of similarity with fresh fruit, which is a key factor in consumer acceptance.

11.7.2 Principles of drying

The drying process involves both the application of heat and the removal of water from food. This requires knowledge of the properties of water, psychrometric parameters, heat and mass transfer aspects.

There are three factors that control the ability of air to remove water from a food: the amount of water vapor present in the air, the air temperature and the amount of air passing through the food. The amount of water vapor present in the air is expressed as absolute humidity (AH) expressed as kg water kg^{-1} air, or as relative humidity (RH). The relationship between the RH, AH and air temperature can be shown through psychometric charts or diagrams.

When food is heated by air, it receives sufficient latent heat to allow the evaporation of water on its surface. The temperature of this air is called dry bulb temperature (DBT). If the temperature sensor in the dryer is constantly moistened by some tissue, heat is removed by the evaporation of the water from the sensor. The temperature is then lower than dry bulb temperature and is therefore called the wet bulb temperature (WBT). The drier the air the greater is its ability to remove moisture, adjusting the imbalance between the moisture in the air and the moisture of the product. Thus, the drier the air the greater the difference between the DBT and WBT. When the air is saturated, or its RH reaches 100%, the DBT and WBT are equal. The difference between the DBT and WBT is also useful in determining the AH and WBT of the air, and can be found in the psychometric chart. The dew point is the temperature at which air becomes saturated with water (100% RH) and any cooling beyond that point results in the condensation of water from the air. When warm air is relatively dry and passes through the surface of the food, the water vapor on the surface of the food spreads across the boundary layer of air around the food and is carried away by moving air. A pressure gradient of water vapor is established between the interior of the moist food and the dry air, providing the driving force for the dehydration of food. The boundary layer is the barrier both for heat transfer and for the removal of water vapor, and its thickness affects the rate of the drying air. A slow rate means that the boundary layer will be thicker, which reduces heat transfer and moisture removal. For any drying process involving air as the gaseous phase introduced for the removal of water, regardless of the drying method, it is important to maintain the DBT at the highest possible level (preventing cooking of the product, burning of the surface, crust formation and losses in sensory and nutrition values), lower relative humidity and much higher air velocity.

Once the drying process starts, the moisture of the product will vary over time. Usually a curve plotted with moisture date of food versus the drying time

represents this profile. However, more careful observation will reveal that when the drying process starts the product surface is heated to the WBT in a short period of stabilization. Then, the water will begin to move inside the product at the same rate at which it is evaporated from the surface in a relatively short period at a constant drying rate. This phase continues until the product attains its critical moisture content, at which point the drying rate starts to decrease until it reaches equilibrium with the moisture content of the drying air. After this it is no longer possible to remove water from the product using this process. All structural changes and most of the sensory changes occur at the end of the constant drying rate period because the product temperature, which was WBT initially, moves toward DBT. This occurs because the rate of water flow from the interior to the surface of the food becomes lower than the rate at which it evaporates into the air which circulates through the food; thus, the surface becomes drier.

11.7.3 Principal equipment

Dehydration of fruits by forced air circulation can be carried out in several ways. Various types of equipment have been developed, the use of which is dependent on the characteristics of the fruits to be dried, the type of processing facility, the volume to be dehydrated, and especially on the attributes of final dried product. Industrial dehydration in small or large trays with forced air circulation is the most common means of dehydration on the market. The trays are very flexible, and are easy to construct and maintain. The equipment consists of a cabinet with trays where the product is dehydrated. The trays are usually perforated, allowing air to flow parallel to the tray, both below and above. The product, usually in pieces or slices, is placed on the trays.

The geometry of the fruit slices is important and the drying time is inversely proportional to the drying area. Purees and pastes are sometimes distributed over a whole tray to dry, but this reduces the efficiency of the process. Fans should ensure air circulation and uniform temperature throughout the cabinet. The uniform distribution of heat throughout the cabin is the biggest challenge for this type of equipment, especially for large dryers. The heating of the air can be carried out by radiators, electric heaters, steam coils, hot water or hot air.

In addition to the geometry of the product, the speed and rate of air flow over the product is also important in the control of the air temperature, which should usually be around 60–80°C, and its relative humidity. The best temperature profile for specific fruits in the dryer should be determined by previous technological experience. Some products are sensitive to the formation of a crust on the surface due to protein denaturation, shrinkage and the presence of dry fibrous material. This may cause the formation of a sealing surface on the fruit, which prevents drying of the interior. In these cases the fruits should be dried gradually by using lower temperatures at the beginning of the process.

The incorporation of explosion puffing into a hot-air dehydration process facilitates faster dehydration and leads to a final product with a highly porous structure that is capable of rapid rehydration. Explosion puffing has been

successful with banana slices (Saca and Lozano, 1992), as well as with temperate fruits such as apples and blueberries (Somogyi and Luh, 1986),

The process starts with conventional hot-air drying of the fruit pieces until a certain water content is attained. Then the pieces are placed in a closed chamber where the pressure is increased by means of superheated steam (Saca and Lozano, 1992). After some time, the pressure is suddenly released by opening the lid of the chamber. Due to the high temperature and the sudden decompression, most of the remaining water in the particles almost instantaneously evaporates. Then the solids are placed back in the air drying equipment to achieve the desired final water content. Due to the highly porous structure of the solids after puffing, the drying time is reduced notably. The key parameters that affect this process are the initial water content of the particles entering the explosion puffing process, the temperature and pressure of the steam in the chamber, and the time inside the chamber (Ratti and Mujumdar, 2004). The optimal initial water content for banana slices was judged to be between 27 and 38% (Saca and Lozano, 1992). In this case it was found that the explosion puffing process decreased the drying time of the whole process by 25%, with an increase of 10.3% to 46.8% in the porosity of the product. This increase in porosity means that the product can be easily rehydrated.

Osmotic dehydration

The preservation of food by osmotic pressure is a very old technique. To some extent, products such as jams, jellies and marmalades have benefitted from this technique for many years. However, what must be examined is the osmotic pre-dehydration of fruits when these are immersed in a concentrated solution of solutes (usually sugars and salt). Osmotic dehydration is unlikely to ensure biochemical and microbial stability, but the low thermal stress involved in the process can minimize the negative effects of other dehydration techniques resulting in better organoleptic and sensory characteristics or at least in a reduction in time and cost of some processes. Thus, it is common for osmotic dehydration to be carried out in association with other processes such as lyophilization, freezing, and drying with forced air.

The mechanism of osmotic dehydration consists of the immersion of the product in concentrated solutions of salt, sugars, or a combination of the two. The cell wall of the food acts as a semi-permeable membrane allowing diffusion of water from the product to the solution on a larger scale than the diffusion of solid solution into this product. The diffusion of solid components of the food to the solution also occurs; however, this is usually negligible and has very little effect on characteristics such as texture or flavor. The process also allows the adjustment of the chemical composition of the fruit by reducing the amount of water, adding solutes or ingredients of interest from a nutritional or sensorial point of view, or incorporating antioxidant additives or preservatives.

The selection of the osmotic agent depends on its ability to lower the water activity, the taste and texture that result, its cost and its safety. Sugar has practically no effect on microorganisms and has a solely osmotic action. In contrast, when a high moisture content is involved, NaCl can effectively reduce the water activity

of the system, and as a result is responsible for the decrease in the solubility of O_2 in the water (reducing problems caused by the oxidation and growth of aerobic microorganisms), and incurs lower cost. However, NaCl also facilitates the loss of some soluble components, is unable to destroy toxins and can transmit undesirable odors if impure.

The main factors influencing osmotic dehydration are the concentration of solutes in the osmotic solution, the molecular weight of the solute, the immersion time, temperature, ratio of fruit to solution, the contact between the solution and fruit and the pressure system, and the pH of the solution.

Souza Neto *et al.* (2004a, 2004b) described dehydration processes for mangoes of 'Coité' variety and Cantaloupe melons using the osmotic process complemented by tray dehydration. Mature fruits that were free of disease were selected, washed, peeled, and cut into 3 cm cubes and steam blanched (100°C/2 min). The fruits were immersed in a 55°Brix sucrose syrup with citric acid (pH 3.0) and sodium benzoate (0.1%) added in a ratio of 1:4 (w/w) for mangoes and 1:2 for melons. For mangoes, the process was carried out at 65°C for four hours followed by tray dehydration at 65°C for 16 hours. For melons the immersion time was five hours and the tray dehydration lasted 12 hours at 65°C. The yield of the process based on the amount of fresh mangoes or melons was ten per cent.

Spray drying by atomization

Spray drying is a method in which food material is sprayed in the form of a suspension of droplets (atomized particles) whose diameter is typically less than 0.25 mm. Drying takes just after a few seconds and the process is relatively inexpensive and offers a high yield compared to other methods of drying.

The drying air is heated with gas by indirect steam, by electric resistance or by a combination of these media. The hot air is moved through the inlet and outlet fans of the equipment. The drying chamber is generally of a cylindrical shape, tapered at the base. The chamber length varies widely, from 4 m for equipment that evaporates around 80 kg of water per hour, up to 20 m for equipment that evaporates over 1 m^3/h of water. At the outlet of the equipment, there is usually a cyclone where separation of the dehydrated product from the moist air occurs.

The temperature of the drying air at the entrance of the equipment is between 180 and 230°C, while at the output it varies from 50 to 100°C. Although the temperature of the air which is in contact with the product is high, the speed of the process ensures the integrity of the sensorial and nutritional characteristics of the food to be dehydrated. At the beginning of drying, the actual temperature of the product is relatively low, close to the wet bulb temperature of the incoming air. As drying progresses, the product becomes partially dehydrated and increasingly resistant to the changes caused by hot air (most degradation reactions slow down with the decrease in moisture content). In fact, when drops leave the atomizer their surface dries quickly and at a constant rate. Solidified solutes and suspended solids quickly form a crust on the surface of the particle, resulting in a decrease in the diameter of the particle. When the crust solidifies, it creates increased water resistance and the drying rate decreases.

The spray process should be fast enough that the particle loses moisture before reaching the surface of the equipment and adhering to it. Thus the drying rate should take into consideration the time at which the droplets leave the atomizer and hit the wall of the equipment. The main process parameters are the path and speed of the particles (determining the required drying time), the temperature of the drying air, the heat transfer coefficient and the drop diameter.

Tanaka (2007) studied the influence of dehydration by spray drying on the stability of ascorbic acid in acerola juice. The level of ascorbic acid determined by High Performance Liquid Chromatography decreased by 80.65% over the duration of the process.

Angel *et al.* (2009) studied the spray-drying of passion fruit juice using lactose–maltodextrin blends as the support material. Results showed that the lowest values of the moisture content and hygroscopicity were reached in the temperature range of 188–190°C and at 12:5% (w/v) concentration of lactose–maltodextrin. The best vitamin C retention level occurred at 180°C and air pressure of 0.2 MPa.

Saiger (2008) carried out studies on guava powder production using a pilot scale spray dryer and evaluated its nutrient retention. The research showed that 170°C was the most appropriate temperature, resulting in minimum vitamin C loss. Vitamin C deterioration was dependent on both temperature and water activity, although experimentally it was proved that water activity plays a more significant role than temperature. First order reaction was used to describe the deterioration of vitamin C in the spray drying process. It was concluded that a fast reduction of moisture content at higher temperature helped to maintain vitamin C content.

Dehydration in drum dryers

Drum dryers are one or more hollow cylinders heated internally, which rotate on their horizontal axis. The cylinders are usually heated by steam, and their temperature is controlled. The fruit, usually in liquid (juice) or paste (puree) form, is added to the surface of the cylinder, forming a film on the stainless steel drum. The film thickness is controlled immediately after the addition of the product which dries as the drum rotates at a pre-determined speed. Once the rotating is completed, the product should be dried and the drum scraped by blades. The dehydrated product takes the form of a film or a granular powder that is usually ground to a uniform size.

Pua *et al.* (2009) studied the optimization of drum drying processing parameters for production of jackfruit (*Artocarpus heterophyllus*) powder using response surface methodology. The results indicated that both steam pressure and rotation speed of the drum drastically ($p \leq 0.05$) affected the overall quality and acceptability of final product. Moisture content and water activity decreased considerably ($p \leq 0.05$) when drum temperature was increased, as did the desired sensory attributes related to Quantitative Descriptive Analysis scores. 336 kPa steam pressure and 1.2 rpm rotation speed were recommended as the optimum drum drying conditions for providing high quality jackfruit powder.

