Postharvest Physiology and Biochemistry of Fruits and Vegetables

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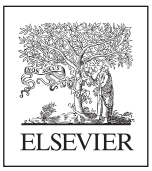

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Foreword

The science behind the postharvest physiology and biochemistry of fruits and vegetables has undergone a major transition in the last few decades, mainly with the advent of incisive molecular and biochemical approaches, and has provided a new understanding and unique regulation of postharvest biology, making it a discipline in itself. Fruits and vegetables supplement diets with health-promoting concoctions of vitamins, antioxidants, minerals, and fiber. There is consumer demand for high nutritional quality foods, especially nutrition-dense fruits and vegetables. Also, postharvest losses of fruits and vegetables is a major obstacle to food security and leads to a large economic loss during storage and long-distance shipping/transportation. Substantial improvement of the nutritional content of fruits and vegetables in addition to longer shelf-life will likely require new germplasm generated using advanced biotechnological approaches. In the process, scientific understanding of the regulation of biochemical and molecular events critical for prolonging the fruit postharvest life together with enhancement of quality attributes including vitamins, proteins, flavor, aroma, and texture, has advanced further. Ethylene as a "ripening" plant hormone and promoter of leaf senescence has attracted the attention of many investigators, revealing important facets of its biosynthesis and action. However, it is becoming clear that other plant hormones also play important roles in fruit physiology and ripening—a field still in its infancy.

With new developments, new technologies, and new knowledge a need arises for high-level to-go-to single-source information for their dissemination. This book purports to achieve this goal, providing condensed information, from one end of the fruit physiology/biochemistry and biotechnology spectrum to the biotechnology intervention for improving postharvest life and fruit nutrition and stress. In addition to the Introduction, the new information in this book is spread across 21 chapters, covering a wide range of subjects authored by world-class scientists. The authors and editor have done an excellent job of condensing the available literature, provided valuable literature, and the contributions together provide knowledge and technologies that can be applied to improve and develop new and robust germplasm. Subjects tackled include human health aspects of fruits and vegetables, texture, transpiration, morphology and anatomy, photosynthesis, respiration, fruit growth and development, ripening and senescence, flavor and aroma, stress responses, ethylene, pigments, phenolic compounds, carbohydrates, organic acids, lipids, proteins, enzymes, vitamins, minerals, modified and controlled atmospheres, to biotechnology of horticultural crops.

This book is timely and I hope it will be read widely by both the initiated and uninitiated, experts and industry, and become an important source of knowledge in this field to reference libraries and research centers around the globe.

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CHAPTER 1
Introduction

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1.1 INTRODUCTION

Fruits and vegetables are very significant to human nutrition, and are highly recommended for a healthy diet. More than 700 million metric tons of fruits are produced in the world each year. The greatest annual fruit harvest in the world occurs in Asia, followed by the Americas, Africa, Europe, and Oceania. China alone produces about 300 million metric tons of fruits annually. The most popular classes of fruits include bananas and apples, followed by grapes and oranges. About 1223 million metric tons of vegetables and melons were produced worldwide, mostly in Asia followed by Europe, the Americas, and Oceania. China is the leading producer, with a production volume of nearly 170 million metric tons, followed by India with approximately 40 million metric tons of fresh vegetables. Horticultural commodities contribute very significantly to the economy of many countries. For example, the total farm income from vegetables in the United States is $20,588,841,000, and the value of US product shipments of frozen vegetables is $9.1 billion.

Fresh fruits and vegetables and their processed products have become essential and important human dietary choices in recent years, primarily because of several epidemiological studies showing various health benefits associated with their consumption. The nutritional and food qualities of fruits and vegetables are a result of the accumulation of components derived from the intricate biochemical pathways. In an era where the consumption of fruits and vegetable is essential for human nutrition and health, postharvest science gets a new meaning.

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However, about 30%–60% of total production of fruits and vegetables are lost and wasted, mostly due to improper postharvest handling. In some developing countries, losses and waste have been estimated at 50% in tomatoes, 49% in carrots, 62% in lettuce, 20%–80% in bananas, 50%–100% in papaya, 43% in avocados, 27% in grapes, etc. It is evident that these losses and waste are very high and significant in terms of nutrition in a world suffering from hunger, mostly due to poor utilization of efforts during production and management before and after harvest and, ultimately, enormous economic losses, especially for countries where agriculture is an important economic sector. Postharvest losses and waste are a huge challenge, because they are not only the result of losses and waste of nutrients in a world still suffering from severe hunger, but also the wasting of other very important resources such as land, water, energy, chemicals, and the environmental problems created by the wasted commodities. Many factors contribute to postharvest losses and waste, including biological, microbiological, and environmental. Proper postharvest handling and management are essential to preserve quality and to reduce losses and waste. Proper handling of perishable horticultural commodities requires the proper understanding of factors that lead to biological changes and mechanisms of maturation, ripening, and senescence.

Fruits and vegetables share several common structural and nutritional properties and characteristic differences due to differences in their biochemical composition. Fruits, in general, are attractive organs for vectors involved in seed dispersal, and thus have evolved features such as enhanced color, attractive flavor, and taste. Consequently, the developmental and biochemical processes within a fruit are programmed to achieve this goal.

Suitability for end use, including storage capability, shelf-life potential, and acceptability for processing either minimal or secondary processing, are very much determined by the physiological and biochemical characteristics of the commodity. Selection of a certain cultivar of any horticulture commodity for its suitability for any postharvest treatment normally requires a complete analysis of all the physiological and biochemical characteristics that define the suitability of the commodity for the desired use. For example, in selecting butterhead lettuce for fresh-cut use, it was established that cultivars having both lower respiration rates and lower sensitivity to high carbon dioxide (CO_2) injury were the most suitable. Most often there is more than one physiological characteristic that determines the overall acceptability of a certain cultivar to a particular postharvest treatment. If all the characteristics required are identified when selecting new cultivars, then there is a greater chance that the specific cultivar will have a consistent acceptability for the specific postharvest treatment to be used over the long term. Horticulture commodity quality is determined by the physiological and biochemical characteristics of the commodity, and therefore, genetic transformation platforms may provide avenues to accelerate quality improvement for fresh storage and processing uses in the future, once the molecular mechanisms for quality are better understood.

Fresh horticultural commodities are living organs capable of continuing their life processes after detaching from the plant. They perform a series of metabolic pathways in order to obtain the required energy to preserve their life, and therefore, physiological and biochemical processes are carried out during their whole postharvest life. Being living organisms, fresh horticultural commodities respire and consequently generate heat. When respiring, they take up oxygen and give off CO_2. After harvest, fruits and vegetables enter into different phases that lead to senescence and death. In the case of botanical fruits, they prepare their tissue for seed dispersal. Several changes take place in the tissue in its pathway to become attractive to the seed dispersers. Changes in cell wall composition and structure result in tissue softening. Changes in pigments result in color development (yellow/orange/red/purple) generally concomitantly with chlorophyll degradation and synthesis and development of other pigments such as carotenoids and flavonoids. Changes in sugar content are an important process in all fruits and vegetables, especially in starchy fruits where an increase in sugars is presented in spite of the sugar consumption by the respiratory process. Changes in flavor (taste and aroma) compounds are common during ripening and senescence. The ethylene volatile is a well-known plant hormone and it has been named the "ripening hormone" because of its immense importance in the physiological and biochemical processes, especially those that lead to ripening and senescence.

Knowledge of the biochemical and physiological profile of fresh horticultural commodities is a very important tool to assist in optimizing the use of postharvest technologies and proper commercial utilization of the commodity. The understanding of the biochemical and physiological bases of quality retention in fruits and vegetables provides good guidance for the maintenance of these commodities and for the proper use of postharvest methods and techniques. Examples of these are the sensitivity to chilling injury, mineral difficiency, heat, very low oxygen, very high CO_2, among others. Therefore, it is important to define the characteristics of the horticulture commodity using existing information and then develop possible strategies to preserve quality and to enhance postharvest life, as defined by different criteria, such as sensory quality, nutritional quality, and functional quality. Postharvest management of fruits and vegetables requires a thorough knowledge of their nature, physiology, and responses to the surrounding environment, such as temperature and relative humidity (RH), composition of the atmosphere, and metabolic products such as ethylene, to which they are subjected to from harvest to consumption, since each product behaves differently depending on its nature and the management conditions. To apply an appropriate postharvest management to these products, it is necessary to understand the main biological aspects that favor their preservation. Lack of this knowledge is the major cause of quality deterioration, high consumer prices, and the heavy losses that occur during the commercialization and distribution of fruits and vegetables.

1.2 THE IMPORTANCE OF FRUITS AND VEGETABLES IN HUMAN NUTRITION AND HEALTH

The trade in fresh fruits and vegetables is increasing worldwide because of the importance of these products for the human diet and health. They provide variety and flavor to dishes and meet several essential nutritional requirements, such as ascorbic acid (vitamin C), in addition to other vitamins such as A, B6, thiamine, riboflavin, and folic acid, some of which humans cannot synthesize, and need to be obtained from the diet. Fruits and vegetables are important sources of carbohydrates and minerals. The dietary fiber provided by fruits and vegetables has been increasingly incorporated into the human diet in order to minimize some diseases related to modern lifestyle. Other important constituents include pigments such as carotenoids, flavonoids, and other polyphenols, and other phytonutrients. For this reason, there is a worldwide trend to increase the consumption of fruits and vegetables.

There is strong evidence that fruit and vegetable consumption can prevent a number of chronic noncommunicable diseases, including cardiovascular diseases (CVDs), diabetes, obesity, cancer, and respiratory conditions, mostly due to the very important phytochemicals they contain. Phytochemicals are bioactive nonnutrient plant compounds found in fruits, vegetables, grains, and other plant foods, and have been linked to reductions in the risk of major chronic diseases. They are almost ubiquitous in plant-derived foods and inherently have more subtle effects than nutrients. Phytochemicals can accumulate in relatively high amounts in plants and appear to have a myriad of supplemental roles in a plant's life cycle. Although these secondary metabolites account for the bioactive chemicals responsible for medicinal actions in humans, they are actually produced to provide the plant itself with unique survival or adaptive strategies. Phytochemicals can provide protection against abiotic stresses such as UV-B irradiation, temperature extremes, low water potential, or mineral deficiency. One of the most versatile groups of phytochemicals, carotenoids, protect chloroplasts from photodegradation by absorbing high-energy quanta, while also scavenging free radicals and reactive oxygen species. Flavonols, another important group of phytochemicals, as well as providing protection against the damaging effects of UV-B, are also involved in promoting the growth of pollen tubes in the style to facilitate fertilization of the ovule. Other phytochemical groups such as lignans, terpenoids, and isoflavonoids also play important defense roles against pathogen and insect attack.

Consumers are increasingly becoming aware of the disease-preventive and health-restoring roles of fruits and vegetables, because of which they are classified by some as functional foods. Many quality components are also regarded as important functional food ingredients (nutraceuticals) that include soluble and insoluble fibers; color pigments such as chlorophylls, anthocyanins, and carotenoids; several polyphenolic components; and sulfur-containing components in crucifer and *Allium* vegetables. Fruits in general contain large amounts of fibrous materials such as cellulose and pectin. The breakdown of these large

polymers into smaller water-soluble components during ripening leads to fruit softening. Anthocyanins are the major color components in several horticultural commodities such as grapes, strawberries, blueberries, apples, and plums. Carotenoids, such as β-carotene, luteion, β-cryptoxanthin, lycopene, among others, are the major color components in several horticultural commodities such as tomatoes, mangoes, and papayas, and these components provide health benefits to consumers through their antioxidant properties and ability to influence metabolic processes within the human body. Vegetables such as asparagus are rich in glutathione, another component in the antioxidant defense system. Lipid content is quite low in fruits and vegetables; however, very few fruits, such as avocado, nuts, and olives store large amounts of triacylglycerols (oils). The amounts of proteins are usually low in most fruits and vegetables.

The nutritional value of horticultural commodities is influenced by the very nature of the product whose composition may vary for genetic reasons, crop development conditions, maturity at harvest, and postharvest handling to which they are subjected to before being consumed.

Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by several internal as well as external factors, such as physical damage, extended storage duration, high temperatures, chilling injury of chilling-sensitive crops, low RH, among others.

Many aspects of the secondary metabolism after harvest of fruits and vegetables are still not fully understood. However, extensive efforts are underway in order to get an insight into the physiology and biochemistry of fruits and vegetables and the relation between their functional-nutraceutical properties and human health.

1.3 FRUIT AND VEGETABLE DEVELOPMENT

Fruits and vegetables belong to various plant structures. Fruits are part of the female reproductive organs of plants that produce seeds, that is, the ovary is developed and mature. For their part, vegetables can consist of fruits, flowers, leaves, stems, buds, roots, and tubers. This great variety of structures and composition greatly influences their management during harvest, postharvest, and commercialization.