Lyophilization

This process consists of the dehydration of a frozen product by sublimation of ice, which is retained in a condenser. The product is initially frozen below its eutectic point, generally outside the lyophilizer. Once the product is frozen it enters the equipment and is deposited in one of several trays inside the machine. The shape of the product can vary widely, from fruit pieces or whole fruit (usually small, such as cherries and raspberries), to pastes, purees and juices usually triturated after freezing (to increase the surface area). Once inside the lyophilizer, a vacuum system is created, reaching pressure below the triple point of water. On the trays containing the product a heated fluid is circulated, usually ethylene glycol, which provides the latent heat of sublimation by conduction to the product. Other forms of heating by radiation such as infrared light and microwaves can also be used. The temperature profile at the base of the trays is controlled, ensuring the maximum drying speed, without thawing the product. In general the temperature at the base of the tray reaches 30 to 40°C, reaching up to 70°C in some situations. A low pressure system ensures that the product remains in solid state. As the product is dried, the water vapor sublimates, creating sublimation conditions which progress through the product. Channels left by the sublimed ice facilitate the exit of the remaining water. The water vapor then adheres to the surface of the condenser which remains at temperatures around −70°C. The efficiency of this cooling system is essential to ensure the success of the process because when the ice sublimates it generates an internal pressure in the system that could cause thawing of the food. Not all ice is removed from the product by sublimation. After approximately 95% of water is evaporated, a step that takes up 33% to 50% of the total process time, the drying is completed by a desiccation process.

Lyophilization is a relatively expensive process. However, when the dried product is reconstituted with water, some fruits have a spongy structure which closely resembles the original structure of the fresh fruit. Moreover, rehydration is faster and more homogeneous, and the product has improved color, texture and nutritional value compared to other methods of drying.

The main process parameters are the pressure in the chamber, the temperature of the plates, the temperature of the condenser, the arrangement of equipment for heat transfer (conduction, radiation) and the thickness of the product or its geometry.

Guava and mango puree, passion fruit and pineapple juice have been dehydrated in a process that combines vacuum-puffing with freeze-drying as described by Moy (1971). Samples were initially frozen in blast freezers at −40°C or −18°C with 1.22–4.88 kg/m^2 and then vacuum-puffed and freeze-dried on aluminum trays (50μ Hg and 48.9–54.4°C shell temperature for 6–9 hours) to 1–2% moisture. In general, the addition of sucrose to create a mixture of 40°±5° Brix resulted in a stable 'puffed' structure, and several permissible calcium salts and silica at 0.1–0.5% (w/w) added to the mixture before drying improved the free-flowing properties of the dried product.

Menezes *et al.* (2008) evaluated some nutrients found in lyophilized açaí pulp. The results of the analyses showed that the powder product is highly calorific

mainly due to its high lipid content (40.75%), with a total of 489.39 Kcal/100g lyophilized pulp. The total carbohydrate content was 42.53% ± 3.56% and the protein content was 8.13 g ± 0.63/100 g. An evaluation of the mineral profile revealed abundant potassium (900 mg/100 g lyophilized açaí) and calcium (330 mg/100 g lyophilized açaí). A significant concentration of magnesium was also observed (124.4 mg/100 g lyophilized açaí), but only a small amount of iron (4.5 mg/100 g lyophilized açaí). Based on the nutritional evaluation it was possible to conclude that lyophilization can be an excellent alternative means of açai pulp preservation, resulting in a product with high nutritional value.

11.7.4 Calculation of drying rate

Unlike other food processing technologies, such as commercial sterilization of foods of low acidity, dehydration can be used by micro, small, medium and large companies. What is required is knowledge about the process, drying behavior, critical moisture content and its equilibrium in the fruit. When a dehydrated fruit is commercially obtained it is possible to estimate the drying time, regardless of weather conditions. This allows better control over the inventory of raw materials and the productive capacity of the company. The heat transfer is expressed by:

$$Q = h_s A (T_a - T_s) \quad [11.6]$$

where,

Q = rate of heat transfer (J s^{-1})
h_s = convective coefficient of heat transfer on the surface (W m^{-2} K^{-1})
A = surface area available for drying (m^2)
T_a = average temperature of dry bulb temperature of drying air (°C)
T_s = average temperature of wet bulb temperature of drying air (°C).

The mass transfer is expressed by:

$$-m_c = k_g A (H_s - H_a) \quad [11.7]$$

where,

m_c = drying rate (kg s^{-1})
kg = coefficient of mass transfer (kg m^{-2} s^{-1})
H_s = moisture content of saturated air on the surface of the product (kg H_2O/kg dry air)
H_a = moisture content of air (kg H_2O/kg dry air).

Equilibrium between the heat transfer rate for food and the mass transfer rate (moisture loss from the fruit), with a constant drying rate, can be expressed as:

$$-m_c = h_c A (T_a - T_s)/\lambda \quad [11.8]$$

where,

λ = latent heat of vaporization in the wet bulb temperature (J kg^{-1}).

The heat transfer coefficient on the surface (h_c) is related to the rate of mass flow of air through the following equations:

$$h_c = 14{,}3\ G^{0,8} \text{ for parallel air flow} \quad [11.9]$$

and

$$h_c = 24{,}2\ G^{0,37} \text{ for perpendicular air flow} \quad [11.10]$$

Transition temperature in drying process

The total content of water is not a reliable indicator of the shelf life of a food, since the water is associated with the non-aqueous constituent of the food at different levels, and thus its availability to promote physical, chemical and biological reactions can display wide variation. In general, the lower the water activity of a product, the greater the possibility of preservation (Silva *et al.*, 2009).

The concept of phase transitions complements the understanding of the stability of the food: while water activity means the amount of water energy available to process changes, the transition temperature of these changes occurs only if the molecules involved are able to travel to the action site. The phase transition state is a form of matter that maintains the structure, energy (enthalpy) and volume of a liquid, but the modifications in energy and volume brought about by temperature (specific heat and thermal expansion coefficient, respectively) are similar in magnitude to those of a crystalline solid. The most important characteristic change in the amorphous state involves the transition of a solid from a glassy state to a rubbery or gummy state or vice versa. It occurs in so-called glass transition temperature (T_g). T_g is not a clearly located point, but defines the center of a region of ±20°C or higher in which the transformation occurs. Below the T_g, the material is in a solid state that is rigid but brittle. When the temperature increases to above the T_g the material becomes soft or rubbery, with a decrease in viscosity which consequently increases molecular mobility (Silva *et al.*, 2009). In dried and semi-dried foods molecular mobility can cause cracks and structural collapse during drying and hence promote the Maillard reaction, enhancing processes of crystallization and recrystallization as well as starch degradation, among other problems.

Most foods are hydrophilic in character, and display a great deal of compatibility with water molecules, which are the main plasticizer in food. As seen in Table 11.6, the T_g value of water is very low and even the presence of small traces of water in food causes significant decreases in the T_g value of the food. This effect in a tropical fruit and fruit juice is also shown in Table 11.6. The prediction of a decrease in T_g value as a result of the plasticizing effect of water may be useful in evaluating the effects of food composition on the T_g, as these related changes could affect the shelf life of the food.

Table 11.6 Glass transition temperature (Tg) for anhydrous compounds, water, tropical fruit and fruit juice

Composite	T_g (°C)	Composite	T_g (°C)
Fructose	5	Maltodextrin M040 (DE 5)	188
Glucose	31	Maltodextrin M100 (DE 10)	160
Lactose	101	Maltodextrin M250 (DE 25)	121
Maltose	43	Starch dried whey	83.1
Sucrose	52	Water	−135
Starch	243	Banana	−35
Gluten	39.3	Pineapple juice	−37

Source: Adapted from Collares *et al.* (2002)

11.8 Manufacture of fruit beverages and purees

The marketing of fresh fruits over long distances is very difficult because the fruits are perishable foods. The postharvest losses of fruits are estimated to vary from 15 to 50% of the total production in some countries. Moreover, most of the fruits are seasonal and hence are available as fresh fruit for only a few months in a year. Thus, the production of frozen pulp has become an attractive alternative to fresh fruit and can supply the market in the periods between harvests (Bueno *et al.*, 2002). Fruit pulp can be defined as 'the unfermented, not concentrated, not diluted, pulpy fruit product obtained by means of appropriate technological process, having a minimum of total solids obtained from the edible part of fruit' (Brazil, 2000). The fruit pulp must be obtained from fresh, healthy and mature fruits, and must maintain the inherent physical, chemical and organoleptic characteristics of fresh fruit (Brazil, 2000).

The technological processes employed for the preservation and manufacture of fruit pulp are operations such as pasteurization, freezing, acidification, and in some cases, irradiation, all of which have been used by industries for obtaining pulp. Fruit parameters such as pH indicate whether more than one process technology needs to be employed: a low-acid pulp, for example, requires pasteurization in addition to refrigeration or freezing, which may depend on the availability of aseptic packaging or sealing after heat treatment. Another important aspect to be considered along with the pH of the pulp is the final packaging of the product, which may or may not be airtight. If the packaging is not airtight, the addition of chemical preservatives may be necessary.

11.8.1 Extraction of pulp/juice

The two most widely used pieces of equipment for the extraction of juice and pulp are the extractor and the hydraulic press. Pneumatic presses also exist, but the hydraulic types are still the most common. There are several models and sizes, but what is very important, alongside a high level of efficiency, is that all parts in contact with the raw materials should be constructed primarily of acid-resistant stainless steel, since most fruits are acidic. Due to the variation in fruit size and

shape, most of this equipment is not suitable for certain fruits such as passion fruit or açai, for which specific equipment must be designed.

Regardless of the extractor type, larger fruits must be reduced in size in order to extract the pulp or juice. The equipment most commonly used for this purpose is a hammer mill or knife mill, as illustrated in Fig. 11.1.

What differentiates one extractor from another is its size, the perforated openings in the drum and the blades which spin inside the extractor and act as scraper knives or brushes. The fruit is introduced into the central part of the drum, which rotates at high speed, throwing the fruit against the walls of the drum. What passes through the holes in the drum is collected as pulp, while that which cannot pass remains in the drum and consequently ends up being removed as waste, including peel, stones, seeds and other dense materials. Less pulpy juices can be obtained by using a drum with smaller openings. Thus thick pulps need openings in the drum of between 1.0 and 1.5 mm, while for viscous juices these openings are between 0.5 and 1.0 mm in size. To prevent clogging, and for low viscosity juices, this operation is usually performed in two or three steps, first using a coarse screen with larger holes, then with a screen with smaller holes. In this case, smaller equipment can be used in the second step, since the bulky portion has been collected in the first extractor. The second device is commonly referred to as the finisher. Another parameter mostly overlooked by processors is the speed and incline of the blades within the extractor. Very high speeds do not allow the pulp to pass easily through the holes and hence it may be discarded as waste material. Inclining the blades could increase the pressure of the pulp on the holes, thereby

Fig. 11.1 Mill knife used to crumble fruit for juice extractor (courtesy of Mecamau).

permitting better yields. Most extractors allow the drum to be changed so that the pulp extraction can be modified according to the nature of the fruit and the desired end product. Figure 11.2 shows a traditional extractor for fruits in general.

Presses

In this case the fruit is squeezed against either a nylon canvas or a stainless steel screen. The pressure can be varied to allow a greater or lesser extraction of juice. Higher quality juice is produced when the application of high pressure is avoided, since this procedure usually leads to undesirable compounds contained in the peel, skin or seeds of the plant being included in the juice. In general, the pressing of fruit with seeds should be avoided because the seeds can cause bitter substances to pass into the juice. Plate XIV (in the colour section between pages 238 and 239) shows a line for pineapple juice extraction, including selection, washing and juice extraction in sequence.

Selection of the extraction process

If the peel of the fruit contains bitter substances, juice is not extracted in the press, unless the peel is removed before extraction. However, if the peel of the fruit does not contain bitter substances, the fruit can be pressed lightly, followed by the removal of the juice. If the fruit has small seeds, the juice can be extracted in a removing device, with or without peel depending on type. If the fruit contains a stone, this must first be removed, with juice extraction then carried out in the press.

Fig. 11.2 Double fruit juice extractor (courtesy of Tropical Food Machinery).

Use of filter aids

Filter aids are basically inert substances, which are added to pulp to facilitate the extraction of juice. These materials create drainage channels in the material to be filtered and are common in the process of extraction by pressing. The aids can be as simple as rice hulls (these must be sterilized before usage), or more complex materials such as diatomaceous earth. The correct use of filter aids allows larger and faster operations in the process cycle, further juice clarification and easier cleaning of equipment after processing.

11.8.2 Clarification and filtration of juices

Not all juices need to undergo clarification or harsh filtration, depending on consumer expectations of a juice with high fiber content and suspended solids. Turbidity in juices is the result of a complex system involving pectins, gums, cellulose, hemicellulose and proteins. A colloidal particle contains within it positively charged protein particles, while on the outside it contains negatively charged particles of soluble or insoluble pectin. In general, emulsifiers may contain 25% of lipids. PVPP (polivinylpyrolidone) is a complexing agent commonly used to destabilize colloidal systems, leading to precipitation of colloidal material.

The juice clarification processes may be physical (sedimentation, centrifugation or filtration), chemical (application of bentonite, gelatin, sand, with or without pretreatment with enzymes), biochemical (application of enzymes, followed by centrifugation or filtration) or a combination of any of these processes. The enzymes normally used in these processes are pectinesterase, polygalacturonase, pectin-transeliminase, cellulase and hemicellulase.

The filtration rate of a juice is affected by the filter area, the amount of filter aid material, the filtering time, the pressure applied to the system, the type of material, its viscosity and its temperature. The most widely used equipment for the filtration of juices is the hydraulic press, horizontal filters, vacuum filters, ultra filtration and micro filtration.

11.8.3 Use of membrane technology in fruit juices

The main advantage of membrane technology is that it does not require heat and hence results in better retention of volatiles, and of the nutritional and sensory characteristics of fruit juices. Usually the process involves filtration or reverse osmosis of fruit juices through a membrane which is selected for its pore size and for the pressure which it applies, which facilitates the transfer of some components in fruit juices. However, as this technology does not involve the latent heat of phase change, only a very small amount of heat is needed.

Moreover, the simplicity of facilities brings down the processing cost, thanks to the lower labor requirements (and consequent expenditure), and to the absence of boilers and other utilities associated with heat treatments.

The process by which the application of membranes is carried out is defined by the average size of the pores in the membrane and the pressure applied to the

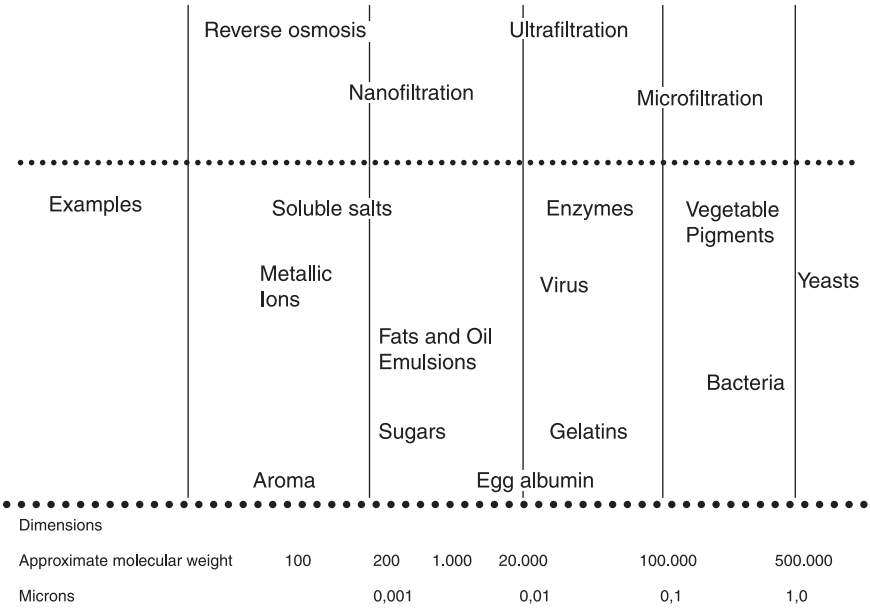

Fig. 11.3 Characteristics of the filtration process (adapted from Fellows, 2006).

system. Figure 11.3 presents the characteristics of various types of filtering in accordance with the type of technology or membrane process used and the types of compounds which could be retained in the process, while Table 11.7 presents a comparison between the concentration processes involving evaporation and those involving reverse osmosis.

For the use of a material as a membrane to be viable, it must possess certain characteristics. These include high selectivity, high permeability, and good thermal stability in combination with mechanical and chemical resistance. Initially, cellulose acetate membranes were used, which had limitations in terms of temperature (no higher than 50°C) and pH (3.0 to 8.0), and were relatively susceptible to microbial agents and chlorine. Later, the second generation of membranes were made up of polymers (polysulfone and polyethersulfone) which could support up to 125°C and pH ranges of 1 to 13, and were also tolerant to 200 ppm of available chlorine. However, these membranes were not able to support the high levels of applied pressure required for an ultrafiltration function. The third generation of membranes, consisting of inorganic materials and minerals, displayed high thermal resistance (up to 350°C), and could be used in a wide range of pH, in high concentrations (2000 ppm) of chlorine and at high pressures; however, the high cost of these membranes, combined with their low resistance to mechanical impact, limited their use.

Some important desirable characteristics for membranes are a high ratio of permeable area to volume, low manufacturing cost, ease of cleaning and ease of

Table 11.7 Comparison between processes of reverse osmosis and evaporation

Parameter	Reverse osmosis	Evaporation
Steam consumption	0	250–500 kg 1000 L^{-1} water removed
Electrical energy consumption	10 kWh (continuous) and 20 kWh (batch) per 1000 L of water removed	~ 5kW h^{-1} 1000 L^{-1} of water removed
Energy use (kW/h)	3.6 (6–12% solids) 8.8 (6–18% solids) 9.6 (60–20% solids)	1 Effect 387 (6 to 50% solids) 2 Effects 90 (6 to 50% solids) 7 Effects 60 (60 to 50% solids)
Workforce	4 h day^{-1}	Two operators during the whole operation at boiler and evaporator
Consumption of cooling water	0–29.300 kJ (solid) and 0–58.600 kJ (batch) per 1000 L of water removed	5.1 to 1.2 ×106 kJ 1000 L^{-1} water removed
Plant dimensions Economic plant size	6.000 L day^{-1} economic plant size or more, with no upper limit	80.000–100.000 L day^{-1}
Considerations on the final product	Maximum 30% soluble solids Capacity varies with the concentration	Up to 60% soluble solids

Source: Fellows (2006)

replacement in the system. The main configurations of membranes are tubular, hollow fiber and spiral-plate frame. Tubular and hollow fiber configuration membranes are most commonly used for fruit juices.

In fruit juice processing, the major applications of membrane technology are the clarification of fruit juice – usually apple – using microfiltration or ultrafiltration; concentration using reverse osmosis; deacidification and discoloration by nanofiltration and cold sterilization with micro or ultrafiltration, followed by aseptic filling. These processes, when coupled with aseptic packaging, lead to cold sterilization of the product.

The presence of pectin, cellulose, hemicelluloses, protein and starch in juices gives them a thick consistency, and the final product becomes cloudy. The clarification of fruit juices reduces their turbidity, and depectinization results in juices behaving as Newtonian fluids, while cloudy and pulpy juices exhibit non-Newtonian behavior.

The use of membranes in the clarification process can replace operations such as centrifugation, coagulation, sedimentation and filtration with only one operation of micro or ultrafiltration, thus reducing the processing time by two to four hours which in turn increases the juice yield to 95–99% (Cheryan, 1998). Additional advantages of membrane processing are that lower volumes of waste are generated, reducing processing costs, the scale of processing can be easily reduced or enlarged, and enzymes can be recovered (Matta, 1999).

Most of the work carried out on membrane processing refers to apple juice; however, several authors have reported its use with other tropical fruits such as pineapple (Jiraratananon *et al.*, 1997; Vaillant *et al.*, 2001; Carneiro *et al.*, 2002; Barros *et al.*, 2004), acerola (Matta, 1999; Barros *et al.*, 2004; Wang *et al.*, 2005), camu-camu (Rodrigues, 2002), guava (Chan and Chiang, 1992; Chopda and Barrett, 2001), orange (Hernandez *et al.*, 1995; Venturini Filho *et al.*, 2003), mango (Vaillant *et al.*, 2001), passion fruit (Jiraratananon and Chanachai, 1996; Vaillant *et al.*, 1999; Vaillant *et al.*, 2001), watermelon (Miranda, 2005), tamarind (Watanabe *et al.*, 2006), tangerine (Chamchong and Noomhorm, 1991; Vaillant *et al.*, 2001) and umbu (Bruyas, 2004; Watanabe *et al.*, 2006b). Table 11.8 presents some applications of membrane technology for tropical fruit juices.

In these studies the operational parameters are quite variable and are dependent on the type of membrane, including its shape and pore size. The problem in membrane processing is always a reduction in permeable flux with time, which is caused by fouling of the membrane by thick pulp or pulp that becomes more concentrated during the process. In order to avoid these problems, most authors have applied enzymatic treatments to the fruit juices and pulps prior to clarification. The main objective is to hydrolyze pectin, cellulose and other polysaccharides that constitute the structural material of the cell walls of fruit. The enzymes pectinases, cellulases and hemicellulases are commonly used and these are applied separately or in a combined form together with membrane processing (Mahler, 1997; Bobbio and Bobbio, 2001).

Most of the studies on membrane processing deal with the application of microfiltration membranes in juices, and examine the resulting physico-chemical characteristics such as pH, acidity, content of soluble solids, color, juice yield, tannin content (for cashew, for example) and viscosity as well as reporting the results of microbiological analysis and finally, the effects of the process variables, such as permeable flux, volumetric concentration factor and transmembrane pressure. Very few studies provide quantification data for flavonoids and/or carotenoids in the permeate and the retentate. The retention of carotenoids and related compounds such as lycopene in membrane processing may constitute an added advantage when the goal is the concentration of these compounds in the retentate. Clareto (2007) reported the retention of 100% of lycopene from enzymatically hydrolyzed guava pulp (using 5 mg $100\,g^{-1}$ Pectinex® 100L per 30 minutes) through tubular ceramic microfiltration membranes of 0.2 μm pore size, transmembrane pressure of 2, 2 bar and flows of the order of 100 kg $h^{-1}m^{-2}$.

The retention of tannins in microfiltered and ultrafiltered cashew apple juice can ensure better product acceptance, and also provides an excellent source of vitamin C. Barato (2008) achieved 97% retention of tannins in nanofiltered cashew apple juice and 71% retention for microfiltered juice using a polyethersulfone membrane. Gasparetto *et al.* (2007) reported the removal of tannins from microfiltered (0.2 μm pore size, ceramic membranes) cashew apple juice, the concentration of which decreased from 0.34% in the fresh juice to 0.02% in clarified juice. The greatest resistance to flow was due to fouling (80% of the resistance to flow).

Table 11.8 Use of membrane technology for fruit juices

Juice	Enzymes	Membrane type	Operating condition	Results	Author (year)
Umbu	Pectinex Ultra SP-L 0.01% 1 h at 35°C	MF model MD 020 TP 2N, Microdyn-NADIR GmbH, polypropylene, tubular, area 0.038 m^2, pore diameter 0.2 µm	P_{TM} 1.1 bar; v 6 m s^{-1}; FC 2.2	The final cumulative flow increased from 63 to 75 $Lh^{-1}m^{-2}$ with the enzyme under the same operating conditions	Ushikubo (2007)
Orange	–	UF Koch-Glitsch Italia Srl, PVDF, MWCO 15 kDa, area 0.23 m^2	P_{TM} 0.85 bar; v 800 Lh^{-1}; T 25°C; FC 6	Average flow 15 $Lh^{-1}m^{-2}$	Cassano *et al.* (2003)
Melon	Rapidas Tropical Cloud 0.01–0.015% 1 h at 35°C	MF with Membralox with IP19-40, ceramic, tubular multichannel, area 0.24 m^2, pore diameter 0.2 µm	P_{TM} 1.5 bar; v 7 m s^{-1}	Average flow of 80 $Lh^{-1}m^{-2}$ was influenced by enzyme treatment	Vaillant *et al.* (2005)
Pineapple	Pectinex SP-L Celuclast 0.03% 1 h at 30°C	Koch Membrane Systems, tubular, polyethersulfone, area 0.05 m^2, pore diameter 0.3 µm	P_{TM} 1 bar; T 25°C; v 6 m s^{-1}	Average flow of 100 $Lh^{-1}m^{-2}$ was influenced by enzyme treatment	Carneiro *et al.* (2002)
Camu-camu	Pectinex Ultra SP-L 0.01% 30min/35°C	PROTOSEP IV, polyethersulfone, tubular, area 0.05 m^2, pore diameter 0.3 µm, 30–80 kDa	P_{TM} 2 bar; t 240min	44% reduction in viscosity with treatment enzyme; tubular MF membrane showed better mean flow (40.9 $Lh^{-1}m^{-2}$ against 17–33)	Rodrigues (2002)
		LAB UNIT M-20 – DDS, plate and frame, area 0.72 m^2, pore diameter 50 kDa; 100 kDa; 0.1 µm	P_{TM} 3 bar; t 30 min		
Cashew	Tanase 0.1% 1 h at 30°C	PROTOSEP IV, polyethersulfone, tubular, area 0.05 m^2, pore diameter 0.3 µm	P_{TM} 2 bar; T 30°C, FC 3	The enzymatic treatment resulted in no change in the product. The average stabilized flow obtained was 70 $Lh^{-1}m^{-2}$	Campos *et al.* (2002)

P_{TM}: transmembrane pressure; FC: final concentration

Thus, the challenge for membrane processing technology is to produce high quality clarified juices with high nutritional value, which means obtaining juices and beverages with high levels of vitamin C, carotenoids and anthocyanins. The problem lies in the transfer of anthocyanins, and especially of carotenoids, through the membranes. The enzyme treatments need to be more efficient at the relatively low temperatures employed in processes involving filtration membranes. The application of surface-active agents is effective only at high concentrations, while the use of non-polar solvents seems to be a viable alternative, but these need to be removed at the end of the filtration process.