The developmental processes in fruits and vegetables are influenced by fertilization, and the hormonal changes induced in the ovary leading to gene expression and biochemical changes resulting in the characteristic fruit that may vary in ontogeny, form, structure, and quality. Fruits originate from different parts of the ovary. Pome fruits such as apple and pear develop from the thalamus of the flower. The ovary wall (mesocarp) in drupes, such as cherries, peaches, plums, and apricots, develops into the fruit enclosing a single seed. Berry fruits, such as tomato and grape, possess the seeds embedded in a jelly-like pectinaceous matrix, with the ovary wall developing into the flesh of the

fruit. Citrus fruits belong to the class known as hesperidium, where the ovary wall develops as a protective structure surrounding the juice-filled locules that are the edible part of the fruit. The seeds in strawberry are located outside the fruit, and it is the receptacle of the ovary (central portion) that develops into the edible part. Most vegetables are leaves, petioles, or stems containing chlorophyll, or roots, tubers, or fruits that predominantly contain storage components such as starch. Examples of these include potato and eggplant (Solanaceae), gourds (Cucurbitaceae), several types of yams (Dioscoreaceae and Araceae), vegetables of leaf and flower origin (cabbage, broccoli, cauliflower—Cruciferae), and unripe fruits of leguminous plants such as peas and beans (Leguminosae).

Edible plants have been classified in many ways. Fruits and vegetables have been classified also in several ways using different criteria, such as botanical, agronomic, gastronomic, and according to how they are handled after harvest. The botanical definitions of fruit refers to the mature ovary of a plant, which contains its seeds, the covering tissue around the seeds, and any closely connected tissue derived from the floral parts. This is the botanical fruit, which may or may not be edible. However, in the sense of food, fruit refers to the edible part of a plant that may consist of the seeds and their surrounding tissues, which can be fleshy such as berries and cucurbits, or dry, papery, leathery, and woody such as legumes and nuts. On the other hand, in the sense of food, the term vegetable refers to any edible part(s) of the plant, such as the root (carrot), tuber (potato), bulb (onion), stems (celery), leaves (spinach), flower bud (globe artichoke), or fruit (cucumber, tomato). In this latter case, fruit is included as a subset of vegetables.

Fruit development is generally divided into three major stages: growth, maturation, and ripening/senescence. The growth period of fruits begins with cell division and continues with cell enlargement until the final size is attained. Maturation begins usually just prior to the end of growth and it is the stage of development that leads to the attainment of the physiological maturity of the fruit, which is the point of the fruit development in which this reaches the full functional capacity to continue ontogeny and ripe normally. Senescence is the period at which predominantly catabolic processes take place leading to tissue degradation and death. Maturity has been divided into two general categories, that is, physiological maturity and horticultural maturity. Physiological maturity is the stage after which a fruit is capable of further development or ripening on or off (in the case of climacteric fruits) the plant. Horticultural maturity refers to the stage of development when the plant or plant part possesses the quality prerequisites for use by consumers for a particular purpose.

Different stages of maturity and ripening are recognized, including: (1) "Mature," which is derived from the Latin word "maturus," meaning ripen, and refers to the stage of fruit development which ensures attainment of maximum edible quality at the completion of the ripening process. (2) "Maturation" refers to the developmental process by which the fruit attains

maturity. It is the transient phase of development from near completion of physical growth to attainment of physiological maturity. There are different stages of maturation, for example, immature, mature, optimally mature, and over mature. (3) "Ripe," on the other hand, is derived from the Saxon word "ripi," which means gather or reap, which is the condition of maximum edible quality attained by the fruit following harvest. (4) "Ripening" involves a series of changes occurring during the early stages of senescence of fruits in which the structure and composition of unripe fruit are altered and become acceptable for consumption. Ripening is a complex physiological process resulting in coloring, softening, sweetening, and increase in number and intensity of aroma compounds. The associated physiological or biochemical changes during ripening include increased rate of respiration and ethylene production, loss of chlorophyll and degradation of chloroplast, synthesis or appearance of other pigments such as carotenoids or flavonoids, synthesis of cromoplats, continued expansion of cells, and conversion of complex metabolites into simple molecules such as aroma volatiles. "Senescence" can be defined as the final stage in the ontogeny of the plant organ during which a series of essentially irreversible events occur which ultimately lead to cellular breakdown and death.

1.4 POSTHARVEST PHYSIOLOGY AND BIOCHEMISTRY

Fruit development, maturation, ripening, and senescence are characterized by several marked physiological and biochemical changes resulting in the coordinated development of complex characteristics. Following pollination and fertilization, the fruit develops in size, several macromolecular components are constructed, and several processes take place, leading to the maturation and ripening processes, which result in the development of proper and particular organoleptic characteristics such as taste, color, and aroma that are important quality-determining features.

Irrespective of the type of commodity, various technologies such as cold storage, modified and controlled atmospheres, and control of some hormones and enzyme actions are commonly used to slow down the metabolic processes, and to prolong the postharvest life of the commodity. Advances in the biochemistry and molecular biology have enabled the development and use of strategies for the preservation of postharvest shelf life and quality of fruits and vegetables.

Several metabolic changes are initiated in fruits and vegetables after harvest. Fruits are generally classified into climacteric or nonclimacteric types on the basis of the pattern of respiration, and responsiveness to externally added ethylene. Climacteric fruits characteristically show a sudden and marked increase in ethylene production and respiration, as shown by the evolution of CO_2. By contrast, nonclimacteric fruits do not show the sudden increase, and commonly emit a considerably reduced level of ethylene. In climacteric fruits

such as apple, pear, banana, papaya, tomato, mango, papaya, and avocado, among others, ethylene evolution can reach up to 30−500 ppm/kg/h (parts per million, μL/L) during ripening at 20−25°C, whereas in nonclimacteric fruits such as oranges, lemons, strawberries, grapes, pineapples, and cucumbers, among others, ethylene production levels usually range from 0.1 to 0.5 ppm/kg/h, during ripening at 20−25°C. Climacteric fruits respond to external ethylene treatment by an early induction and increased levels of ethylene and CO_2 levels, and an accelerated ripening, in a concentration-dependent manner. Nonclimacteric fruits, on the other hand, show increased respiration and ethylene production in response to external ethylene treatment without showing acceleration in the time required for ripening.

The initiation of ripening in fruits and vegetables on or off the tree is accompanied by the development of several biochemical changes, including changes in color, texture, and flavor. Some of these changes are desirable, especially for the quality of fruits, but some changes may not be desirable for some other commodities, particularly for some vegetables. Therefore, strategies for the preservation of fruits and vegetables after harvest could be different depending on the type of commodity and its end use, and so it is important to understand the biochemical differences between fruits and vegetables, and the different biochemical pathways that operate in these tissues, to develop and use the most appropriate postharvest handling techniques and conditions.

1.5 BIOCHEMICAL PARAMETERS OF HORTICULTURAL CROP QUALITY

At least two major aspects define the quality of a horticultural commodity: the first being the inherent biochemical characteristics that provide color, texture, and taste to the produce, among some other quality components, and the second being the consumer perception.

The quality of horticultural commodities is a complex perception of many attributes determined by the consumer either subjectively and/or objectively. Fruits and vegetables are consumed mainly for their nutritive and health value but also for their variety of shapes, colors, and flavors that make them attractive for food preparations. The brain processes the information received by sight, smell, and touch and instantly compares or associates it with past experiences or with textures, aromas, and flavors stored in its memory. For example, just by looking at the color of a certain horticultural commodity, the consumer can assume its stage of ripeness and other characteristics such as flavor, and nutritional components. In addition to noting the color of the commodity, the consumer commonly uses other senses such as touch to judge firmness, or other perceptible characteristics. Aroma is also used as a quality parameter, especially in some fruits such as guava. Another important quality component of fresh horticultural commodities is their freedom of biotic or nonbiotic contaminants that may affect health.

Fresh fruits and vegetables contain a large percentage of water, which can often exceed 95% by fresh weight. Together with carbohydrates, proteins, lipids, and other substances, water gives rise to a product with very special sensory characteristics. High water content is a determining factor for the consumption of horticultural commodities because it imparts turgidity and freshness that are highly appreciated by the consumer, but in turn, this feature makes the preservation of horticultural commodities very difficult. Once the commodities are separated from the plant they become very susceptible to water loss and wilting. The high water activity of these fresh commodities allows for very high biological activity, and facilitates high microbiological activity.

Fruit ripening is a dynamic transitional period during which several easily perceived changes, such as alterations in color, firmness, sweetness, and acidity, take place. These changes make fruit desirable for human consumption and capable of seed dispersal by birds, animals, and environments. Fruit firmness is associated with several attributes including crispness, mealiness, grittiness, chewiness, succulence and juiciness, fibrousness, toughness, and oiliness. The development of various organoleptic components, such as sweetness, sourness, astringency, bitterness, and production of odor-active volatiles leading to characteristic aroma are mostly related to other ripening changes such as textural modifications. Although most of these changes impart desirable traits to various fruits and vegetables, some might be unacceptable for some types of commodities, such as the development of excessive softening, and the development of off-flavors and off-odors. Excessive softening can enhance the susceptibility to pathogens due to their proneness to solute leakage that provides rich media for their growth, and can result in severe losses during postharvest storage and marketing.

The stage of development in a fruit determines its biochemical composition and the quality defining parameters. Color, caused by different clases of pigments, is perhaps the first parameter that attracts a consumer to a produce. The composition of chlorphylls, carotenoids, flavonoids, and betalaines determines the color quality characteristics of the different horticultural commodities. Anthocyanins are the major color components in many fruits and vegetables such as grapes, blueberries, strawberries, apples, plums, eggplants, and many others. Carotenoids, such as lycopene, β- and α-carotene, luteine, and α- and β-cryptoxanthin, are the major pigments that impart color to many horticultural commodities, such as tomato, carrot, mango, and papaya. Consumers may also associate the depth of color with the taste of the commodity.

Fruit and vegetable texture and the degree of softness are determined by the intactness of the cell walls and membranes, their contents and structures of pectic substances and other fibrous materials, and also related to the amount of water contained in the produce and the ability to retain that water after harvest. The degradation of cell wall and cell membrane components negatively affects the rigidity of the tissue in fruits and vegetables, resulting in tissue

softening that might be preferred for some fruits and vegetables in some instances and some stages, and may not be preferred in other commodities in other instances. Excessive degradation of structure reduces the postharvest life of the commodity and may enhance the development of decay organisms. Ripening of fruits is mainly orchestrated by the biosynthesis of ethylene that triggers a series of biochemical and physiological processes inducing softening. Insoluble pectin levels usually decline during ripening with a concomitant increase in soluble pectin levels. During softening, dissolution of the ordered arrangement of cell wall and middle lamella polysaccharides occurs. As the fruit ripens, a substantial portion of its cell wall pectins is converted to a water-soluble form affecting the texture. The major changes involved in softening are the catabolism of cell walls and the development of an intercellular matrix containing pectins.

Flavor is a very important component of the quality perception, and the degree of ripeness determines the level and types of flavor components such as sugars, acids, phenolic compounds, and odor-active volatiles such as esters and terpenoids emitted from the produce. Aroma is part of the flavor, caused by odor-active volatiles produced by the commodity, and is derived from several types of compounds such as monoterpenes (such as in limes and oranges), ester volatiles (such as ethyl and methyl butyrate in apple, isoamyl acetate in banana), small-chain aldehydes such as hexenal and hexanal (such as in cucumber), among many other odor-active volatiles. In most fruits and vegetables the ripening process is associated with the conversion of stored starch into sugars, and enhanced evolution of flavor components. Fruits and vegetables commonly have a high percentage of organic acids before ripening, which decrease during ripening, leading to changes in flavor. Organic acids are important substrates of respiration. The presence of off-flavors resulting from the development of certain aldehydes (such as acetaldehyde) may negatively impact the quality perception, whereas other aldehydes such as hexanal tend to enhance the green flavor and consumer preference of some vegetables. The evolution of sulfur volatiles in crucifer vegetables such as broccoli and cabbage, and *Allium* vegetables such as onion and garlic, is an important quality component. The evolution of some essential oils in Lamiaceae members such as mint, oregano, and rosemary, also attracts consumers. Aroma volatiles commonly develop at the later stages of ripening and senescence.

1.6 POSTHARVEST FACTORS AFFECTING FRUIT PHYSIOLOGY AND BIOCHEMISTRY

Fresh horticultural commodities are diverse in morphological structure (roots, stems, leaves, flowers, fruits, etc.), composition, and general physiology. Thus, commodity requirements and recommendations for maximum postharvest life vary significantly among these commodities. All fresh horticultural crops are high in water content and are subject to desiccation (wilting, shriveling) and mechanical injury. They are also susceptible to attack by bacteria and fungi,

with consequent pathological breakdown. Biological (internal) causes of deterioration include respiration rate, ethylene production and action, rates of compositional changes in color, texture, flavor, and nutritive value, mechanical injuries, water stress, sprouting and rooting, physiological disorders, and pathological breakdown. The rate of biological deterioration depends on several environmental (external) factors, including temperature, RH, air velocity, and atmospheric composition (concentrations of oxygen, CO_2, and ethylene), and sanitation procedures.