11.9 Manufacture of jams and jellies

Jams and jellies are well established conventional fruit products which are obtained by cooking the fruit, whole or in pieces, or as pulp or juice, with sugar and water. The mixture is then concentrated to a gelatinous consistency with or without the addition of an appropriate gelling agent. The definitions of jams and their characteristics, classification and permitted ingredients may vary according to the country of production and should be subject to the laws of the country where the product is usually marketed.

One of the most important parameters for the classification of jams is the soluble solids content. O'Beirne (1993) states that the minimum soluble solids content in jams should be 60%. According to Smith (1993), jelly is defined by the US Department of Agriculture (USDA)'s identity standard 'as a semisolid food made from not less than 45 parts by weight of fruit juice to each 55 parts of weight of sugar so as to yield to 65° Brix in the finished jelly'. Jams also require the same proportion of fruit in relation to parts of sugar, but in jam manufacture, whole fruit or different components of the fruit may also be used rather than fruit juice alone. A concentration of at least 65% soluble solids is achieved in all jams, with some requiring up to 68% solids so as to obtain the desired organoleptic qualities (Smith, 1993). In some countries, all such products are referred to as 'jellies', but the soluble solids content should be between 60 and 65% or above, according to the legislation in each country. Limits on the addition of preservatives by the Codex Alimentarius (2009) are fixed at 1.000 mg kg^{-1} of sorbates and benzoates and 50 mg/kg of sulphites expressed as residual SO_2 in the final product, except in cases where the original fruit has been treated with sulfite, when a concentration level of 100 mg kg^{-1} may be allowed in the final product. Fumaric and citric acids may be added in sufficient quantities to achieve the desired effect, as can tartaric acid up to a maximum concentration of 3.000 mg kg^{-1} (Codex Alimentarius, 1995).

A high quality jelly should maintain its textural characteristics and when removed from the pot, it should be easy to remove and should be firm. The product should not be very sweet, sticky or viscous, and should retain the flavor and aroma of the original fruit. In general, the use of artificial colors and flavors is not permitted.

The basic ingredients for the manufacture of jelly are: fruit, pectin, acid, sugar and water. The quality of the jam or jelly will depend on the quality of ingredients used and their correct combination, as well as on the order of addition during processing. Figure 11.4 shows the influence of basic jelly components on the degree of gelation. Generally, the addition of acidulants and pectin to compensate for any deficiency in the natural pectin content of the fruit is permitted. The gel is formed only at a pH of around 3, and above pH 3.4 gelation does not occur. The optimum concentration of sugar is about 67.5%, but it is possible to obtain jelly with a high pectin and acid content with less than 60% sugar. The amount of pectin used in jelly manufacture depends on its quality. Lima *et al.* (2010) reported that the ability to form gels depends not only on the type of pectin (methoxylated, acetylated) but principally on its concentration which varies from 0.1 to 1.5% at a pH of between 2.7 and 3.7, and with total soluble solids varying from 64 to 71%. Usually the addition of 1% of pectin is enough to produce a firm jelly (Jackix, 1988).

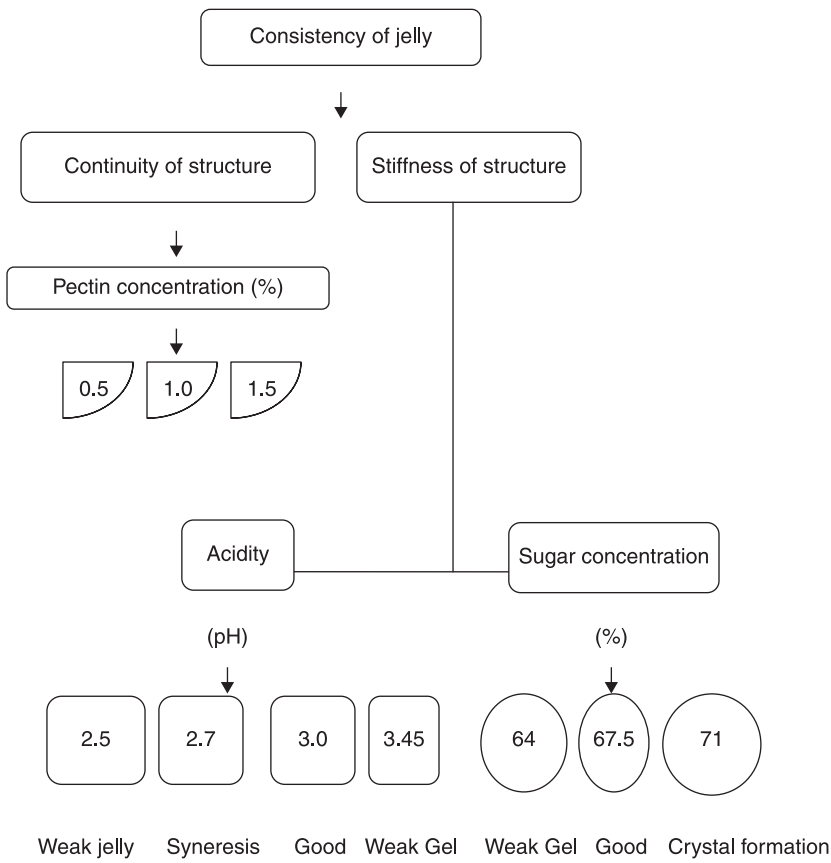

Fig. 11.4 Influence of the basic constituents of a jam on its consistency (from Rauch, 1965).

The addition of sugar affects the balance between pectin and water, which destabilizes pectin conglomerates, forming a fiber network (the gel), which consequently facilitates liquid aggregation. The density and the matrix of this network are affected by the pectin content while the rigidity of the structure is affected by the concentration of sugar and the level of acidity. The acid hardens the fiber network, but high acidity affects the elasticity because the pectin is hydrolysed. The total acidity of the jelly should be around 0.5 to 0.8; acidity higher than one per cent leads to syneresis or liquid exudates from the jelly (Torrezan, 2003).

Jams and jellies can also be made by using a combination of various fruits. Acosta *et al.* (2008) used response surface methodology to evaluate the effects of sweetener, low methoxyl (LM) pectin and calcium content on the overall acceptability of a tropical mixed fruit (pineapple, banana and passion fruit) jelly as judged by 100 consumers. Calcium concentration had a significant effect on overall acceptability but LM pectin and sweetener concentration did not. Abdullah and Cheng (2001) used the same methodology to determine the optimum ratio of pineapple, papaya and carambola in the formulation of a reduced calorie tropical mixed fruit jam. A contour plot of the sensory attributes of these jams showed that the formulations containing 3.5 to 37.7% papaya, 0 to 15% carambola and 61.5 to 96.5% pineapple produced acceptable products.

Figure 11.5 shows the basic flowchart for obtaining jellies. The sequence of steps in the manufacture of jams and jellies is based on the characteristics of the product. Jams and jellies can be obtained from fresh fruit, from frozen fruit such as fruit pulp, or from puree or fruit juices. A comprehensive description of processing operations for producing jellies from fresh fruit is provided below.

11.9.1 Raw material and fruit preparation for jellies

Special considerations must be taken into account for some fruits for the purposes of industrialization and the preparation of jellies in particular. In the case of guava (*Psidium guajava* L.), for example, the best fruits for industrialized products are those from cultivars with red pulp, fewer seeds and stone cells, higher acidity, a pleasant flavor and high ascorbic acid content.

For the production of passion fruit (*Passiflora edulis* Sims) jelly, both varieties of fruits – yellow (*P. edulis* form Flavicarpa) and purple (*P. edulis* Sims) – can be used; however, fruits of the yellow variety are more commonly used (Teixeira *et al.*, 1995). The fruit must be ripe, with a pulp pH of between 2.85 and 3.05, acidity (expressed as citric acid) close to 4.5% and the minimum soluble solids content about 38%.

For the production of mango (*Mangifera indica* L.) jelly, fruits of the local variety may be used provided these are juicy, rich in color and flavor with no terpenic odor, contain soft pulp and have a good fibrous consistency. In Brazil, fruits of the 'Charlotte' cultivar can be used: these tend to be rich in color, flavor and aroma and are characterized by excellent yields and a soluble solids content varying from 18 to 20%. However, the variety 'Tommy Atkins' which is relatively

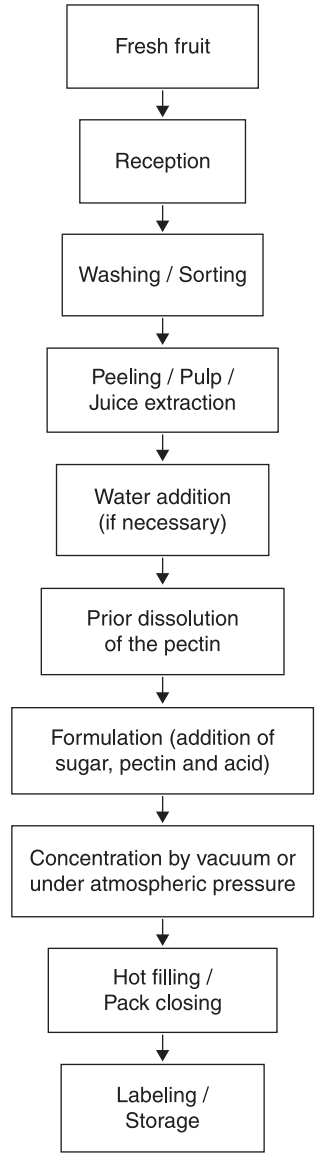

Fig. 11.5 Basic processing operations for fruit jam and jellies.

poor in taste and color compared to the 'Charlotte' variety but is juicy and firm with medium fiber content, may also be used either individually or in combination with other fruits (Pinto *et al.*, 2002).

Pineapple (*Ananas comosus* L. Merrill) jelly is made from the clarified juice obtained from the pineapple cultivars 'Smooth Cayenne' and 'Perola'. The juice

should be obtained from ripe fruit in order to retain better sensory characteristics (Medina *et al.*, 1987).

The papaya (*Carica papaya*) varieties 'Solo' and 'Taiwan' are most widely used in industrial processing due to their pleasing aroma and flavor. Fruits for processing are usually harvested at the half-ripe stage of maturity and are ripened in suitable chambers kept at a controlled temperature (25°C) and humidity (85 to 90%) (Medina *et al.*, 1995).

The most common variety of banana used for industrial processing belongs to the 'Cavendish' group since these are less astringent. The starch content should be about 2% and sugars should vary between 17.5 and 19.0% (Nogueira and Torrezan, 1999). Jams and jellies obtained from oranges can be prepared from either the fruit or juice concentrate. The minimum soluble solids content should be 11% and Brix/acidity ratio should be in the range of 11.5 to 18.0 (Filgueiras *et al.*, 1985).