Temperature is the most important environmental factor that influences the deterioration of horticultural commodities. Temperatures outside the optimal range for each different commodity can cause rapid deterioration. Many nonchilling sensitive perishable horticultural commodities can be maintained for the longest duration at temperatures immediately above their freezing point (at near 0°C). However, many commodities, especially all those of tropical origin and many of those of subtropical origin can be injured when exposed to temperatures between 0°C and 18°C. Commodities that are slightly sensitive to low-temperature injury, such as "Hass" avocado, oranges, and melons, are kept best at about 3−7°C, while commodities highly sensitive to low-temperature injury, such as mango, papaya, and banana, are best kept at temperatures around 8°C and 15°C.

At temperatures above the optimum, the rate of deterioration increases two- to threefolds for every 10°C rise in temperature. Temperature significantly influences how other internal and external factors affect the commodity. It has dramatic effects on respiration, ethylene production, enzymes activities, spore germination, growth rate of pathogens, etc. The freezing point of all perishable horticultural commodities is between −3°C and −0.5°C, and the disruption caused by freezing usually results in immediate collapse of the tissues, total loss of cellular integrity, and losses of some of the components of the commodity.

As indicated previously, many commodities (mainly those of tropical and subtropical origin) respond unfavorably to storage at low temperatures well above their freezing points, but below a critical temperature (between 0°C and 15°C depending on the commodity, maturity stage, and duration of exposure to the temperature) termed as the chilling threshold temperature or lowest safe temperature. Chilling injury is manifested in a variety of symptoms including surface and internal discoloration, pitting, water soaking, failure to ripen, uneven ripening, development of off-flavors, and accelerated susceptibility to pathogen attack. Low-temperature storage-induced chilling injury may be more subtle in nature than development of visual defects in fresh horticultural commodities. In many cases, these subtle effects can be noted as changes in processing quality induced by low storage temperatures. A good example is potato, which is known to sweeten in response to low-temperature storage. Consequently, the storage temperature recommendations for potatoes vary significantly according to the end-use of the product. At low temperatures, the

storage starch is converted into sugars and therefore, potato will tend to be susceptible to unacceptable levels of browning under normal chipping conditions. Another consequence of this sweetening is on the pasting quality attributes when the potatoes are being used for potato flour starch manufacture. This is another of many examples indicating the importance of understanding the biochemical and physiological responses of the commodity to the different handling methods and factors.

Very high temperatures can cause severe injury to perishable horticultural commodities. Transpiration is vital to maintain optimal growth temperatures when the product is connected to the plant. However, after harvest, the lack of the protective effects of transpiration and direct sources of heat can rapidly heat tissues to above the thermal death point of their cells, leading to localized bleaching or necrosis, shown as sunburn or sunscald, and may even lead to general collapse and death of the tissue. High temperatures lead to an alteration of gene expression, and fruit ripening can sometimes be either accelerated, delayed, or even disrupted, depending on the type of commodity, temperature, and exposure duration. Cell wall-degrading enzymes and ethylene production are frequently the most disrupted by heat treatments, and their appearance is delayed, and their activities affected following heating. However, postharvest heat treatments are commercially used after harvest for the control of insects, pathogens, and ripening in some heat-resistant horticultural commodities. Several treatments are established based on hot air and hot water, and some are established as legal quarantine treatments. For example, a legal treatment very much used commercially in several countries as a quarantine treatment for mango consists of immersion of mango fruit in water at 46.1°C for 65–90 min depending on fruit weight.

RH, which refers to the moisture content (as water vapor) of the atmosphere, expressed as a percentage of the amount of moisture that can be retained by the atmosphere (moisture-holding capacity) at a given temperature and pressure without condensation, affects the water loss of fresh horticultural commodities and several other components and processes. RH can also influence decay development, incidence and severity of some physiological disorders, losses of some water-soluble components from the commodity such as vitamin C, and the uniformity of fruit ripening.

The response of a fresh horticulture commodity to the different levels of gases, such as oxygen and CO_2, determines whether the commodity can be maintained in a modified or controlled atmosphere, the optimal atmosphere that can be used, and whether a certain commodity can be maintained with another commodity, even if they are closely related botanically.

1.7 MOLECULAR BIOLOGY AND BIOTECHNOLOGY

The economic consequences of fruit softening, especially of tomato fruit, have led to a considerable interest of geneticists, physiologists, biochemists, and

molecular biologists to investigate and understand their molecular basis over the last five decades. The last 50 years have seen excellent research and a significant increase in our understanding of the biochemical changes associated with fruit textural modifications. Emerging recombinant DNA technologies, including reverse genetics, have begun to provide some answers. Excellent research has been conducted to describe cell wall chemistry and relations of various families of cell wall-modifying enzymes to the developmentally regulated softening of horticultural commodities during maturation, ripening, and senescence. Therefore, various postharvest factors affecting structural deterioration of horticultural commodities and the potential of chemical or genetic means to reduce softening and deterioration are implemented to better preserve commodities and to maximize the high textural integrity, and maintain quality and extend postharvest life. The long shelf-life tomato cultivars currently cultivated and commercialized all over the world is a clear example of these efforts.

It was also observed later that a strategy to enhance the shelf life of horticultural commodities could be adopted through regulation of endogenous ethylene production and action. Research has shown that this could be achieved by use of genetically modified (GM) crops where gene expression of key enzymes responsible for ripening, like polygalacturonase, ethylene forming enzyme, and 1-aminocyclopropane-1-carboxylic acid synthase, is achieved by means of antisense RNAs. However, adoption of this technology has so far been deterred due to safety issue apprehensions associated with GM crops.

1.8 RESEARCH ON POSTHARVEST PHYSIOLOGY AND BIOCHEMISTRY

Research in the area of postharvest physiology and biochemistry of horticultural commodities has been very active during the last 5–6 decades, leading to very significant advances, especially those initial ones led by Jacob Biale at the University of California, Los Angeles, on fruit ripening and the climacteric during the 1950s and 1960s, and those led by Shang Fa Yang at the University of California in Davis during the 1980s on ethylene biosynthesis. However, research in developing countries is still very limited, with a shortage of trained researchers and very limited infrastructure and funds. Traditionally, almost all agricultural research, especially in developing countries, has focused on preharvest factors such as genetic improvement and adaptability of cultivars, fertilization, irrigation, preharvest control of insects and diseases, etc. Recent technological advances to determine optimal maturity at harvest, careful handling, precooling, use of physical treatments in addition to pesticides for insect and disease control, and other procedures to control deterioration, refrigerated storage and transport, controlled and modified atmospheres for storage, transport and packaging, and control of maturation and ripening, irradiation, packing, specific handling techniques for different products, transport, marketing, etc., have all been mostly based on excellent research and understanding of

physiological and biochemical phenomena, and have resulted in a significant increase in the number, duration, and availability of fresh horticultural products in the world markets. However, the proper use of postharvest technologies still requires more application of current postharvest physiology and biochemistry knowledge to further improve the use of these technologies, and the proper management systems of fresh commodities. While some postharvest losses in the quality and quantity of fruits and vegetables can be minimized using currently available information, further reduction of these losses and further quality improvement and expansion of marketing opportunities will require new discoveries based on increased research efforts on biology and postharvest physiology, biochemistry, and technology of horticultural commodities.

1.9 CONCLUSIONS

Fresh horticultural commodities are of great importance for the human diet, nutrition, health, and wellbeing. They are very diverse in anatomical, morphological, and physiological characteristics, and in shape, form, color, taste, aroma, etc., and therefore they have diverse requirements for optimum postharvest handling. Perishable horticultural commodities suffer the most losses and waste among all types of food classes. Reduction of losses and waste of horticultural commodities is essential, not only to increase food availability but also to prevent the wasting of very important resources such as land, water, energy, and chemicals, and to reduce environmental problems resulting from these wastes. The development of proper postharvest handling and management techniques requires adequate understanding of the mechanisms involved in the development, maturation, ripening, and senescence of horticultural commodities.

This textbook provides knowledge on postharvest physiology and biochemistry of fruits and vegetables and includes important new advances in these subjects. The book adopts a thematic style where the different chapters have been written by experts in their respective fields. The book is directed not only to undergraduate and graduate students and professors, but also to professionals related to food and agricultural and nutritional careers and to all those interested in the understanding of the postharvest biology of horticultural crops. Each chapter is intended to be simple and explanatory in order to be read easily. The topics covered as individual chapters are as follows: contribution of fruits and vegetables to human nutrition and health, photosynthesis, respiration, biology and biochemistry of ethylene, morphology and anatomy, fruit growth and development, ripening and senescence, transpiration, carbohydrates, organic acids, pigments, phenolic compounds, lipids, texture, proteins, enzymes, vitamins, minerals, flavors and aromas, physiological responses to stress, physiological and biochemical effects of modified and controlled atmospheres, and biotechnology of horticultural commodities.

The book provides the fundamental knowledge on the biological, chemical, and biochemical changes that occur in fruits and vegetables from their very emergence till their death to the target audience. The first part of the book depicts health issues concerning the contributions of fruits and vegetables on human health, encompassing how fruits and vegetables fight diseases such as cancer, cardiovascular disease, diabetes, and obesity. The second part deals with the metabolic processes involved in fruit growth and development: photosynthesis, respiration, and ethylene. This part starts with photosynthesis as the process that explains how sunlight energy is converted to chemical energy to form sugars that are stored in the plant and eventually used during fruit growth and developmental events occurring during the whole life of the plant and fruits and converted into many other components. This chapter is finalized by a discussion on "photosynthesis in fruits," a topic very rarely discussed in the literature, much less in the postharvest literature. Respiration is a physiological process that utilizes the sugars produced during the process of photosynthesis, and has great importance during the postharvest handling of horticultural crops, thus the book addresses this topic considering the closed relationship between photosynthesis and postharvest life and respiration. Ethylene, which is considered as the ripening hormone, is discussed in a full chapter given its immense relevance during the whole life of horticultural commodities.

Another part of the book deals with the developmental aspects during the whole life of fruits and vegeatbles. The chapter on "fruit growth and development" addresses how plant hormones affect fruit development, encompassing fruit ripening and senescence.

The book also discusses important issues such as the morphology and anatomy of fruits and vegetables, beginning with the structural organization of the whole plant and the fruit structure, and botanical classification.

Another part of the book discusses the chemical composition and compositional changes that occur in fruits and vegetables after harvest. Different chemical compounds and their changes during ripening and storage of fruits and vegetables are discussed in relation to nutritional quality. The different compositional changes discussed include carbohydrates, organic acids, pigments, phenolic compounds, lipids, texture components, proteins, enzymes, vitamins, minerals, and flavor and aroma components.

Another section illustrates the physiology of fruits and vegetables under stress conditions, basically chilling injury, low oxygen, and high CO_2 stresses.

The last part of the book discusses biotechnological methods to control different fruit and vegetable quality traits such as softening, quality, and nutritional value of fruits.

The most valuable feature of the book is its simple style and explanatory format. The concepts are well developed and easy to understand, covering a wide range of interrelated themes. In this way, the book can be adopted as a

textbook to support different courses not only for undergraduate and postgraduate students, lecturers, and researchers, but also for technicians and professional consultants interested in the understanding of the fundamental knowledge of postharvest biology of horticultural commodities.

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CHAPTER 2

Contribution of Fruits and Vegetables to Human Nutrition and Health

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2.1 INTRODUCTION

Clinical and epidemiological studies have demonstrated an inverse association between fruit and vegetable consumption and chronic diseases, including different types of cancer, and cardiovascular and neurodegenerative diseases. There is mounting evidence that people who consume sufficient quantities of fruit and vegetables are at lower risk of these diseases. It is estimated that about one-third of cancer cases and up to half of cardiovascular disease (CVD) rates are diet related. Therefore, interest in the health benefits of fruit and vegetable consumption is increasing. In addition, the interest in understanding the type, number, and action mechanism of the different components of fruits and vegetables that confer nutritional and health benefits is also increasing.

Fruits and vegetables are rich sources of some micronutrients (vitamins, minerals), fibers, and a wide array of phytochemicals that individually, or in combination, benefit human health. There are many biologically plausible reasons for this potentially protective association, including the fact that many of the phytochemicals act as antioxidants, anticarcinogens, and immunomodulators.

Phytochemicals, which possess anticarcinogenic properties, are referred to as chemopreventive agents, molecules able to reverse, suppress, or prevent either the initial phase of carcinogenesis or the progression of neoplastic cells of cancer (Fig. 2.1). Based on their mechanisms of action the phytochemicals

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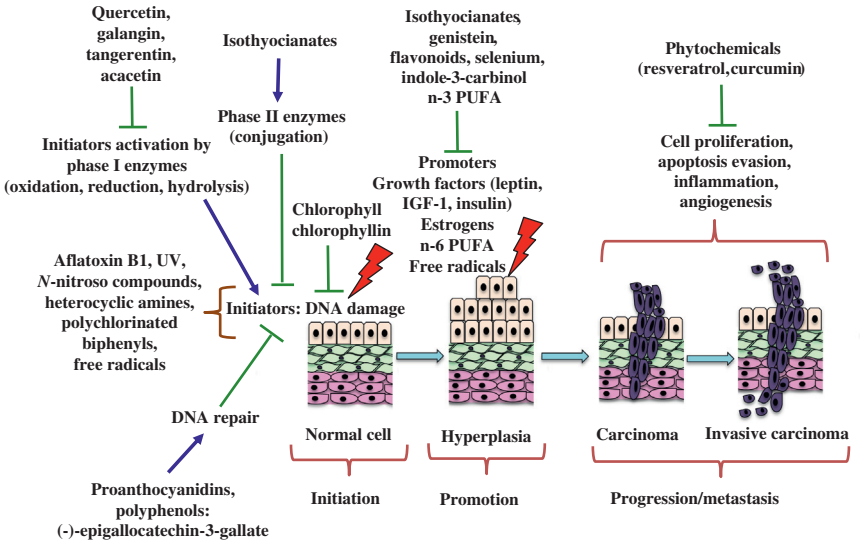

FIGURE 2.1

Schematic representation of inhibition of carcinogenesis by phytochemicals. Carcinogenesis is a multistep process, including: (1) initiation: the first step which consists of a single exposure to carcinogen and appears to involve DNA damage (mutation); (2) promotion: which involves multiple exposure to agents (promoters) that do not damage DNA directly and induce cell proliferation such as growth factors; (3) progression/metastasis: which involves the conversion of benign lesions to malignant and the ability to invade cancer cells. Each step in carcinogenesis can be inhibited by phytochemicals through different mechanisms.

present in a diet rich in fruits and vegetables have been classified in terms of their ability to block the initiation stage of carcinogenesis (cancer-blocking agents) or to suppress (cancer-suppressing agents) the proliferative capacity of preneoplastic lesions in the stages of tumor promotion and progression (Fig. 2.1). The blocking agents are based on their antioxidant activity and the capacity to scavenge free radicals. Among the most investigated antioxidant agents against cancer are some vitamins such as C, A, and E, flavonoids, and phenolic acids (Table 2.1), which account for 60% and 30%, respectively, of the dietary (poly)phenolic compounds, and pigments such as carotenoids, chlorophylls, and betalains. The suppressing agents are able to suppress or eliminate tumor cells by interfering with cell cycle regulation, signal transduction pathways, transcriptional regulation, and inhibition of cyclooxygenase activity, suppression of oncogenes and tumor formation, and induction of apoptosis of cancer cells (Fig. 2.1).

Health authorities worldwide, such as the World Health Organization (WHO), promote high consumption of fruits and vegetables, recommending a daily intake of more than 400 g per person. Many of the putative chemoprotective phytochemicals in fruits and vegetables are colored (due to different pigments). The guidelines are based on selecting 1 serving daily of fruits and vegetables from each of seven color classes (red, yellow-green, red-purple, orange, orange-yellow, green, white-green), so that a variety of phytochemicals is consumed.

Table 2.1 Sources and Potential Effects on Human Health of Some Phytochemicals and Dietary Constituents

Phytochemical	Sources	Potential Effects on Human Health
1. Ascorbic acid (vitamin C)		
Ascorbic acid	Broccoli, cabbage, cantaloupe, citrus fruits, guava, kiwifruit, leafy greens, peppers, pineapples, potato, strawberry, tomato, watermelon	Cardiovascular disease, healthy immune system, scurvy prevention, wound healing
2. Carotenoids		
α-Carotene	Apricots, broccoli, Brussels sprouts, cabbage, cantaloupe, carrots, green beans, kale, kiwifruit, lettuce, lima beans, mango, papaya, peaches, peas, prunes, spinach, squash, sweet potato	Atherosclerosis, coronary artery disease, ischemic, stroke, tumor growth
β-Carotene	Dark green vegetables (such as collards, broccoli, spinach, turnip greens, Swiss chard), orange vegetables (such as carrots, pumpkins, sweet potato), orange-flesh fruits (such as apricot, cantaloupe, mango, nectarine, orange, papaya, peach, persimmon, pineapple), red pepper, tomato	Cancer, cataracts, coronary artery disease, chronic fatigue, ischemic stroke, heart disease, night blindness prevention, provitamin A activity, psoriasis
Lycopene	Autumn olive, Brazilian guava, papaya, tomato, watermelon, red grapefruit	Atherosclerosis, breast and prostate cancer, heart disease, male infertility
Xanthophylls (β-cryptoxanthin, lutein, zeaxanthin)	Cantaloupe, corn, okra, spinach, summer squash, turnip greens, sweet corn	Atherosclerosis, cancer, macular degeneration
3. Dietary fiber		
Fiber	Most fruits and vegetables, pulses (legumes), and nuts	Diabetes, heart disease, colorectal cancer
4. Folate		
Folicin or folic acid	Dark green vegetables (such as spinach, mustard greens, butterhead lettuce, romaine lettuce, broccoli, Brussels sprouts, okra), legumes (such as lentils, chickpeas, green peas), asparagus	Birth defects, cancer, heart disease

(*Continued*)

Table 2.1 (Continued)

Phytochemical	Sources	Potential Effects on Human Health
5. Oganosulfur compounds		
Allicin, diallyl sulfide, glucosinolates, indoles, isothiocyanates	Broccoli, Brussels sprouts, chives, garlic, horseradish, leeks, mustard green, onions	Blood pressure, cancer, hypercholesterolemia, diabetes
6. Phenolics		
6.1 Flavonoids		
Anthocyanidins (cyanidin, malvidin, delphinidin, pelargonidin, peonidin, petunidin)	Red, blue, and purple fruits (apple, blueberry, blackberry, cranberry, grape, nectarine, peach, plum, prune, pomegranate, raspberry, strawberry)	Heart disease, cancer initiation, diabetes, cataracts, blood pressure, allergies
Flavan-3-ols (epicatechin, epigallocatechin, catechin, gallocatechin)	Apples, apricots, blackberries, plums, raspberries, strawberries, cherries	Platelet aggregation, cancer
Flavanones (hesperetin, naringenin, eriodictyol)	Citrus such as oranges, grapefruits, lemons, limes, tangerines	Cancer
Flavones (luteolin, apigenin, chrysin)	Artichoke, celeriac, celery, guava, parsley, peppers, rutabaga, spinach	Allergies, cancer, heart disease
Flavonols (kaempferol, myricetin, quercetin, rutin)	Broccoli, cranberry, kale, lettuce, onions, peppers, snap bean, apples, cherry, berries	Cancer initiation, capillary protectant, heart disease
Isoflavonoids (genistein, daizein, glycetein, formonetin)	Soy beans	Breast cancer, cardiovascular diseases, osteoporosis
6.2 Phenolic acids		
Hydroxybenzoic acids (gallic acid, protocatechuic acid, syringe acid, vanillic acid)	Black olive, black raspberry, carrot, dates (dried, fresh), green chicory, kiwi, mushrooms, red chicory, strawberry	Endothelial dysfunction, hypertension
Hydroxycinnamic acids (caffeic acid, ferulic acid, sinapic acid, chlorogenic, acid, coumaric acid)	Apple, blueberry, broccoli, cabbage, carrot, cherry, cranberry, eggplant, grapes, lemon, pear, orange, grapefruit, peach, potato, spinach	Atherosclerosis, antimicrobial effect, antiinflammatory, cancer, osteogenic
6.3 Tannins		
Proanthocyanidins	Apple, cranberry, grape, pomegranate	Cancer

(*Continued*)

Table 2.1 (Continued)

Phytochemical	Sources	Potential Effects on Human Health
7. Monoterpenes		
Limonene	Citrus such as grapefruit and tangerine	Cancer
8. Isoprenoids (lipophilic vitamins)		
Vitamin E (tocopherols)	Avocado, nuts (such as almonds, cashew nuts, filberts, macadamia nut, peanuts, pistachio, walnuts), lentils, chickpeas, green leafy vegetables	Cancer, diabetes, heart disease, immune system, LDL oxidation
Vitamin K	Crucifers (such as broccoli, Brussels sprouts, cabbage), green onions, lentils, nuts, leafy greens	Osteoporosis, synthesis of procoagulant factors

2.2 HEALTH-PROMOTING COMPONENTS IN FRUITS AND VEGETABLES

Fruits and vegetables contain thousands of phytochemicals belonging to different classes, such as fibers, pigments (such as chlorophylls, carotenoids, flavonoids, betalains), phenolic compounds, and micronutrients (vitamins and minerals). This section briefly describes the most important phytochemicals, minerals, and vitamins for human health, present in fruits and vegetables.

2.2.1 Dietary Fiber

Dietary fiber is the edible part of plants composed of polysaccharides, oligosaccharides, lignin, and associated plant substances, which are resistant to the activity of human small intestinal enzymes, but are finally fermented by colonic microflora. The plant fiber can be structurally associated to the cell wall such as pectins, celluloses, and hemicellulose. Pectins are abundant in fruits, accounting for up to 40% of the total cell wall polysaccharides. The pectins are a group of polymers rich in galacturonic acid. Cellulose is a cell wall polymer of β-1,4-linked glucose units. Hemicellulose is a crosslinking glycan; the most common hemicellulose polymer is xyloglucan (cellulose of a backbone of β-1,4-linked glucose, but with lateral chains of the pentose xylose: α-1,6 linked). These xylosyl residues can be modified with galactose, arabinose, and/or fucose. There are other types of structural nonpolysaccharide fibers like lignin or nonstructural polysaccharides such as gums and mucilage. Lignin is one of the most abundant biopolymers in nature, present in secondary cell walls, and associated with the xylem vessels, however, in fruits and

vegetables, its content is relatively low. This is an aromatic heteropolymer formed by the association of three hydroxycinnamyl alcohol derivatives (*p*-coumaryl, coniferyl, and sinapyl alcohols). The health benefits linked to the formation of a gel matrix that reduces the concentration and absorption of harmful biliary acids and other potential carcinogenic compounds present in feces are based on the increases in fecal mass. This is because there is a soluble fiber form that stimulates intestinal motility, weight and volume of the bolus, and intestinal transit time, contrary to the insoluble fibers like cellulose, hemicellulose, and lignin. Currently the recommendations for adult dietary fiber intake generally fall in the range of 20–35 g/day.

2.2.2 Vitamins

These are considered micronutrients because they are organic molecules required in low or trace amounts for a normal human metabolism and consequently healthy development. These molecules are not synthesized in adequate quantities by humans and must be acquired from the diet, especially from fruits and vegetables, although there are important variations in content among species and cultivars. Vitamins can be classified according to their solubility in water (complex B and vitamin C or ascorbic acid), and in fat (vitamins A or retinol, D, E, and K). The complex B is composed of B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B9 (folate/folic acid), biotin, choline, and B12 (cyanocobalamin). Vitamins B12 and D do not occur in fruits and vegetables.

The B1 vitamin (thiamine) is required as a coenzyme precursor (thiamine phosphate) for the metabolism of carbohydrates. Legumes are an important source of thiamine, and are heat labile, because they can lose between 25% and 40% during cooking. Riboflavin or B2 is a precursor of the coenzymes flavin adenine dinucleotide and flavin adenine mononucleotide, important in bioenergetic processes of mitochondria, and green vegetables are especially rich in it. The B3 vitamin (niacin), also known as nicotinic acid, is the precursor of NADH, NAD, NAD^+, and NADP, and these coenzymes are essential bioenergetics and redox reactions of metabolism. These vitamins can be synthesized by human tryptophan amino acid. Almonds are an important source of B3, but rare in most fruits or vegetables, except Cape gooseberry and avocado. The pantothenic acid (B5) occurs widely in peas, beans, nuts, broccoli, mushrooms, potatoes, and sweet potatoes, and is the precursor to coenzyme-A, which is important for the metabolism of carbohydrates and lipids (triacylglycerides and cholesterol). Vitamin B6 is a precursor of the coenzyme pyridoxal phosphate required in transamination, decarboxylation, and deamination reactions. This vitamin can be found in appreciable amounts in grapes, spinach, beans, bananas, cabbage, cauliflower, sweet potatoes, prunes, and avocados. Biotin acts in metabolic reactions of deamination of amino acids and decarboxylation–carboxylation. It is relatively stable during cooking, processing and storage of fresh, canned and frozen fruits and vegetables. Vitamin B9, or folic acid, is very important for normal reproduction and growth

because it is the precursor of constituents for DNA and RNA synthesis, and metabolism of some amino acids like serine, tyrosine, glutamic acid, histidine, and choline. Folic acid is present in green fruits and vegetables.

Vitamin C, or ascorbic acid, has antioxidant and acidic properties due to the presence of a 2,3-enediol moiety. This vitamin is synthesized only by plants using L-galactose or galacturonic acid as precursors. Fruits and vegetables contribute about 90% of the vitamin C requirement depending on the region and the amounts of fruits and vegetables consumed. Fruits, such as tropical species, and leafy vegetables, are rich in vitamin C, including rosehip, jujube and guava, persimmon, strawberry, kiwifruit, peppers, and citrus fruit, among others, and vegetables such as spinach, broccoli and cabbage, etc. This vitamin is heat labile and important losses can occur with heating.