Fruits used for the manufacture of jams and jellies should be sufficiently mature and at ripe stage, when the best color, aroma and flavor are retained, and when the sugar and pectin content is high. Slightly green fruit have a higher pectin content than very ripe fruit, as it is during ripening of the fruit that pectin is degraded into pectic acid, without forming a gel. In order to obtain high quality products with desirable organoleptic characteristics, the use of a mixture of ripe fruit with better flavor, taste and color and more green fruit with higher levels of pectin is recommended. The most suitable fruits for the processing of jams and jellies should be rich in pectin and acid, but these compounds may also be complemented with acid or commercial pectin. Table 11.9 presents a classification of fruits according to their pectin content and acidity level.

For fruits which are not mentioned in Table 11.9, a simple, rapid qualitative test can be performed in order to determine the presence of pectin in fruit juice. The test involves taking two to three spoons of alcohol, to which an equal amount of fruit juice is carefully added. The mixture is then lightly agitated by shaking the container. The mixture is left to stand and is observed after one minute. If the fruit juice is rich in pectin, it will form a solid mass; if it is moderately rich, the mass will be broken into two or three pieces; and if it is low in pectin, it will break into small chunks.

The initial stages of fruit preparation for processing are basically the same as those which have been described above. The following steps are specific to jelly processing.

11.9.2 Addition of ingredients

The type of sugar usually employed in the manufacture of jams and jellies depends on the producing country, and includes sucrose from cane sugar, glucose, glucose syrups, invert sugars, fructose, 'brown sugar', cane molasses and honey (O'Beirne, 1993). Jams and jellies can even be prepared with artificial sweeteners.

During cooking in an acidic medium, sucrose is hydrolysed and partially degraded into glucose and fructose. This partial inversion of sucrose is necessary to prevent the formation of crystals which might occur during storage. However,

Table 11.9 Classification of some tropical fruit according to pectin and acidity

	Pectin			Acidity		
Fruit	Rich	Average	Poor	High	Medium	Low
Pineapple			x	x		
Acerola			x		x	
Araçá (purple)	x			x		
Banana (water or midget)		x				x
Cajá manga			x	x		
Cashew			x		x	
Carambola (acid)			x		x	
Carambola (sweet)			x			x
Custard apple			x		x	
Guava (red ripe and time)	x				x	
Jabuticaba (common)			x		x	
Jabuticaba (ponhema)			x	x		
Jabuticaba (sabará) with peel		x		x		
Jabuticaba (sabará), shelled			x			x
Orange (bay and pear): whole fruit	x			x		
Lemon (Citron and Sicilian)	x			x		
Papaya			x			x
Mango (sword)		x		x		
Mango (Espadão holy and Alexandrian)	x			x		
Passion fruit (yellow and purple)			x	x		
Quince	x				x	
Pitanga		x		x		
Uvaia			x	x		

Source: Adapted from Jackix (1988)

when there is a need to adjust the final concentration of total soluble solids to above 65%, it is important to replace part of the sucrose content with maize glucose or invert sugar (a mixture of glucose, fructose and sucrose).

The addition of glucose or invert sugar is always recommended in the case of vacuum processing or when improvement in color and reduction in sweetness is desired. The replacement of sucrose with glucose or invert sugar, up to a maximum of 15% of the total sugar content, can improve product color and shine, slow the crystallization process, and reduce excessive sweetness.

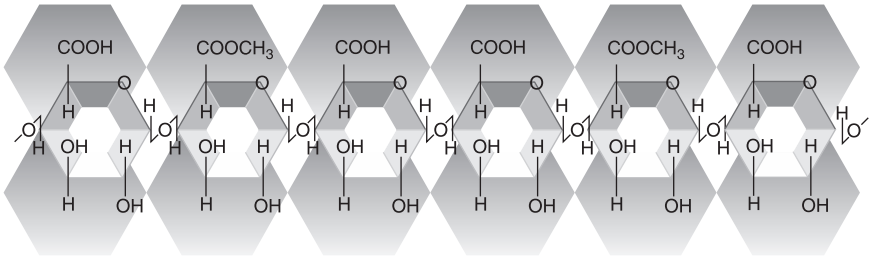

Fig. 11.6 Illustration of the pectin chain.

The solid sugar used must be in powder form and should be sifted before it is added so as to prevent the introduction of foreign materials such as threads from bags, metal pieces, etc., which might be present in the sugar. Sugar should be added slowly in order to avoid any caramelization during cooking.

The other essential ingredient for jam and jelly preparation is pectin. Chemically, pectins are a complex mixture of polysaccharides of which galacturonans are the main component. Pectin is a linear polymer made up of repeated units of α-(1-4)-linked D-galacturonic acid, forming a long polygalacturonic chain (Fig. 11.6). In their molecular structure, the carboxylic acids of galacturonic monomers may or may not be esterified with methanol, or even acetic acid (Mesbahi *et al.*, 2005, Lima *et al.*, 2010). Pectin is obtained by aqueous extraction of a mixture of the appropriate parts of the plant material, usually citrus fruits and apples. Commercially, pectin is available in powder form or as a concentrate.

The ratio of esterified acid groups to the total number of acidic groups defines the degree of esterification (DE) or degree of methoxylation (DM) of pectin. The DE strongly influences the time and temperature required for gelation (El-Nawawi and Heikel, 1997). Pectins can be high (Fig. 11.7b) or low methoxyl (Fig. 11.7a) and the low-methoxyl pectins may contain amide groups. High methoxyl pectins are characterized by a DM of more than 50%, are gelled at concentrations of 60–80% soluble solids and at a pH of between 2.8 and 3.8. Low methoxyl pectin has a DM below 50% and can form gels at concentrations of soluble solids varying from 10 to 70% and at a pH between 2.8 and 6.0, but only in the presence of polyvalent ions such as calcium and magnesium (Soler, 1991).

The temperature at which the mixture begins to form a gel during the cooling process depends on the degree of esterification of the pectin. According to Soler (1991), high methoxyl pectin is classified commercially into three groups with respect to temperature and rate of gelation:

- Slow gelation pectin: the degree of esterification is 60–65% and the gel forms at temperatures of between 45 and 60°C.
- Medium gelation pectin: the degree of esterification is 60–65% and the gel forms at temperatures of between 55 and 75°C.
- Fast gelation pectin: the degree of esterification is 70–76% and the gel forms at temperatures of between 75 and 85°C.

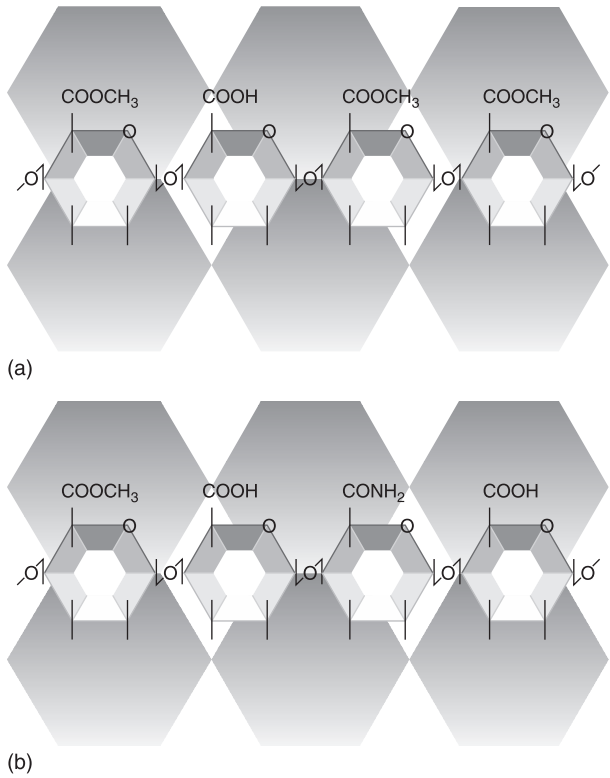

Fig. 11.7 Illustration of pectin chain (a) high methoxyl and (b) low methoxyl.

Each of these pectins has a different optimum pH, usually between 2.8 and 4.2, and is used for different applications. Fast gelation pectins are used in products that contain fruit pieces or peels. Slow gelation pectins are used for standard jams and jellies, or those packed in large containers, as they create homogeneous gels, and avoid premature gelation, which hinders the filling of packages.

Low methoxyl pectins are often used in diet products because these do not require higher sugar content for gel formation. An alternative means of producing a gel without the use of sucrose is to use low methoxyl (LM) pectin in combination with calcium (Thakur *et al.*, 1997).

The addition of pectin is a very important step in the processing of jams and jellies. The pectin must all be dissolved in the material before processing begins in order to obtain the desired effect and to ensure high quality products. In the case of cooking carried out at atmospheric pressure, the pectin must be added at about the halfway point of the total cooking time. This avoids any deterioration in pectin quality that could be caused by excessive cooking. However, in vacuum processing, pectin can be added at the beginning of the process, along with the other ingredients.

The pectin must be dissolved before it is added to the concentrator. Initially, one part of dry pectin should be mixed with four parts of sugar. Water heated to 65–70°C should be added to this mixture slowly, and the mixture should be vigorously stirred mechanically (for small quantities an industrial blender can be used) until a homogeneous solution with no lumps is formed. The maximum concentration of pectin by weight in this solution should be four per cent to ensure that it is fully dissolved.

Acidulants are added with the aim of lowering the pH, which is important for proper gelling and enhancement of the natural flavor of the fruit. To achieve proper gelation, the final pH should be between 3.0 and 3.2. For most fruits, this pH is not reached by using simply fruit, pectin and sugar and thus it is necessary to add organic acids which are natural constituents of fruits, such as citric acid or tartaric acid. The most commonly used acid is citric; however, each acid has a different impact in terms of lowering pH and adding to the taste of the product. An example of this is phosphoric acid, which is about four times stronger than the citrus in relation to its capacity to lower pH, but the resultant taste is only ten per cent more acidic (Jackix, 1988).

If the addition of acids is not carried out at the correct point, irreversible damage can be caused to the gelling power of the pectin, and hence to the final product. The acid should be added at the end of the process and, if possible, immediately before packaging, especially in processing at atmospheric pressure. However, in vacuum processing, the addition of acid can occur at any stage of the processing since the working temperature is lower and consequently does not promote pectin hydrolysis.

11.9.3 Concentration and packaging

Jams and jellies can be prepared using two basic methods: cooking at atmospheric pressure or under vacuum conditions. The equipment used in these two methods is shown in Plate XVa and XVb, respectively (in the color section between pages 238 and 239), and Fig. 11.8 shows a gas pan that can be a viable alternative to small food processors as it does not incur the high financial costs involved in running boilers for steam generation.

Concentration at atmospheric pressure is carried out in open steam jacketed pans, with a mechanical stirrer. To increase production, multiple units, rather than larger containers, should be used, as the latter reduces heat transfer and increases cooking time. The concentration time depends on several factors, including the relationship between the volume of the evaporator and that of the available heating surface, the thermal conductivity of the device and product, the temperature of the heating surface and the difference in °Brix between the processed material at the beginning and at the end of the process.

Very long periods of cooking can cause caramelization of sugar, resulting in darkening of the product, excessive inversion of sucrose, flavor loss, pectin degradation and excessive expenditures of time and energy. Moreover, if the cooking process is too short, it may cause little or no inversion of sucrose and

Fig. 11.8 Open pan with gas cooking facility and with mixer, used for the manufacture of jams and jellies (courtesy of Incal).

incomplete absorption of sugar by the fruit. This can then lead to osmotic processes, which consequently break the gel matrix during storage, resulting in a poor quality product, as well as decreasing the final concentration of soluble solids (Soler, 1991).

Vacuum concentration can be continuous or discontinuous, depending on the type of equipment used. All the ingredients are initially combined in a pot and then transported to the concentrator. The concentration temperature is about 50–60°C. After cooking, the jelly can be heated in the same concentrator, at a temperature of 85–90°C, or can be removed and heated in another heating vessel before packaging.

The end point of processing of jams and jellies can be determined by various methods: the main parameter to be measured is the refractive index. This index indicates the concentration of soluble solids, and can be measured by manual or automated refractometers. In a manual refractometer, the refractive index must be read using a representative sample of the lot at a temperature of 20°C to avoid changes, or if this is not possible, the readings should be corrected taking the temperature of the reading into account. The automatic refractometers are attached to the cooking equipment and continuously monitor the °Brix of the product throughout the process.