Vitamin E corresponds to tocopherols and tocotrienols that have aromatic rings with a hydroxyl group that can donate hydrogen atoms to reduce reactive oxygen species (ROS), which are considered as antioxidants. The best-known isomers of tocopherols are α, β, γ, and Δ, based on the number and position of methyl groups in the ring, with α-tocopherol being the most active form. The most important sources of this vitamin are oily seeds, nuts, avocados, and olives, but it is also present in low quantities in broccoli and leafy vegetables. Vitamin K is important for blood coagulation and bone health because it promotes the carboxylation of osteocalcin and several proteins involved in coagulation (factors II, VII, IX, and X), and enhances the calcium fixation activity of these proteins. This vitamin is abundant in lettuce, spinach, cauliflower, and cabbage, but can also be produced by intestinal microflora.

2.2.3 Minerals

Minerals are important for the human diet as micronutrients because they are essential in many biological activities for normal cellular functions. They have a role in the synthesis and structural stabilization of proteins and nucleic acids. Vegetables and fruits are sources of manganese (Mn), copper (Cu), iron (Fe), zinc (Zn), sodium (Na), sulfur (S), and selenium (Se). Manganese is a cofactor of superoxide dismutase. At a physiological level it maintains brain function and reproduction, required for glycemia control, and as part of bone structure. Manganese is a cofactor in the function of antioxidant enzymes, such as those in the mitochondria. Spinach is a good source of this mineral. Copper is a metal involved in redox reactions of the oxidative defense system and other enzymes like ceruloplasmin, cytochrome c oxidase, tyrosinase, and dopamine-β-hydroxylase. In addition, it is necessary for the formation of hemoglobin. Grains, legumes, nuts, and soybean are important sources of copper. Iron and copper participate in redox reactions, and structure of heme-containing proteins, electron transport chain, and microsomal electron transport protein. The importance of these minerals in human health is based on the effect of deficiency, reduction of cognitive functions, delayed growth, alterations in bone mineralization, and diminished immune response.

Nuts, vegetables (such as parsley, broccoli, kale, turnip greens, and collards), and legumes are good sources of these minerals. Phytochemicals such as phytic acid, oxalic acid, and tannins reduce their absorption, but vitamin C increases it. The mineral zinc has catalytic functions as enzymatic cofactor, antioxidant functions, modulating immune response, intestinal digestion, reproduction, and wound healing. Fruits are poor sources of zinc; good sources include parsley, pecans, and walnuts. Sodium is important for the regulation of blood pressure (BP) and electrolyte balance. Fruits are poor sources of sodium, but some vegetables such as artichoke, broccoli, carrot, celery, radish, and sweet potato are good sources. Sulfur is a micronutrient important in the synthesis of cysteine and methionine amino acids, where the thiol groups are mediators of redox reactions. The vegetables that contain important quantities of sulfur compounds, like thiocyanates and isothiocyanates, are from the order Brassicales. Selenium is an important mineral present in the metabolites hydrogen selenide, methylselenol, and selenomethionine, that are able to regulate gene expression, protect DNA from damage, and enhance the repair and regulation of the cell cycle and apoptosis.

2.2.4 Carotenoids

This is a group of fat-soluble molecules (terpenoids) responsible for the yellow, orange, and red colors of some fruits and vegetables (such as apricot, mango, citrus, papaya, watermelon, tomatoes, peppers, carrots). They are formed by eight isoprene units and derived from isopentenyl diphosphate. These plant pigments are important for the process of photosynthesis. Carotenoids are either oxygenated like xantophylls (zeaxanthin and lutein) or carotenoid hydrocarbons like lycopene and β-carotene, which differ in thermal stability. Those that have an unsubstituted β-ring with 11-carbon polyene chain have provitamin A activity, like α-carotene, β-carotene, and cryptoxanthin. Carotenoids have conjugated double bonds within their structure, which confer an antioxidant property due to singlet oxygen quenching, which is able to destroy peroxyl radicals. These molecules have received great attention because of their antitumor properties, especially in breast and prostate cancers, involving antioxidant, antiproliferative, and modulation of immune functions.

2.2.5 Phytoesterols

These are molecules structurally similar to cholesterol that reduce the intestinal cholesterol absorption and low-density lipoprotein (LDL)-cholesterol levels in serum due to their low bioavailability and capacity to inhibit the absorption of cholesterol. The National Cholesterol Education Program (NCEP) Adult Treatment Panel III recommended the consumption of 2 g of phytosterols daily for reducing LDL-cholesterol and CVD risk. Phytosterols are found, like glycosides, in ester or free forms, in vegetable oils, nuts, seeds, legumes, wheat germ, bran, fruits, and vegetables.

2.2.6 Phenolic Compounds

Phenolic compounds are the most numerous group of phytochemicals in plants. They include phenolic acids, flavonoids (flavonols, flavones, flavanols, flavanones, and anthocyanins), stilbenes, and lignans, of which flavonoids and phenolic acids account for 60% and 30%, respectively, of the dietary polyphenols. These molecules exert an antioxidant activity and effects on tumor development and carcinogenesis at the cellular level in processes of detoxification, signaling cascades (MAPKinases, p53, NF-κB, PI3K) involved in cell growth and death. Flavonoids are compounds that share the same common skeleton of diphenylpropanes (C6−C3−C6) and contain phenolic hydroxyl groups attached to ring structures that confer antioxidant activity as reducing agents, hydrogen donators, singlet oxygen quenchers, superoxide radical scavengers, and metal chelators. Phenolic acids are composed of hydroxycinnamic and hydroxybenzoic acids. They also present antioxidant activity as chelators and free radical scavengers. The most studied compound is gallic acid, the precursor of many tannins, while cinnamic acid is the precursor of all the hydroxycinnamic acids. Most of the polyphenolic compounds are present as esters, polymers, or glycosides that can be absorbed in these forms or be hydrolyzed by intestinal enzymes or colonic bacteria.

2.3 CONTRIBUTION OF FRUIT AND VEGETABLE CONSUMPTION TO THE PREVENTION OF VARIOUS DISEASES

2.3.1 Cancer

It is estimated, based on epidemiological studies, that improving nutrition and physical activity-related factors can prevent many cancers, around 27% (in low-income countries), 30% (in middle-income countries), to 34%−39% (in high-income countries). The World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR), through comprehensive analysis mainly based in systematic literature reviews and meta-analysis of observational epidemiologic studies, case−control studies, and cohort studies, published a second expert panel report in 2007, about food, nutrition, physical activity, and cancer prevention. This expert panel report of WCRF/AICR shows that the evidence about fruit and vegetable consumption probably protects against some cancers (Table 2.2). Nonstarchy vegetables probably decrease risk of cancers of the mouth, pharynx, and larynx, and those of the esophagus and stomach. There is limited evidence suggesting that they also decrease the risk from cancers of the nasopharynx, lung, colorectal, ovary, and endometrium. Allium vegetables probably decrease the risk of stomach cancer. Garlic, an allium vegetable, probably decreases the risk of colorectal cancer. Fruits probably decrease the risk of cancers of the mouth, pharynx, and larynx, and of the esophagus, lung, and stomach. There is limited evidence suggesting that fruits also protect against nasopharynx, pancreas, liver, and colorectal

Table 2.2 Fruit and Vegetable Foods and Phytochemicals That Can Provide Decreased Risk (↓↓) or Convinced Increased Risk (↑↑↑↑) of Several Types of Cancer, According to WCRF/AICR Second Expert Panel Report, 2007

	Type of Cancer						
Foods or Phytochemicals	**Mouth, Pharynx, Larynx**	**Esophagus**	**Lung**	**Stomach**	**Pancreas**	**Colorectal**	**Prostate**
Foods containing dietary fiber						↓↓	
Nonstarchy vegetables	↓↓	↓↓		↓↓			
Allium vegetables				↓↓			
Garlic						↓↓	
Fruits	↓↓	↓↓	↓↓	↓↓			
Foods containing folate					↓↓		
Foods containing carotenoids	↓↓		↓↓				
Foods containing β-carotene		↓↓					
Foods containing lycopene							↓↓
Foods containing vitamin C		↓↓					
Foods containing selenium							↓↓
Selenium supplements							
β-carotene supplements[a]			↑↑↑↑				

[a] *In current smokers.*

Source: Modified from World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR), 2007.

cancers. The chemopreventive properties of vegetables, fruits, and pulses against some types of cancers are attributed to some micronutrients considered as markers for consumption of vegetables, fruits, and pulses (legumes). For example, foods containing carotenoids probably protect against cancers of the mouth, pharynx, larynx, and lung; whereas evidence of consumption of foods containing β-carotene and lycopene suggests that they probably protect against esophageal and prostate cancer, respectively. Convincing evidence indicates that β-carotene supplement intake increases the risk of lung cancer among smoking-exposed populations. This paradoxical effect could be explained by at least two reasons generated by experimental data in ferrets: (1) a high dose of β-carotene increases phase I enzyme, which results in reduced retinoic acid and parallel reduced retinoic signaling, and increased cell proliferation; and

(2) eccentric cleavage of β-carotene metabolites facilitating the binding of smoke-derived carcinogens to DNA. It seems that deleterious effects of β-carotene are dose-dependent and supplementation could promote a very high intake of this phytochemical. Therefore, WCRF/AICR recommend, as public health goals, an average consumption of at least 600 g of nonstarchy vegetables and fruits daily, and an average of at least 25 g of nonstarch polysaccharide from relatively unprocessed cereals (grains) and/or pulses (legumes), and other foods that are a natural source of dietary fiber. On the other hand, despite the well-described quercetin mechanisms of action, there is limited evidence suggesting that consumption of foods containing this flavonoid, such as apples, tea, and onions, protect against lung cancer.

2.3.1.1 CHEMOPREVENTION OF CARCINOGENESIS BY PHYTOCHEMICALS

Carcinogenesis is a very complex process, defined by the course in which a normal cell becomes malignant. It can be summarized in three steps: (1) initiation, (2) promotion, and (3) progression/metastasis (Fig. 2.1). In the first step, initiation occurs when either chemical (as polycyclic hydrocarbon) or physical (as ultraviolet (UV) radiation) stimuli damage cell DNA which can produce mutations in genes involved in proliferation (oncogenes) or in DNA repair, cell death, and inhibition of proliferation (tumor suppressor genes). After initiation, the promotion step consists of the proliferation of cells with mutations. Finally, the progression/metastasis step consists of the acquisition of several characteristics that permit the tumor to survive and invade adjacent tissue (progression) and even far away tissues through blood and lymph (metastasis). As is shown in Fig. 2.1, phytochemicals could act in each step of carcinogenesis.

In the first step of carcinogenesis, initiation, the activation of chemical carcinogens (initiators) (aflatoxin B1, benzo(a)pyrene, 2-naphthylamine) by cytochrome P450 phase I enzymes is a key point of this process. Carcinogens bind DNA and form adducts which distort DNA structure and disrupt its replication, and this could cause mistranslation. Carcinogens can also break DNA, and thus they can generate mutation and deletion of genetic material. Flavonols such as quercetin, galangin, and targerentin (flavone) inhibit phase I enzymes such as CYP1A2 and thus inhibit initiator activation.

On the other hand, it is well documented that organosulfur compounds, such as isothiocyanates, found in Brassica vegetables, increase liver phase II detoxification enzymes, catalyze the conjugation of chemical carcinogens, and promote their elimination through urine or bile. Main phase II enzymes are NAD(P)H:quinone oxireductase, UDP-glucoronosyl transferases, glutathione *S*-transferases, and sulfotransferases. Phase II enzyme expression genes are regulated by erythroid-2-related factor 2 (Nrf2), which binds antioxidant response element present in the promoter sequence of phase II genes.

Another mechanism of phytochemicals against the initiation process is the activation of DNA repair. UV-induced DNA damage consists of the formation

of pyrimidine dimers, which is contrasted by epigallocatechin-3-gallate (EGCG) through nucleotide excision repair and interleukin 12-mediated mechanisms. Finally, some phytochemicals, such as chlorophyll and its food-grade derivative chlorophyllin, interact directly with carcinogens such as aflatoxin B and benzo(a)pyrene and form a complex. This complex reduces carcinogen bioavailability. Chlorophyll and chlorophyllin can also inhibit the initiation process through inhibition of phase I enzymes and induction of phase II enzymes.

Different events occur in the promotion and progression/metastasis steps of carcinogenesis that could be regulated by phytochemicals. The hallmark characteristic in the promotion step is cell proliferation which is stimulated by hormones, growth factors, free radicals, and other environmental agents called promoters. In this step, phytochemicals can act as modulators of hormones such as genistein and isothiocyanates that modulate estrogen receptors α and β, a key factor in the promotion of premenopausal breast cancer. In some cases phytochemicals act on cell-signaling pathways, such as curcumin blocking phosphorylation of tyrosine kinases, and serine/threonine kinases, key proteins in promoting cell growth.

In the progression/carcinogenesis step, cancer cells have different strategies to grow, invade, and survive. Cancer cells promote their own uncontrolled proliferation, inflammation, and angiogenesis, evade cell death, invade other tissues, and become multidrug resistant, and phytochemicals can act in all these different processes. As an example, resveratrol, the most important stilbene, mainly present in red wine and grapes, and curcumin, the major yellow pigment present in turmeric, both with demonstrated chemopreventive properties, are able to inhibit the proliferation of a wide range of human cancer cells (ovarian, breast, prostate, liver, uterine, leukemia, lung, gastric, colorectal), and to suppresses carcinogenesis in several organ sites (head and neck, liver, thyroid, stomach, colorectal, pancreas, prostate, renal, bone, skin, breast, lung), by controlling cell cycle progression, apoptosis, inflammation, angiogenesis, invasion, and metastasis.

2.3.2 Cardiovascular Disease

CVD is the number one cause of death in developed and developing countries, and prevention is at the top of the public health agenda. In 2008, 17 million deaths worldwide were due to CVD, which represents 48% of noncommunicable disease deaths. Numerous epidemiological studies around the world have demonstrated evidence that diets rich in fruits and vegetables prevented CVD and reduced mortality from CVD. The positive effect has been accomplished by 3 servings of vegetables and fruits, and the relative risk (RR) can be minimized to a great extent by enhancing the vegetable and fruit consumption by up to 10 servings/day. The inverse relationship between vegetable intake and CVD was more evident with smokers consuming at least 2.5 servings of fruits and vegetables per day in comparison with less than 1 serving/day. A high

fruit and vegetable intake has shown a significant inverse association with CVD risk factors such as systolic BP, total cholesterol and LDL-cholesterol, and explained 48% of the protective effect. Legume consumption was also significantly and inversely associated with CVD, lowering the risk by about 11%. In the Mediterranean Diet Prevention (PREDIMED) study, a randomized, controlled trial including 7447 obese men and women with a mean age of 67 years at high risk for CVD, 50% of the participants had type II diabetes mellitus (T2DM), more than 70% had dyslipidemia, and more than 80% had hypertension. After a median follow-up of 4.8 years the study showed that a Mediterranean diet supplemented with extra virgin olive oil or a mix of nuts (almonds, walnuts, and hazelnuts) reduced the incidence of CVD (myocardial infarction, stroke, or cardiovascular death) by 30% in the olive oil group (hazard ratio adjusted [HR_{adj}] = 0.70, 95% confidence interval [CI] 0.54−0.92) and 28% in the nut group (HR_{adj} = 0.72; 95% CI 0.54−0.96) compared to the control group that was instructed to eat a low-fat diet (Estruch et al., 2013). Moreover, in the prospective part of the PREDIMED study, the baseline intake of fruit was inversely associated with all causes of mortality (HR for the fifth compared with the first quintile = 0.59 [95% CI 0.44−0.78]) and the associations were stronger for CVD mortality than other causes of death (Buil-Cosiales et al., 2014).

On the other hand, it is well documented that high consumption of fruits and vegetables is inversely associated with the risk of coronary heart disease (CHD). A meta-analysis that included 23 cohort studies involving 937,665 participants and 18,047 patients with CHD, concluded that a fruit and vegetable intake of more than 5 servings/day was significantly associated with a lower risk of CHD in Western populations, but not in Asian populations. This meta-analysis found that in dose−response studies the RR of CHD decreased by 12%, 16%, and 18% for daily 477 g of total fruit and vegetables, 300 g of fruit, and 400 g of vegetable, respectively (Gan et al., 2015).

It has been reported that death attributed to CVD and CHD showed strong and consistent reductions with increasing nut/peanut consumption. Moreover, ischemic heart disease (IHD) is inversely associated with 28 g of nut consumption 4-weekly (reduction of 24%) and nonfatal IHD (reduction of 22%). Likewise, a 27% reduced risk of all-cause mortality for 1 serving of nuts per day and a 39% risk for CVD mortality per daily serving of nuts (Grosso et al., 2015). However, as the authors pointed out, confounding factors such as body mass index, smoking status, increased intake of fruits and vegetables, as well as intake of alcohol, have to be taken into account when considering the findings.

Nuts and peanuts have beneficial effects on lipids, lipoproteins, and various CHD risk factors, including oxidation, inflammation, endothelial function, and arterial stiffness. Daily nut consumption decreased by 5% and 7%, total cholesterol and LDL-cholesterol, respectively, and improved LDL-cholesterol to high-density lipoprotein (HDL)-cholesterol ratio. Moreover, pistachio consumption reduced LDL-cholesterol in patients with prediabetes.

The LDL-cholesterol-lowering response of nut and peanut consumption studies is not only from changes in the fatty acid profile of the diet. Thus, nuts and peanuts contain other bioactive compounds that explain their multiple cardiovascular benefits such as plant protein, arginine, fiber, potassium, calcium, magnesium, tocopherols, phytosterols, phenolic compounds, and resveratrol. Nuts and peanuts are food sources of cardioprotective components and if routinely incorporated in a healthy diet, the population risk of CHD would therefore be expected to decrease markedly. Some biological mechanisms have been proposed to explain these protective effects by using in vivo models such as inhibition of lipid oxidation, increase of antioxidant capacity of serum or plasma, protection against oxidation of cholesterol and other lipids in cell membrane, reduction in oxidative stress, antiinflammatory effect, prevention of platelet aggregation, reduction of vascular tone, synthesis induction of glutathione, endothelial NO synthase, and inducible NO synthase.

The consumption of avocado, a food rich in mono-unsaturated fatty acids, reduces both total cholesterol and LDL-cholesterol while preserving the level of HDL.

Lycopene from tomato fruit was found to prevent the oxidation of LDL-cholesterol and to reduce the risk of developing atherosclerosis and CHD disease, and daily consumption of tomato products providing at least 40 mg of lycopene was reported to be enough to substantially reduce LDL oxidation. Lycopene is recognized as the most efficient singlet oxygen quencher among biological carotenoids. Lycopene has also been reported to increase gap-junctional communication between cells and to induce the synthesis of connexin-43.

Anthocyanin, proanthocyanidin, flavanone, flavone, and flavanol consumption is inversely associated with the risk of CVD. An average increase of 10−20 mg/day of flavonol intake was associated with 5%−14% decrease in the risk for developing CVD. The biological mechanisms by which flavonoids may exert this effect are antioxidant, antiinflammatory, and vasodilatory properties.

2.3.2.1 HYPERTENSION

High blood pressure is a continuous, consistent, and independent risk factor for CVD that is modifiable through lifestyle. Indeed, a moderate average BP reduction of 3.5 and 2.0 mmHg for systolic and diastolic BP, respectively, was associated with a 24% reduction in stroke. It is consistently shown that diet plays a major role in BP control. Several studies clearly showed that consumption of vegetables, fruit, and low-fat dairy products lowered BP. Nut consumption reduces systolic BP among participants without T2DM (−1.3 mmHg, 95% CI −2.3 to −0.22 mmHg), whereas pistachios had the strongest effect on systolic (−1.8 mmHg, 95% CI −3.0 to −0.7 mmHg) and diastolic BP (−0.8 mmHg, 95% CI −1.4 to −0.2 mmHg). Even more, it was shown that a vegetarian diet

is associated with a reduction in mean systolic BP (−4.8 mmHg, 95% CI −6.6 to −3.1 mmHg) and diastolic BP (−2.2, 95% CI −3.5 to −1.1 mmHg) (Mohammadifard et al., 2015).

The BP-lowering effects of fruits and vegetables may involve several mechanisms. Oxidative stress could a play a role in the pathogenesis of hypertension and antioxidants may increase the bioavailability of NO by decreasing endogenous oxidant formation. Another possibility may be the increased intake of potassium. Potassium has been shown to promote vasodilation, decrease renin and renal sodium reabsorption, reduce reactive oxygen production, and reduce platelet aggregation. In addition, the inhibition of angiotensin-converting enzyme activity by fruit and vegetables is another possibility.

2.3.2.2 CHRONIC HEART FAILURE

Chronic heart failure (CHF) has a prevalence of about 80% among individuals of more than 80 years of age worldwide, and is seen as the end stage of CVD and the final pathway of diseases such as hypertension and CVD. CHF has many causes, but oxidative stress has also been proposed as a risk factor. Furthermore, the total antioxidant capacity measured in the diet is inversely associated with CHF. A diet rich in fruit and vegetables has been associated with reduced incidence of CHF by up to 22%.

2.3.3 Diabetes Mellitus

The prevalence of T2DM is increasing worldwide. In 2013, 382 million cases were reported, and this number is expected to rise to 592 million by 2035. Because T2DM is characterized by either resistance to the blood glucose-regulating hormone insulin or its relative deficiency, it has been proposed that antioxidants may play a role in increasing insulin sensitivity. Thus, fruit and vegetable consumption in patients with T2DM improve glucose and glycated hemoglobin levels, and increase serum antioxidant compounds (vitamin C and reduced glutathione), and reduce markers of oxidative stress and inflammation such as DNA oxidation and lipid peroxidation and IL-6 serum levels. Moreover, several studies have shown that the consumption of fruit, vegetables, nuts, and whole grain intake of antioxidants reduce the RR of T2DM by 13%−15% (Wu et al., 2015; Bazzano et al., 2008; Hamer and Chida, 2007). However, fruit and vegetable consumption and its effects on T2DM risk are inconsistent (Villegas et al., 2008). It has been proposed that this inconsistency between studies examining the association between fruit and vegetable intake and the risk of T2DM could be due to the extent of measurement error associated with the food frequency questionnaire overestimating fruit and vegetable consumption. In addition, these studies include consumption of fruit juices that contain important sugar content, especially fructose that contributes to the development of T2DM and insulin resistance (Bazzano et al., 2008).

Experimental evidence showed that consumption of prickly pear cladodes (nopal) could decrease blood glucose levels (Frati et al., 1990). The intake of

broiled *Opuntia* stems for 10 days improved glucose control in a small group of adults with T2DM (Frati-Munari et al., 1990). The rise in serum glucose levels which follows the intake of a sugar load (oral glucose tolerance test) was lower with previous ingestion of *Opuntia* stems compared to if the sugar was ingested alone (Frati et al., 1990). In patients with T2DM, the ingestion of some species of nopal (*Opuntia streptacantha*, *Opuntia ficus-indica*) in fasting conditions is generally followed by a decrease in serum glucose and serum insulin levels (Frati, 1992). These positive health effects of *Opuntia* stems might be associated with dietary fibers, since similar results can be achieved by *Plantago psyllium* or other sources of dietary fibers (Frati, 1992). Ingestion of raw and cooked *Opuntia ficus-indica* extracts resulted in beneficial effects on total cholesterol, without any secondary effect on glucose and lipoprotein amounts in blood (Cárdenas Medellín et al., 1998).

Some flavonoids, such as procyanidins, have antidiabetic properties because they improve altered glucose and oxidative metabolism of diabetic states (Pinent et al., 2004). Extract of grape seed procyanidins administered orally to streptozotocin (STZ)-induced diabetic rats resulted in an antihyperglycemic effect, which was significantly increased if procyanidin administration was accompanied by a low insulin dose (Pinent et al., 2004). The antihyperglycemic effect of procyanidins may be partially due to the insulinomimetic activity of procyanidins on insulin-sensitive cell lines. Similar results have been reported using guava leaves that have an antihyperglycemic, antihyperlipidemic, and antioxidant activity attributed to their phenolic compounds such as flavones, gallic, and ellagic derivatives, cyanidin-glucoside, pentacyclic triterpenoids, guiajaverin, and quercetin (Díaz de Cerio et al., 2016). The leaf extract of guava has traditionally been used for the treatment of diabetes in East Asia and Africa. In Japan there is a guava leaf tea containing the aqueous leaf extract from guava which has been approved as one of the "foods for specified health uses" and which is now commercially available. This has been shown to reduce postprandial blood glucose and to improve hyperglycemia, hyperinsulinemia, hypoadiponectinemia, hypertriglyceridemia, and hypercholesterolemia using STZ-induced "type I diabetes mellitus (DMI)" murine models (50−800 mg/kg) (Ojewole, 2005; Deguchi and Miyazaki, 2010). Moreover, the leaf aqueous extract of *Psidium guava* exhibits a hypotensive property as observed in the hypertensive Dahl salt-sensitive rats which were used to investigate the antihypertensive effect of the plant's extract (50−800 mg/kg). Intravenous administration was administered producing dose-dependent, significant reductions ($P < .05-.001$) in systemic arterial BP and heart rates (Ojewole, 2005). In spite of these findings using guava plant, little is known regarding the therapeutic activity in human clinical trials as well as its underlying mechanism of action and safety using guava leaves.

One of the possible mechanisms involved in the protective effect of flavonoids on T2DM is their antioxidant activity to protect tissues against ROS and lipid peroxidation. In addition, flavonoids are able to activate pathways that lead to

antiinflammation, improvement of endothelial function, and reduction in blood cholesterol concentrations.

2.3.4 Overweight and Obesity

WHO estimates that more than 1.9 billion adults and more than 42 million of children under 5 years old are overweight worldwide, indicating its prevalence in all age groups. Obesity is characterized by the accumulation of excess fat in adipose tissues. It is considered a major public health issue, especially in most developed countries for its wide spread across population groups, as well as its contribution to the development of chronic diseases, particularly CVDs, T2DM, some types of cancer (i.e., colorectal, breast) resulting from a sedentary lifestyle, unhealthy dietary practices, high energy intake, and low energy expenditure.