Table 11.10 Boiling point data of typical mixtures of fruit juice and sugar at different altitudes

Soluble solids (°Brix)	Boiling point (°C)				
	Sea level	500 m	1000 m	1500 m	2000 m
50	102.2	100.5	98.8	97.1	95.4
60	103.7	102.2	100.3	98.6	96.9
62	104.1	102.4	100.7	99.0	97.3
64	104.6	102.9	101.2	99.5	97.8
66	105.1	103.4	101.7	100.0	98.3
68	105.7	104.0	102.3	100.6	98.9
70	106.4	104.7	103.0	101.3	99.6
72	107.3	105.5	103.0	102.1	100.4
74	108.3	106.6	104.8	103.1	101.4

Source: Filgueiras *et al.* (1985)

The end of the process can also be determined by controlling the temperature of boiling jam at atmospheric pressure. This is not the best method as it does not guarantee accurate results; however, it can be used when refractometers to measure the soluble solids content are not available. The temperatures are fixed at different levels according to the soluble solids content and the degree of sucrose inversion. Table 11.10 presents some data regarding the adjustment of boiling temperatures according to concentration of soluble solids.

The final yield of the manufacture of jellies can be calculated as detailed below (Vendruscolo, 1989):

$$P_D = \frac{\left(100 \times P_A\right) + \left(P_F \times B_F\right)}{B_D} \quad [17]$$

Where,

P_D = Weight of jelly to be obtained, in Kg;
P_A = Weight of the sugar used in the formulation, in Kg;
P_F = Weight of fruit pulp or juice in the formulation, in Kg;
B_F = Soluble solids content of fruit pulp or juice, in degrees Brix;
B_D = Desired concentration of soluble solids in jam, in degrees Brix.

The containers used for jelly packaging show a high degree of variation in size and shape. Glass is the most commonly used material, but tinned cans coated with varnish and plastic containers may also be used.

Prior to filling, the glass jars should be washed with hot detergent solution and rinsed with hot water, which aids sanitation and prevents thermal shock. The

bottles are transported upside down, and are automatically turned to the correct position and inspected before filling.

In the case of jellies produced by vacuum processing, the temperature of the jelly must be increased to 85°C prior to filling so as to prevent the development of osmophilic fungi and yeasts.

However, jellies processed at atmospheric pressure must be cooled to 85°C in order to achieve the following necessary conditions: correct gelation, satisfactory homogeneous distribution, minimization of weight variations in filling due to density variation, minimization of the risk of glass breakage due to thermal shock and minimization of browning, inversion and pectin hydrolysis (Jackix, 1988). Super-cooling should be avoided as it carries the risk of pre-gelation and microbiological recontamination.

After filling, the jars are transported to cap sealing machines. Internally painted metal lids, fitted with ring seals, can be used; the jars have a typical finish on the edge that allows hermetic sealing and subsequent disinfection of the free space. The caps are put on manually or mechanically, and then fastened onto the edge of the jars. Screw caps are sealed by an internal rubber gasket which closes on the edge of the container. Another closure system is the application of the cap to the jar while the space is filled by a jet of steam.

When steam injection is not used during closure of the jars, it is necessary to disinfect the space, and to cool the product sufficiently to form a partial vacuum, followed as quickly as possible by a gradual cooling, so as to avoid thermal shock. This can be achieved by turning the jars upside down immediately after filling, and returning them to the normal position after a few minutes.

When the product is hotter than 85°C, no extra heat treatment is required during the closure process, since the product, having been heat treated through cooking, also heats the packaging material. However, if the temperature is below 85°C, the product must be heat treated. Some factories use a continuous sterilizer, in which jam jars are loaded onto a wire mat and placed in a water tank at an adequate temperature. Usually a temperature of 82°C and a duration of 30 minutes is sufficient.

After the heat treatment, the product is cooled, labeled, packaged and sent for storage and distribution.

11.10 Heat treatments applied to fruit products

Heating is undoubtedly the most commonly used technology for preserving food in general, including tropical and subtropical fruit products. It allows the inactivation of agents associated with food deterioration and also of pathogenic microorganisms which may cause risk to the consumer. As stated above, microorganisms and enzymes are able to act in a certain temperature range around the optimum temperature for activity. The optimum temperature for the activity of most pathogenic and deteriorating microorganisms, as well as deteriorating enzymes, is around the normal environmental temperature, i.e, between 20°C to 40°C. Heat may promote damage to microorganisms and enzymes, by changing the structure of

proteins and other compounds and by catalyzing reactions that may negatively affect the metabolism and homeostasis of the microorganisms and enzymes.

11.10.1 Types of thermal processes

The means chosen for the transmission of heat to a particular product is dependent upon the type of product, i.e., whether it is liquid or solid, and also if it has been previously packaged or not. Generally, thermal processes can be classified as sterilization, pasteurization or blanching. Sterilization aims to eliminate all pathogenic microorganisms, including spores, and also those that cause deterioration. It generally involves high intensity treatments in terms of duration of treatment and temperature, which is usually between 110°C and 150°C.

The main aim of pasteurization is to eliminate vegetative cells of pathogenic microorganisms and some of those responsible for deterioration. Elimination of spores, however, is more limited with this treatment. It generally involves temperatures of between 60°C and 100°C in order to assure product safety and quality for a limited period of shelf life. Blanching is a heat treatment commonly used as a preliminary operation for decreasing microbial load, inactivating enzymes and eliminating oxygen from the food tissue. The selection criteria for any of these treatments are mainly based on the product characteristics, as outlined above, and on the objective of the treatment, including the desired shelf life. The low pH of acidic products, including most fruits, usually acts as a natural inhibitory factor to avoid the germination of spores and growth of most pathogenic microorganisms. In these cases, a lower intensity treatment such as pasteurization can be sufficient to preserve the product even for long periods, provided the product is hermetically packed to avoid further re-contamination. Although the treatment is not strictly sterilization, in that it does not eliminate all spores of pathogenic and deteriorating microorganisms, this thermal treatment when used in such cases is nevertheless named commercial sterilization. This is because of its ability to ensure the elimination of deteriorating and pathogenic vegetative cells and to avoid the germination of spores thanks to the intrinsic characteristics of the product and the storage conditions. Some spores of some highly thermo resistant microorganisms (known as thermodurics) may survive at the high temperatures employed in pasteurization or even sterilization, but are usually unable to grow under storage conditions. Some other microorganisms known as thermophilics may even develop during mild pasteurization because they have optimum activity at elevated temperatures (40°C to 70°C).

In order to classify the products according to their acidity with the aim of defining the type of thermal treatment required for preservation (pasteurization or sterilization), a reference pH of 4.5 or 4.6 was defined as previously considered. Below this pH the products are classified as acidic (medium or high), meaning that most spores of pathogenic and deteriorating microorganisms cannot germinate. For those products pasteurization is generally sufficient to eliminate vegetative cells of most common microbiota. However, for products with low acidity, sterilization is usually required to preserve the products throughout

storage. The reference pH was based on *Clostridium botulinum*, a highly heat resistant anaerobic microorganism that can produce potentially lethal toxins, but whose spores cannot develop below pH 4.6.

11.10.2 Process design and optimization

The design of the thermal process is usually based on the thermal resistance kinetics parameters of a target (generally pathogenic) microorganism, with higher resistance among the common microbiota of the product. The thermal resistance parameters used for the characterization of the target microorganism are the D-value and the F-value. The D-value is the duration of treatment at a certain temperature required to ensure the reduction of one logarithmic cycle (or 90%) in the microbial population. Z represents the temperature variation required to decrease the D-value by 90%. In the case of *Clostridium botulinum*, the D-value is 0.2 minutes at 121.1°C and Z is 7.7°C. For that reason, for most canned products with low pH the duration of the sterilization process is 15 min. This length of time is set to ensure a reduction significantly above 12D in the microbial population. For instance, if there were a population of 10^{12} of *Clostridium botulinum* spores in the product inside a can, probability dictates that only one spore would survive for the duration of a 12D process, i.e. 2.91 min. In the case of *Clostridium botulinum*, a process time equivalent to 12D is known as death time or thermal death, and is represented by F. When expressed based on 121.1°C, this term is expressed as F_0.

The ideal approach regarding the design of a thermal process such as those used for the preservation of tropical and subtropical fruit products would be to characterize the thermal resistance of the microorganism with the highest resistance which can impair the safety of the product. A predetermined population of the target microorganism could then be inoculated into the product, and by applying thermal shocks of increasing intensity, based on the binomial time and temperature, it would be possible to estimate the parameters D and Z. As a result, a process specifically designed for the product could be established, which would also take into account an extra safety margin. Such an approach has the advantage of ensuring the safety of the product while also avoiding overprocessing, in order to maintain the sensory and nutritional quality at as high a level as possible.

For products with high or medium acidity levels, such as most fruits, the pasteurization process can be designed in order to partially destroy vegetative cells of pathogenic microorganisms and most deteriorating microorganisms too. As discussed above, a temperature below 100°C is usually employed. The spores may survive the process but remain unable to germinate due to the pH of the product. Since some deteriorating microorganisms survive thermal pasteurization, complementary processes are usually used in combination with thermal treatment, such as refrigeration, addition of preservatives, and the use of vacuum packaging to ensure an anaerobic environment. The shelf life of a pasteurized product is generally determined by thermal treatment conditions, by the complementary processes employed and by the storage conditions.

A similar method to that outlined for sterilization can also be used for pasteurization: again, a target microorganism can be selected to design the process, based on its thermal resistance and the kinetics parameters. Generally a 5D reduction is required for pasteurization, exemplified by the FDA requirement for juices and nectars processed in or exported to the United States of America (U.S. Food and Drug Administration, 2004).

The pasteurization process is traditionally classified into slow or fast pasteurization. The process known as Low temperature Large Time (LTLT) employs temperatures of between 60°C and 75°C over a long time period (10 to 30 minutes.). Fast pasteurization is achieved through the process known as High Temperature Short Time (HTST), in which temperatures from 80°C to 95°C and time between 5 and 30 seconds are employed. More recently the concept of UHT (Ultra High Temperature), originally developed for milk, has been adopted for liquids based on fruits, particularly juices, nectars and purees and fruit-based beverages in general. The process employs high temperatures (often above 100°C) for a very short time (less than five seconds) mainly in order to preserve the nutrients and overall quality of the product, to optimize the utilization of energy and to increase the process yield. The process is commonly combined with a parallel and independent system to sterilize the packaging material. In this type of procedure, the heat treated product is cooled and then placed into the packaging in an aseptic environment, before the packages are vacuum sealed. This approach, whereby a higher temperature and shorter time is preferred over the equivalent procedure with lower temperature and longer time, is based not only on the increase in the process yield, but also, and more importantly, on the improved preservation of nutrients such as vitamins that results. This can be explained by the Arrhenius model according to Equation 11.3: this was used above with reference to freezing, and specifically regarding the effect of the temperature on deteriorating reactions, and can be used analogously for the effects of heating. The higher activation energy required to destroy vitamins in comparison to that necessary to inactivate microorganisms explains how the higher temperature still allows nutrient preservation while ensuring higher microbial stability and safety for the products.

One very common and traditional method of pasteurization or commercial sterilization for fruit liquid products is the hot fill as considered for jams. Just after the thermal treatment, while the product is still hot due to the process conditions, it is poured into a pre-sanitized container, normally glass bottles or metal cans or barrels. The container is then turned upside down in order to allow the product to come into contact with all parts of the container, enabling further decontamination of the inside of the bottle or can. The containers are then rapidly cooled down, normally with the use of a heat exchanger to speed up the operation and avoid further scorching of the product.

In order to ensure that the thermal treatment required for the sterilization or pasteurization process is applied to the entire product, the study of heat transfer or penetration is necessary. In the case of canned products, this is carried out by inserting a thermocouple inside the so-called 'cold point' of the product, i.e. the point which requires the longest to achieve the set temperature of the process as a result of heat transfer from the retort to the product. In this sense, by following the temperature

increase at that point and knowing the D and Z-value of the reference microorganism, it is possible to determine when the expected F value is achieved, with the aim of ensuring the required level of microbiological safety. In the case of heat treatments for fluid products using a heat exchanger, it is similarly possible to insert thermocouples along the processing line to totalize the overall F contribution regarding the conditions set for pasteurization and the thermal kinetics parameters for microbial inactivation.

Although most fruit products are acidic and can therefore be preserved using pasteurization or low intensity commercial sterilization, as stated above, one concern regarding tropical fruits in particular is the presence of fungal spores that can often prove resistant to thermal preservation processes and are potentially able to produce mycotoxins such as byssochlamic acid, patulin and byssotoxin A (Beuchat and Rice, 1979). Among such species are *Byssochlamys fulva*, *Byssochlamys nivea*, *Neosartorya fischeri* and *Talaromyces flavus*. These are extremely heat resistant and are frequently associated with the deterioration of heat treated fruit products (Hocking and Pitt, 1984; Beuchat, 1986). The fungal spores, known as ascospores and related to sexual reproduction, display high resistance not only to high temperature, but also to pH variation, elevated sugar levels, and concentrations of fat and acids, and so on (Tournas, 1994).