Despite the alarming increase in the prevalence of obesity in the world, epidemiologic studies on the relation between fruit and vegetable consumption and weight gain (WG) are still insufficient. In a systematic review, Fogelholm et al. (2012) showed that a high intake of fiber-rich foods and nuts predicted less WG and reduced waist circumference. In fact, a recent analysis from the National Health and Nutrition Examination Survey 2005–10 showed that nut consumers who ate a mean of 44 g of nuts per day had lower body mass index and waist circumference than nonnut consumers (P for both $<.01$) (O'Neil et al., 2015). The mechanism for the beneficial effect of nuts on body weight may be due to their satiating effect and subsequent food compensation (Mattes et al., 2008).

The evidence accumulated indicates that the combination of increased fruit and vegetable intake, together with other dietary recommendations, might promote satiety and weight loss in overweight and obese individuals, because of low-fat but high in water content, indigestible fiber, and soluble dietary fiber, which contribute to reducing the energy intake of meals, reducing the energy intake, and consequently the body weight. It has been proposed that soluble dietary fiber delays gastric emptying of ingested food and forms a gel-like environment in the small intestine that diminishes partly the activity of enzymes involved in the digestion of macronutrients and absorption, prolonging the contact of the nutrients with receptors in the small intestine, such as fructose, causing the release of putative satiety peptides, and causing a hyperosmolar environment in the colon leading, for example, to a decrease in insulin secretion and improved glucose control, attraction of fluids into the gut lumen, and lost interest in further food consumption (Mirmiram et al., 2013).

Vioque et al. (2008) explored the associations between fruit and vegetable intake and WG over a 10-year period in an adult Mediterranean population of 206 aged 15–80 years at baseline in 1994, who participated in a nutrition survey in Valencia, Spain. They concluded that dietary patterns associated with a high intake of fruits and vegetables in Mediterranean

populations may reduce the long-term risk of subsequent WG and obesity among adults.

Svendsen et al. (2007) assessed the effect of increased consumption of vegetables and fruit on body weight, risk factors for CVD, and antioxidant defense in obese patients with sleep-related breathing disorders (SRBD). They concluded that targeted dietary advice to increase the intake of vegetables and fruit among subjects with SRBD contributed to weight reduction and reduced systolic and diastolic BP, but had no effect on antioxidant defense measured by ferric-reducing/antioxidant power assay.

He et al. (2004) examined the changes in intake of fruits and vegetables in relation to risk of obesity and WG among middle-aged women through a prospective cohort study with 12 years of follow-up, conducted in the Nurses' Health Study with a total of 74,063 female nurses aged 38−63 years, who were free of CVD, cancer, and T2DM at baseline in 1984. During the 12-year follow-up, participants tended to gain weight with aging, but those with the largest increase in fruit and vegetable intake had a 24% of lower risk of becoming obese compared with those who had the largest decrease in intake after adjustment for age, physical activity, smoking, total energy intake, and other lifestyle variables. For major WG ($\geq$25 kg), women with the largest increase in intake of fruits and vegetables had a 28% lower risk compared to those in the other extreme group.

Low fruit consumption is considered an important contributor to the global disease burden, and an important attributable risk factor because many clinical studies have evidenced that increased daily consumption of fruits is inversely correlated to WG. However, these researches pointed to the possible obesity effects of fruits in the form of juices, which are low in fiber and rich in simple sugars (sucrose, glucose, or fructose), stimulating hepatic de novo triacylglycerides biosynthesis and very-low-density lipoproteins that increase circulation in blood and augment fat mass.

The following possible antiobesity mechanisms proposed are attributable to consumption of whole fruit:

1. Reduction of energy consumption, because adding fruit daily to the diet will reduce overall energy consumption, restricting WG, reduction of fat mass, and control obesity;
2. Presence of fruit micronutrients able to downregulate genes involved in adipocyte generation and differentiation such as vitamins A, E, and C and minerals such as zinc, iron, and calcium;
3. A diet rich in fiber and polyphenols leads to a prevalence of Bacteroidetes and Actinobacteria, which are characteristics of lean individuals, whereas in obese people the presence of Firmicutes and Proteobacteria is increased, however the specific mechanisms responsible for modulatory effects of gut microbial ecology related to fruit consumption on obese individuals are unknown (Sharma et al., 2016);

4. Fruit provides prolonged satiety attributed to dietary fiber. Bes-Rastrollo et al. (2006) assessed the association between fiber intake and fruit and vegetable consumption with the likelihood of WG in a Mediterranean (Spain) population with a cross-sectional analysis of 5094 men and 6613 women in a multipurpose prospective cohort (Seguimiento Universidad de Navarra Study). There was a significant inverse association between total fruit/vegetable consumption and WG, but only among men, and it was more evident among those with a high intake of total fiber, and the benefit of total fiber was more evident among those with a high consumption of fruit and vegetables.

De Carvalho et al. (2006) evaluated the dietary fiber intake of adolescents in the metropolitan area of São Paulo City (Brazil), and the association between low dietary fiber intake with constipation and overweight. The study included 716 adolescents, and evaluation of fiber intake was based on a 24-h daily intake record and a frequency questionnaire. Adolescents who did not eat beans on more than 4 days/week presented a higher risk of fiber intake below that recommended, and dietary fiber intake below that recommended was associated with a greater risk toward overweight in students attending public schooling.

2.3.5 Pulmonary Health

Several studies have shown a positive association between fruit and vegetable intake and pulmonary function. Fruit and vegetable consumption has been suggested to maintain a healthy pulmonary function in well-adult populations, and improve lung function in those with established pulmonary disorders. Phytochemicals, especially of antioxidant potential, have been suggested to be important in protecting the lungs from oxidative stress. Higher fruit intake was found to be consistently associated with lowered mortality from chronic obstructive pulmonary disease (COPD)-related causes. Vitamin E intake did appear to be protective when data were adjusted for age, country, and smoking. Fruit intakes of over 121 g/day and increased vegetable consumption were reported to be associated with significantly reduced COPD.

It has been suggested that reduced antioxidant intake is one critical factor associated with increased susceptibility to asthma, and therefore fruit and vegetable intake has been suggested to reduce it by improving ventilatory function and respiratory symptoms. Fruits and vegetables associated with reduced incidence of asthma included green leafy vegetables (intake of >90 g/day; 22% risk reduction), tomato (intake of >28.2 g/day; 15% risk reduction), carrots (intake of >24.9 g/day; 19% risk reduction), and apples (intake of >31.2 g/day; 10% risk reduction). Moreover, the consumption of fruit and vegetables is associated with reduced occurrence of wheezing and shortness of breath in schoolchildren.

2.3.6 Bone Health

The loss of bone mass is a global epidemic associated with osteoporosis. Fruit and vegetable consumption has been suggested to improve bones. Higher fruit and vegetable intake was associated with improved markers of bone status in males and females ranging between 16 and 83 years old. Tylavsky et al. (2004) showed that fruit and vegetable intake might be important in bone health in white girls ages 8–13 years. The effect was high with 3 servings/day or more and low with less than 3 servings/day, with 4.0 servings (1.6 fruit/2.4 vegetables) in the high group and 1.7 servings (0.6 fruit/1.1 vegetable) in the low group. Girls in the high fruit and vegetable intake group had significantly larger bone area of the whole body and wrist, and higher mineral content for whole body and at the wrist. A study of 1407 premenopausal farm women from five rural districts in Japan concluded that fruit and vegetable intake is positively correlated with bone health. In a study conducted with 85 boys and 67 girls, ages 8–20 years in Saskatchewan, Canada, fruit and vegetable intake was reported to be an important independent predictor of accrued total body bone mineral content (BMC) in boys but not in girls. In a study with adolescents ages 12 and 15 years in Northern Ireland ($n = 1345$), 12-year-olds consumed the highest quantity of fruit and a positive association has been demonstrated between bone density and fruit intake.

Oxidative stress contributes to the etiology of osteoporosis through the inhibition of osteoblastic differentiation via extracellular signal-regulated kinases (ERK) and ERK-dependent nuclear factor-κB signaling pathways. Thus antioxidants in fruits and vegetables, including β-carotene, are able to reduce oxidative stress on bone mineral density (BMD), in addition to the potential role of some nutrients such as vitamins C and K that can promote bone cell and structural formation.

Many fruits and vegetables 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 bones. Potassium intake was significantly and linearly associated with markers of bone turnover and femoral BMD. High potassium, magnesium, and calcium content, in addition to antioxidants, phytochemicals, and lower acidity of fruits and vegetables, could be important factors for bone health.

In a study of 40 healthy men and women, with an average age of 63.7 years, who were randomized to either an "alkali" diet (meat plus fruits and vegetables) or an "acid" diet (meat plus cereal grains) (Jajoo et al., 2006), altering the renal net acid excretion over a period of 60 days impacted several biochemical markers of bones turnover and calcium excretion. The acidity of the diet had a significant effect on increasing "urinary N-telopeptide (NTX)", a urinary marker of bone breakdown and increasing the amount of calcium excreted in the urine.

Li et al. (2013) reported a significantly positive association between fruit intakes and BMD and BMC in all participants including boys and girls

(11–14 years), young women (20–34 years including postpartum within 2 weeks), and postmenopausal women (50–70 years), in a cross-sectional study where the mean fruit intake was 185, 206, 380, and 174 g/day in boys, girls, young women, and postmenopausal women, respectively. About 40% of the fruit intake was from the group of apple, pear, peach, pineapple, and plum, and 20% from the group of orange, grapefruit, and lemon. In addition, Liu et al. (2015) reported that a daily increase of 100 g/kcal total fruit intake was associated with 4.5% and 6.4% increases of BMD at whole body, and 3.9% and 4.8% increases at the femoral neck in Honk Kong Chinese men and women aged 65 years and older, respectively. Similar to the study of Li et al. (2013), these authors did not find a significant association between vegetable intake and bone mass.

Shen et al. (2012) proposed a potential osteoprotective mechanism of most commonly consumed fruits and their phytochemicals, such as tomato (lycopene), grape (resveratrol), citrus fruits, berry fruits, dried plum, and apple (polyphenols, flavonoids, phloridzin, and pectin), via antiinflammatory and antioxidant mechanisms, leading to osteoblast mineralization and osteoclast inactivation. They proposed that fruit intake promotes osteoblastogenesis by upregulating RunX2 and osteocalcin, specific genes for osteoblast formation. This process also involves activation of the Wnt signaling pathway through β-catenin that, together with BMP-2 protein, promotes osteoblast differentiation. In addition, IGF-I is correlated positively with bone formation by inhibiting collagen degradation.

Fruits and phytochemicals also downregulate RANKL, a key protein responsible for differentiating monocytes/macrophage precursors into osteoclasts, because RANKL stimulates the expression of osteoclast-specific genes (TRAP, cathepsin K [CATK], calcitonin receptor, and β3-integrin) that consequently degrade bone minerals and collagen matrices. In addition, the inhibition of RANKL will reduce the matrix metalloproteinase (MMP-2 and MMP-9) activity that degrades bone collagen. Finally, downregulation of RANKL can inactivate NFATc1, a calcineurin- and calcium-regulated transcriptional factor that promotes osteoclastogenesis (Shen et al., 2012).

2.3.7 Cataracts and Eye Health

Oxidative mechanisms have been implicated in the etiology of cataracts in humans, and fruit and vegetable intake has been associated with this problem. A study involving 35,724 healthy professional women over the age of 45 years in the United States was conducted to determine the potential association between fruit and vegetable intake and subsequent risk of cataract development over a 10-year follow-up period. RR of developing cataracts during the 10-year study was only slightly reduced in women with the highest intake of fruits and vegetables (10 servings/day) compared to those with the lowest intake (2.6 servings/day). A study of 479 women with an average fruit intake of 2.5 servings/day and average vegetable intake of approximately

4 servings/day also indicated that fruit and vegetable intake did not differ between women with and without nuclear opacities. The authors concluded that multiple aspects of the diet are more important in reducing the risk of cataracts than emphasizing one particular food group or component over another. A study with 98 participants, 68% women, ranging in age between 45 and 73 years, used macular pigment optical density (MPOD) as a marker to correlate diet and serum carotenoid levels with the amount of molecular pigment in the retina and showed that a high ($\geq$5 servings/day) intake of fruit and vegetables was associated with significantly higher MPOD compared to measurement in subjects with lower ($<$3 servings/day) intake.

2.3.8 Arthritis

Dietary antioxidants and antiinflammatory components in food are thought to be important in reducing the risk or improving the course of rheumatoid arthritis (RA), and therefore, fruit and vegetable consumption has been associated with reduced risk of RA. A study of 29,368 married women from the United States, predominately white, average age 61.4 years, for 11 years, indicated that total fruit consumption ($>$83 servings per month) was associated with reduced risk of RA. Oranges were the only individual fruit linked to a reduced incidence of RA, and β-cryptoxanthin, a carotenoid found in this fruit, was consistently highly protective. Total vegetable intake was not associated with reduced incidence of RA, but intake of cruciferous vegetables ($>$11 servings/month), particularly broccoli ($>$3 servings/month), was associated with a moderate effect on RA. In a study where dietary intake for 73 cases was compared to intake of 146 controls (mean age 60−61 years; 70% women) indicated that lower but not statistically significant intake of fruits and vegetables was weakly associated with higher incidence of inflammatory polyarthritis (IP). Subjects with the lowest intake ($<$55.7 mg/day) of vitamin C were three times more likely to develop IP than those with the highest intake ($>$94.9 mg/day). In a related study to determine the relationship with carotenoid intake, the diets of 88 cases were compared to those of 176 controls (mean age 61 years; 69% women), and it was found that intake of vitamin C and dietary carotenoids, particularly β-cryptoxanthin and to a lesser extent, zeaxanthin, were significantly correlated with reduced risk of IP.