The ascospores can stay in a dormant state, before becoming activated by commercial pasteurization methods applied to fruit products, and can then further germinate, grow and visually deteriorate the products by inflating the package, under favorable conditions (Kotzekidou, 1997; Ugwuayi and Obeta, 1999).

Figure 11.9 shows the heat resistance kinetics characterisation of *Byssochlamys nivea*, which has been identified as the most heat resistant microorganism found

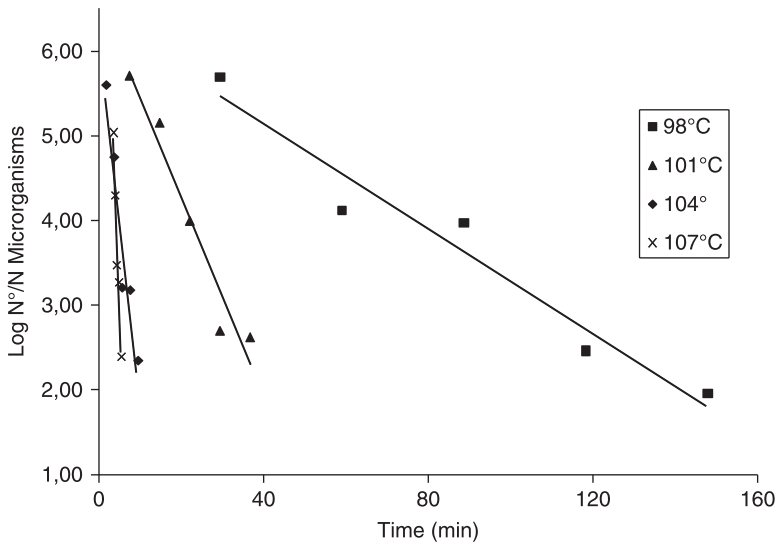

Fig. 11.9 Thermal survival curve for *Byssochlamys nivea* in pineapple nectar (from Ferreira *et al.*, 2011).

in a pineapple nectar industrial line. The high D104°C of 1.5 minutes revealed the problems encountered in using heat treatments to eliminate this microorganism. In order to achieve the required level of 5D reduction recommended for pasteurization, a very long and high intensity process would be required.

Figure 11.10 shows the effect of usual heat treatment (104°C/30 min) on the sensory characteristics of passion fruit nectar, with results taken from samples collected from the same industrial line for nectars production as in Fig. 11.9.

As can be seen from Fig. 11.10, the sensory attributes of passion fruit nectar are already compromised after normal thermal processing, in comparison with the control (fresh nectar). A further increase in the treatment intensity or duration would certainly cause more damage not only to the sensory characteristics of the product, but also to its nutritional value and would furthermore have implications in terms of higher energy demand and significant decrease in process yield.

Contamination by heat resistant molds should be avoided or decreased through the adoption of the correct Good Agricultural Practices, alongside the possible use of combined methods as previously discussed. Finally, in the future, emerging technologies may also help in avoiding this type of contamination.

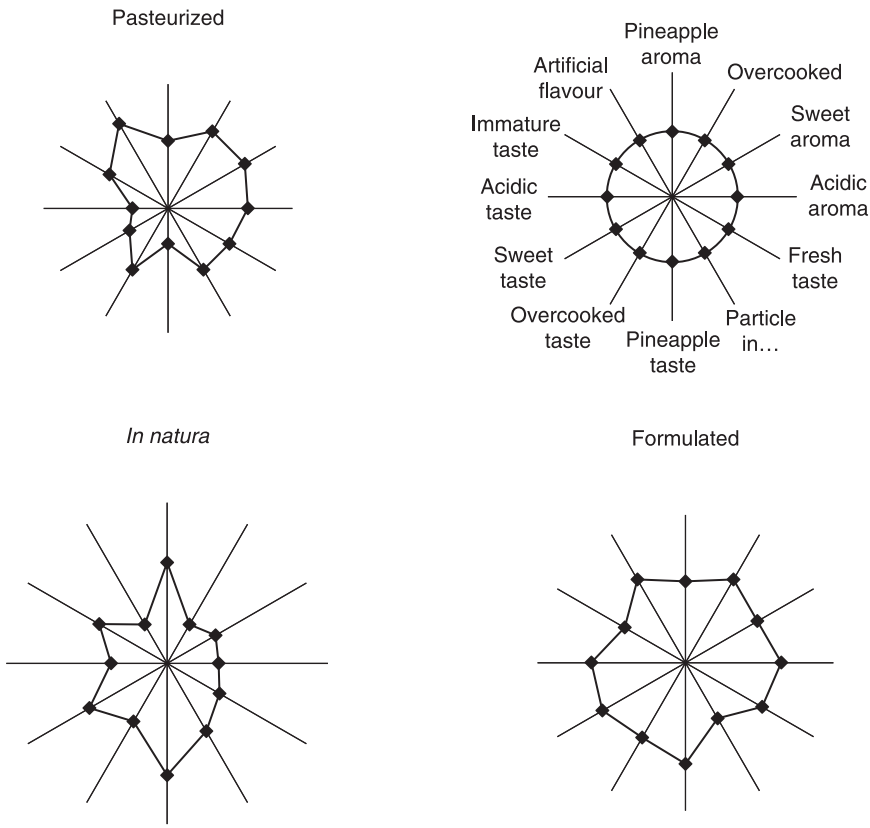

Fig. 11.10 Sun ray plot for the attributes of pineapple nectar sensory profile.

11.11 Non-thermal processes applied to tropical and subtropical fruit processing

As discussed previously, conventional processes for fruit processing are based on the manipulation or adjustment of factors intrinsic to the fruits (chemical composition, pH, water activity and potential redox) or factors extrinsic to the fruits (temperature, environmental gas composition, environmental air relative humidity) as well as implicit factors. For instance, acidification consists of decreasing the pH (to a level below 4.5) in order to avoid germination of pathogenic bacteria spores. Drying and salting consist of a reduction in water activity required for microbial activity and deteriorating reactions. In the same way, refrigeration and freezing are based on reducing the temperature to a level below that required for microorganisms' metabolisms and enzymatic and chemical reactions. Similarly, the principle of thermal treatments is to eliminate or inactivate microorganisms and enzymes by applying heat at temperatures much higher than the optimum for the activity of deteriorating agents.

Although heating is very effective at inactivating or eliminating deteriorating agents, it can also destroy certain nutrients and functional compounds, such as vitamins (vitamins C and B1, for example). Heat also catalyses deteriorating mechanisms such as the Maillard reaction, which involves free carboxylic groups from carbohydrate and amino groups present in proteins and other compounds. These can be beneficial in sensory terms for certain products but may compromise the bioavailability of amino acids, as well as potentially producing toxic substances. Besides, heating can also result in negative sensory changes, involving production of undesirable taste and flavor attributes such as overcooked, bitter or burned, and can also promote changes in color by destroying pigments (such as anthocyanins and carotenoids, which also have nutritional and functional properties) and cause inadequate color compounds to develop, as occurs in fruit browning.

Non-thermal technologies are defined as those that can preserve food using a less aggressive approach, allowing nutritional and sensory properties to be maintained. This is achieved principally through the avoidance of heat treaments or through the use of lower intensity heat treatments, very often in a combined and optimized approach involving other technologies.

Non-thermal technologies generally include emerging technologies such as high pressure, pulse electric fields, ohmic heating, pulse light, and some more traditional technologies such as gamma radiation and microwaving. In particular, the application of high pressure treatments for tropical and subtropical fruits has been studied and is discussed in the next section.

11.11.1 High pressure

High pressure treatment consists of the application of very high levels of pressure to the food, in the range of 100 to 900 MPa (1500 to 9000 Bar). There are two distinct processes involving the application of high pressure that are currently

being investigated: the high hydrostatic pressure (HHP) approach, and the high pressure homogenization approach.

High hydrostatic pressure has been investigated as a non-thermal processing technique to destroy food-borne pathogens and to inactivate enzymes in order to enhance the safety and shelf life of perishable foods (Knorr, 1994), and is already enjoying increasingly widespread commercial use. Usual commercial HHP processing subjects food to pressures of and between 200 and 700 MPa, usually for commercial applications, and normally employing water as the medium for transmitting pressure (San Martín, Barbosa-Cánovas and Swanson, 2002). At ambient temperatures, the application of pressures of between 300 and 500 MPa inactivates the vegetative cells of microorganisms and reduces the activity of enzymes, but still allows the retention of small molecules responsible for taste and color and many vitamins, resulting in a product that can be stored for a considerable time at 4–6°C (Cartlez *et al.*, 1995). In the static system the pre-packaged food is usually inserted into a pressure chamber which is filled with the liquid (generally water) enabling the transmission of pressure to the product.

In the homogenization process, liquid food flows into a system that resembles the normal homogenization process, but faces a differential in pressure throughout the expansion valve which is much higher than that found in conventional homogenization processes (between 100 to 300 MPa in one or two serial stages).

The reason that high pressure can preserve compounds related to food quality while eliminating or inactivating deteriorating agents is its minimal effect on the covalent chemical bonds present in the molecules of compounds such as vitamins, pigments and substances determining flavor. On the other hand, high pressure does affect the chemical bonds responsible for the stabilization of intra- and intermolecular interactions, such as hydrogen bonds, present in proteins and carbohydrates, and electrostatic and Van der Waals interactions, which are also responsible for the stabilization of protein systems.

The first studies on the application of high pressure treatments to food began as early as the end of the nineteenth century, starting with the work of Bert H. Hite on the effect of high pressure on different types of foods, which was then followed by the research of P. W. Bridgman on egg albumen coagulation and the physical properties of water under pressure, published in 1914. However, technological developments remained incipient and restricted to sporadic studies until the end of the twentieth century. This was mainly due to the fact that the major development in heat treatment for food preservation processes took place at the beginning of that century, and brought about the installation of industrial food plants worldwide based on thermal processes.

Only around 1990 did studies carried out primarily in Japan and then later in the United States and Europe show the marketing potential of high pressure treatments for food processing. These studies revealed that high pressure processes allow products to retain fresh characteristics, matching consumer demand for higher quality products.

High pressure proved to be effective in inactivating the vegetative cells of microorganisms, though it was somewhat limited in its ability to destroy spores.

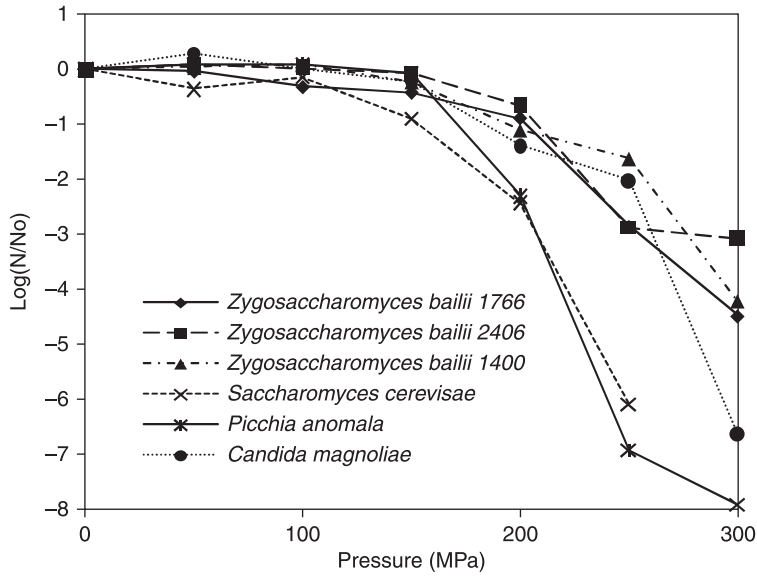

Fig. 11.11 Yeasts' inactivation at different pressure levels in pineapple juice (pressurization time = 10 min) (Source: Rosenthal *et al.*, 2002).

For this reason, commercial pasteurization using high pressure was found to be adequate for acidic foods, including most fruit products, but is not suitable for low-acid foods. Higher quality products can be obtained that have an added advantage of longer shelf life than traditional products. Products such as jellies, jams and juices were among the products initially marketed using this novel technology, first in Japan and later in Europe and the United States.

The resistance of microorganisms to high pressure varies not only between different genera and species, but also within different strains of the same species. Figure 11.11 shows the variation in different strains inoculated into pineapple juice and submitted to high pressure levels of up to 300 MPa for ten minutes. As can be seen from the figure, some species present low resistance to pressure, including *Picchia anomala, Candida magnolia*, and *Saccharomyces cerevisiae*, resulting in inactivation levels of up to seven or eight logarithmic cycles. On the other hand, *Zygossaccharomyces bailii* presented higher resistance and variation among the evaluated strains, resulting in inactivation levels of three to four logarithmic cycles in the initial population count (Rosenthal *et al.*, 2002).

The combined use of high pressure and mild heating may be an alternative means of inactivating spores of highly resistant microorganisms. Figure 11.12 shows the combination of pressure with heat with the aim of inactivating ascospores of *Byssochlamys nivea* originally isolated from pineapple nectar and then re-inoculated into the same type of nectar and into the juice for investigation purposes. As can be seen from Fig. 11.12, combining pressure at 600 MPa with

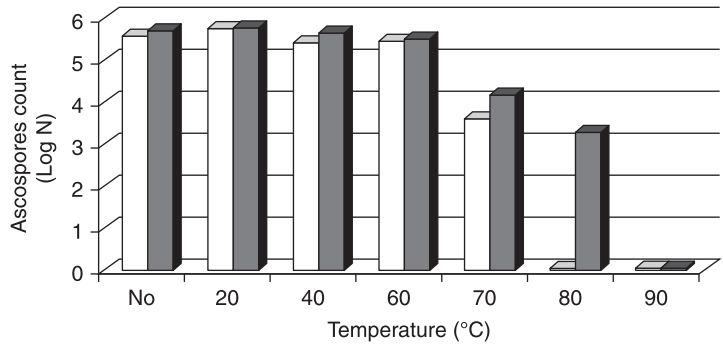

Fig. 11.12 Inactivation of *Byssochlamys nivea* ascopores at 600Mpa for 15 minutes at different temperatures in pineapple juice (□) and pineapple nectar (■). (No) is the initial count of *Byssochlamys nivea* ascospores (Source: Ferreira *et al.*, 2009).

heat treatment led to an increase in the level of inactivation, although a lower inactivation was achieved in the nectar, possibly due to the protective effect of sugars (Ferreira *et al.*, 2009).

Another possible alternative method for increasing the inactivation of spores is the application of pressure in cycles or pulses instead of continuously. Figure 11.13 shows that three pressure cycles lasting five minutes each are more efficient than a single continuous processing cycle of 15 minutes. If the treatment is combined with an increase in temperature, it is possible to achieve a higher level of inactivation of *Byssochlamys nivea* ascospores (Ferreira *et al.*, 2009).

The approach of combined pressure and thermal treatment has led to pressure assisted thermal processing (PATP) being considered as a possible sterilization process for the destruction of spores of pathogenic and deteriorating microorganisms, and has already been approved by U.S. Food and Drug Administration (Barbosa-Cánovas and Juliano, 2008). The process relies on adiabatic heating due to the pressure elevation to achieve the conditions of pressure and temperature necessary for sterilization.

The possibility of maintaining the nutritional and functional quality of tropical fruits using high pressure is exemplified by the work of Tiburski *et al.* (2009) and Shinagawa *et al.* (2009), who studied the effect of high pressure on the antioxidant activity of puree of yellow mombin and papaya respectively, both of which are a rich source of carotenoids. For both products high pressure generally preserved the antioxidant activity, depending on the operational parameters, although under certain conditions reductions of close to 20% compared to the fresh pulp were observed.

The study by Laboissière *et al.* (2007a) on passion fruit juice outlines the ability of high pressure treatments to retain flavor characteristics in the product similar to those of the fresh product, particularly when compared to flavor characteristics of thermally processed products commercialised in the Brazilian

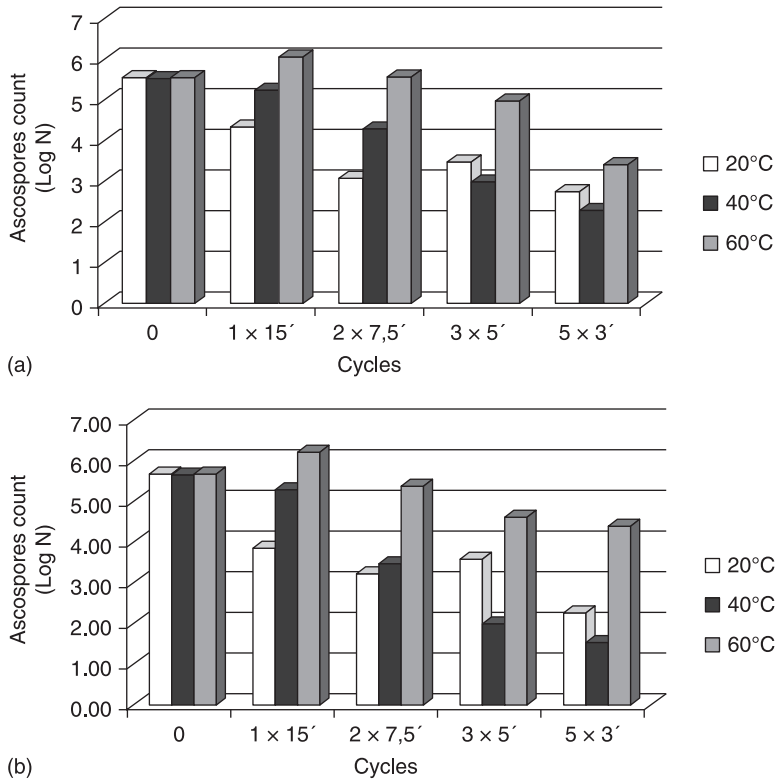

Fig. 11.13 Inactivation of *Byssochlamys nivea* ascospores in pineapple juice (a) and pineapple nectar (b) by high pressure cycles, maximum pressure = 550 MPa at different temperatures (20°C, 40°C and 60°C). (N_0) is the initial count of *Byssochlamys nivea* ascospores (from Ferreira *et al.*, 2009).

market. The results are illustrated in Fig. 11.14, which shows that the trained panel scored the fresh juice (NAT) very similarly to the pressurised sample (HHP), whose attributes were judged to be closer to natural passion fruit than those of marketed products. Conversely, the commercial products presented more attributes such as an artificial, cooked and fermented taste, illustrating the negative effects of thermal processing and the benefits of high pressure.

11.11.2 Pulse electric fields

Most of the emerging non-thermal technologies for food preservation are based on the application of electromagnetic waves or electric current for food preservation. The methods frequently involve the fast generation and transmission of heat to the food, and usually bring about mechanisms that allow the synergistic

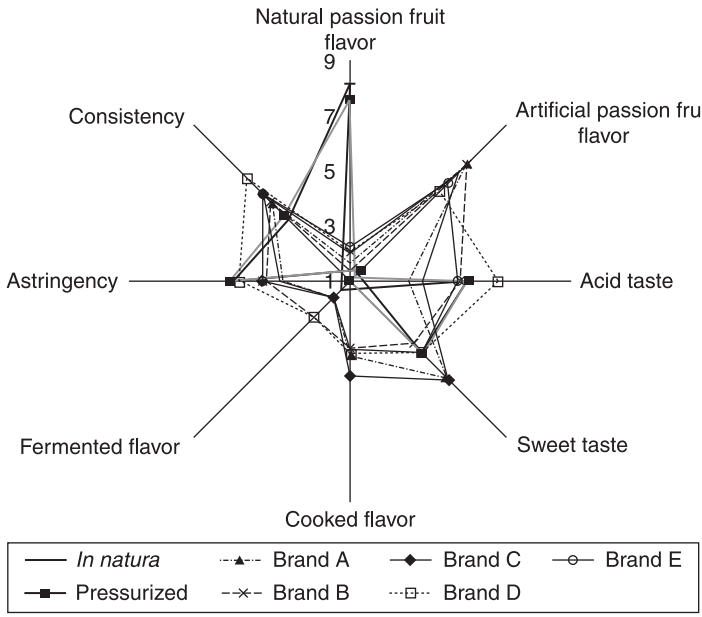

Fig. 11.14 Flavor and consistency attributes used by a quantitative descriptive analysis (QDA) panel to score *in natura* (NAT), pressurised (HHP) and commercial (A to D) yellow passion fruit juices (from Laboissiere *et al.*, 2007a).

inactivation of microorganisms and enzymes. Among the most promising electromagnetic technology for tropical fruit processing are Pulse Electric Fields (PEF).

This technology consists of an electric field generated by an electric circuit in which a range of serial capacitors provide high energy storage, which in turn generates electrostatic induction and electric potential (Rosenthal and Deliza, 2008). The product then flows through this electric field. The level of electric potential usually studied is between 20 and 80 kV/cm, with a pulse duration of 2 to 10 μs. The field leads to the redistribution and polarization of the cellular membranes of the microorganisms, resulting in the formation of pores (electroporation), which lead to the disturbance of cellular homeostasis. Such pores may even result in the leak of intracellular materials, as shown in Fig. 11.15 (Harrison *et al.*, 1995). Studies have shown that PEFs have higher energy efficiency than continuous thermal systems (Qin *et al.*, 1995). One key aspect in determining the efficiency of the system is the design of equipment, including the pulse generator, the treatment chamber and electrodes, which should be designed to minimize the electrolysis effects (U.S. Food and Drug Administration, 2000).

The potential application of PEF for tropical fruits is illustrated by Aguiló-Aguayo *et al.* (2010), who evaluate its use in watermelon juice in comparison to

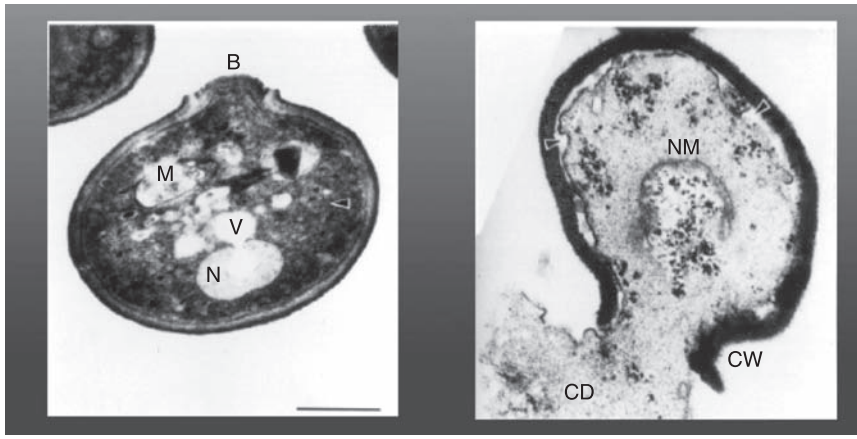

Fig. 11.15 Microphotography of *Saccharomices cerevisiae* cell present in apple juice after treatment with pulse electric fields, in comparison to the control, with evidence of intracellular liquid exudation throughout the membrane porous (Source: Harrison *et al.*, 1997).

heating (90°C for 30 s to 60 s). PEF (35 kV/cm for 1727 μs applying 4 μs pulses at 188 Hz in bipolar mode) proved to be efficient at inactivating the enzymes peroxidase, lipoxygenase and pectin methylesterase, but was limited compared to heating in terms of the inactivation of polygalacturonase. Over 56 days of storage, watermelon juice treated by PEF maintained a brighter red color than the heat treated juice.

11.12 Conclusions

Several conventional and emerging technologies are already used or have potential application in the preservation of tropical and subtropical fruit products and for the manufacture of new products. New technologies may allow further improvement and optimization of preservation processes with the aim of obtaining safe products with high nutritional and sensory quality. The same technologies may also use a limited amount of energy and utilities, minimize residues, and generate less effluent. Knowledge of the individual characteristics of the fruits, including potential contaminant microorganisms that may cause deterioration or health risks to the consumer, deteriorating enzymes, and quality attributes such as functional compounds and sensory attributes, as well as an understanding of the fundamentals of the preservation processes, as presented and discussed in this chapter, allows manufacturers to design processes which maximize benefits while minimizing possible risks.

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Plate XIV (Chapter 11) Line for pineapple juice extraction (courtesy of Tropical Food Machinery).

(a)

(b)

Plate XV (Chapter 11) Steam jacketed pans used in jams and jellies manufacture concentration. (a) Open steam jacketed pan with mechanical stirrer and (b) vacuum concentrator ('Buller') (courtesy of Mecamau).

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