2.3.9 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. Fruits and vegetables are an important source of dietary folate and their consumption has been associated with increased plasma levels of folate. Plasma folate concentration increased by 13%−27% after short-term feeding experiments with fruits and vegetables and red blood cell folate also increases with increasing fruit and vegetable intake (from 1 to 7 servings/day).

2.3.10 Diverticulosis

Diverticulosis affects 50% or more of the population over the age of 60 years in several countries. Fruit and vegetable consumption is inversely associated with the risk of diverticulosis and fiber-rich food intake is an important aspect of its therapy.

2.3.11 Skin Diseases

Skin is the largest organ and is exposed constantly to environmental factors able to induce oxidative stress, such as UV radiation, air pollutants, and chemical oxidants favoring skin aging, an inevitable normal process. However, premature skin aging may occur due to nonhealthy lifestyle (smoking, imbalanced nutrition, excessive caloric restriction, and mental stress). In vitro and in vivo studies suggest that antioxidants regulate the biomarkers associated with premature aging by reducing oxidative stress in skin. Lycopene had a protective effect on the oxidative stress-mediated damage to human skin after irradiation with UV light.

A formulation of a synergistic blend named OptiBerry that contains wild blueberry, bilberry, cranberry, elderberry, strawberry, and raspberry seed extracts developed by Bagchi et al. (2006) showed whole-body antioxidant protection in vitamin E-deficient rats after exposure at 2 atmospheric pressure (atm) for 2 h with hyperbaric oxygen. The animals were supplemented for 8 weeks, reducing significantly "reduced glutathione (GSH)" levels compared to placebo-fed rats. In addition, vascular endothelial growth factor (VEGF) expression induced in keratinocytes by treatment with H_2O_2 and tumor necrosis factor-alpha, an inflammatory cytokine), was inhibited after 12 h of treatment with OptiBerry; and pretreatment of cells with OptiBerry inhibited ROS- and inflammation-induced VEGF protein expression.

Promising results have been obtained to prevent UV-induced skin alterations and premature skin aging by using extracts of pomace from Riesling grapes that showed a dose-dependent inhibitory activity against both enzymes with IC_{50} values of 20.3 and 14.7 μg/mL for collagenase and elastase activity, respectively, with the free phenolic acids fraction being the most active. Human dermal fibroblasts incubated with strawberry extract at 0.5 mg/mL and stressed with H_2O_2 showed an increase in cell viability, a smaller intracellular amount of ROS, and a reduction in membrane lipid peroxidation and DNA damage, which was also able to improve mitochondrial functionality, increase the basal respiration of mitochondria, and promote a regenerative capacity of cells after exposure to prooxidant stimuli. These findings promote the use of natural sources of antioxidants like fruits and plant extracts (green and black tea, carotenoids, coffee) for protecting skin against premature aging, moreover vitamins A, B, C, and E, CoQ10 and its analogues, and flavonoids are currently incorporated into a variety of antiaging skin care systems by oral administration and topical application. However, many controlled clinical studies are needed to determine the efficacy and risks of plant-derived

products in dermatology. Safety aspects, especially related to sensitization and photodermatitis, have to be taken into account for dermatologic disorders and cosmetic purposes.

2.3.12 Neurodegenerative Diseases

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 diseases. Animal and human studies have suggested that consumption of fruits and vegetables has the potential to decrease some age-related processes, primarily due to the antioxidant and antiinflammatory properties of several phytochemicals. In vitro studies have suggested that some classes of phytochemicals also act in cell signaling and thus may protect against aging by mechanisms other than oxidative and inflammatory processes.

Fruit and vegetable extracts have been demonstrated to reverse or retard various age-related cognitive and motor deficits in rats. Strawberry and spinach extracts attenuated age-related cognitive and neuronal decline in rats over 6–15 months, and blueberry extracts were effective in reversing existing cognitive deficits and improving motor function in aged rats. Examination of the brain tissue from these animals showed evidence of reduced inflammatory and oxidative processes in the supplemented groups. A transgenic mice model of Alzheimer's disease fed blueberry extract exhibited cognitive performance equivalent to that of normal nonsupplemented mice and was significantly improved compared to nonsupplemented transgenic mice. Blueberry supplementation in the transgenic mice increased the concentration of cell-signaling kinases thought to be involved in converting short-term memory to long-term memory, and increased other aspects of cell signaling, including increased muscarinic receptor activity that is also known to be important in cognitive function. Aging rats provided with Concord grape juice at low concentration (10%) for 9 weeks improved cognitive performance, while a high (50%) concentration improved motor performance. 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.

It is believed that oxidative stress plays a key role in the development of Alzheimer's disease because of the characteristic lesions associated with free radical damage and the attenuation of these processes with supplementation of some antioxidants. To date, there are no clinical trials that specifically address the role of dietary fruits and vegetables, although there are trials to investigate the association between dietary antioxidants in food and risk of Alzheimer's disease. A study involving 5395 men with an average age of 67.7 years, living in the Netherlands and followed for 6 years, reported that baseline dietary intake of vitamins C and E as well as the use of antioxidant supplements was associated with reduced risk of developing Alzheimer's during

the follow-up period, with a stronger protective effect in subjects who were smokers, and flavonoids and β-carotene intake was protective in smokers but not in nonsmokers.

The mechanisms proposed are based on the antioxidant action of curcumin by increasing glutathione levels, reducing lipid peroxidation in 3-nitropropionic acid (3-NP)-induced neurotoxicity in rats, improving learning and memory. Epigallocatechin gallate is able to protect neuronal cell lines submitted to glucose or glutamate toxicity and age-associated oxidative damage in rat brain by increasing activities of superoxide dismutase, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase. Another mechanism observed with epigallocatechin gallate, genistein, kaempferol, and quercetine, is the inhibition of cholinesterase activity, which improves cognitive functions, like learning and memory. It has also been observed that curcumin grape extracts and resveratrol and epigallocatechin gallate have the capacity to reduce amyloid-β (Aβ) deposition and Aβ protein in preclinical in vitro and animal models.

There are a growing number of evidences about the potential of natural polyphenols against neurodegenerative diseases, despite the application into clinical routine being limited because it is necessary to understand the systemic metabolism, pharmacokinetics, brain bioavailability, local metabolism, and modification in the central nervous system.

2.4 SUMMARY AND CONCLUSIONS

Accumulating evidences demonstrate that fruit and vegetable consumption has health benefits. These benefits of fruits and vegetables are associated with their content of thousands of phytochemicals of diverse classes. Therefore, health organizations recommend the consumption of at least 400 g of fruits and vegetables daily. Intensive research activity is dealing with the identification of the phytochemicals in fruits and vegetables, and their role in human nutrition and health through controlled clinical intervention trials, as well as studies to reveal the mechanisms behind the effect of the different phytochemical components.

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CHAPTER 3
Photosynthesis

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3.1 INTRODUCTION

Plants are the primary source of all terrestrial food, either directly or indirectly: directly as grains, fruits and vegetables, and indirectly from products of animal origin (meat, eggs, and dairy). Also, many medicines, flavors, construction materials, and clothing are subproducts obtained from plants. The biological process responsible for all this primary mass and energy production is called photosynthesis. Its significance is clear when we note that photosynthesis is the biggest "energy-producing industry" on Earth, bigger than the entire global industrial activity (in a thermodynamic sense). Similarly, in the aquatic ecosystems, photosynthesis is the main source of primary production, and therefore, of the important biological products provided by oceans, lakes, and rivers.

Photosynthesis is a biological process that enables plants, algae, and certain bacteria to harvest energy from sunlight. This energy is then utilized not only for plant maintenance, growth, development, and reproduction, but also for plant adaptive responses to environmental conditions.

Photosynthesis converts sunlight, atmospheric carbon dioxide (CO_2), and water to glucose and oxygen. Plants are estimated to fix approximately 1.4×10^{14} kg of CO_2 per year to convert it into carbohydrates (primary energy source). A coarse estimation indicates that 10% is done by higher plants and 90% by inferior plants, algae, and other seaweeds. From the glucose produced by photosynthesis, other carbon-based compounds are synthesized, and finally different plant tissues are formed. In this way, photosynthesis provides organic matter that can be used by animals and fungi as sources of energy and blocks for molecular building. The oxygen released into the atmosphere during

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photosynthesis plays an important role in the process of cellular respiration taking place in almost all living organisms.

The general process of photosynthesis is illustrated in a very simple way as follows:

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O)n + O_2$$

Light induces a complex series of oxidation–reduction reactions in order to transform solar radiation into chemical energy, necessary to complete the photosynthetic process. Water is the reducing agent since it is the source of electrons. This is in gross mode the photosynthetic process of green plants and cyanobacteria, although some bacteria utilize H_2S as the reducing agent instead of H_2O. Two water molecules are oxidized by charge separation reactions to release hydrogen ions which are utilized in an electron transport chain, an associated proton pump, and ATP synthase. As a result, ATP is produced for further formation of carbohydrate molecules from carbon dioxide and water.

Oxygen is released to the atmosphere as a waste product from these light-induced reactions.

During the 1930s, experiments conducted by Van Niel indicated that the O_2 production during photosynthesis comes from water, not from CO_2. This fact was confirmed by using oxygen isotopes both in water and in carbon dioxide, leading to:

$$CO_2 + H_2{}^{18}O \xrightarrow{\text{light}} CH_2O + {}^{18}O_2$$

In summary, photosynthesis consists of two very different reactions (Fig. 3.1): the first reaction needs light, and is called the "light-dependent reaction," where the energy of light is used for the photochemical oxidation of water. With this reaction, the reduced coenzyme NADPH is produced, and O_2 is

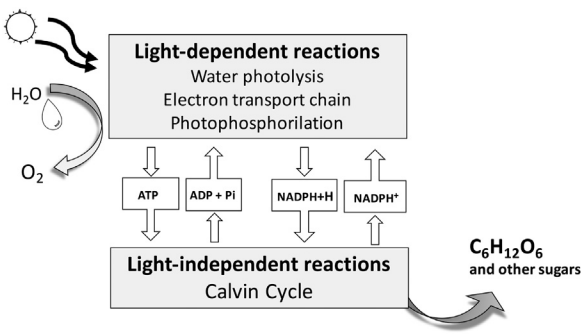

FIGURE 3.1
Light transformation and carbon fixation reactions in photosynthesis.

released. In addition, part of the solar energy that is captured is utilized to obtain ATP from ADP. This process is called "photophosphorylation." A photosensitive protein (chlorophyll pigment) is responsible for light absorption in this reaction.

The second reaction is called the "light-independent reaction," which does not need the presence of light, but can also occur in its presence. In this reaction, the NADPH and ATP previously produced in the light-dependent reaction are used for the fixation of CO_2 into carbohydrates. An enzyme (ribulose bisphosphate carboxylase-oxygenase) is responsible for carbon dioxide fixation, in a metabolic reaction known as the Calvin cycle. These reactions occur when light reaches the leaf, and stomata are open, allowing the entry of CO_2 to photosynthetic cells.

Each requirement for plant growth and development depends on the internal energy balance, which is the result of carbon assimilation in photosynthesis. Carbohydrates derived from photosynthesis are the energy source for plant maintenance (using glucose for the cell respiration process) in the first instance, and then to support metabolic energy needs for growth, when structural carbohydrates like cellulose are also required. Consequently, dry matter is accumulated which is subsequently distributed internally in the plant for the development of new structures such as branches, wood, flowers, and fruits. In this way, dry matter (storage assimilates) and carbon flow derived from photosynthesis are allocated during fruit growth and development. Therefore, fruit development, maturation, ripening, and quality are influenced by the process of photosynthetic activity, which is the reason for the great importance of this process (photosynthesis), not only during the development of horticultural commodities (fruits, vegetables, flowers), but also its consequences during postharvest. Just to mention one example, tree canopy management, which is fundamental to enhancing light interception, and hence, fruit yield, fruit color, fruit contents of several components (such as sugars and pigments), has direct on the activity of the process of photosynthesis.

3.2 THE LEAF AND ITS STRUCTURE

3.2.1 Mesophyll Cells and Stomata

Mesophyll is the internal ground tissue located between the two epidermal cell layers of the leaf; and is composed of two kinds of tissues: the palisade parenchyma, an upper layer of elongated chlorenchyma cells containing large amounts of chloroplasts; and the spongy parenchyma, a lower layer of spherical or ovoid cells with few chloroplasts and very prominent intercellular air spaces (Fig. 3.2).

In order for carbon fixation to occur, atmospheric CO_2 enters the mesophyll through stomata, which are specialized complexes of cells which form tiny pores that function as small valves for gas exchange; these are mostly located in the lower epidermis of the leaf. Stomata open during the day in the vast

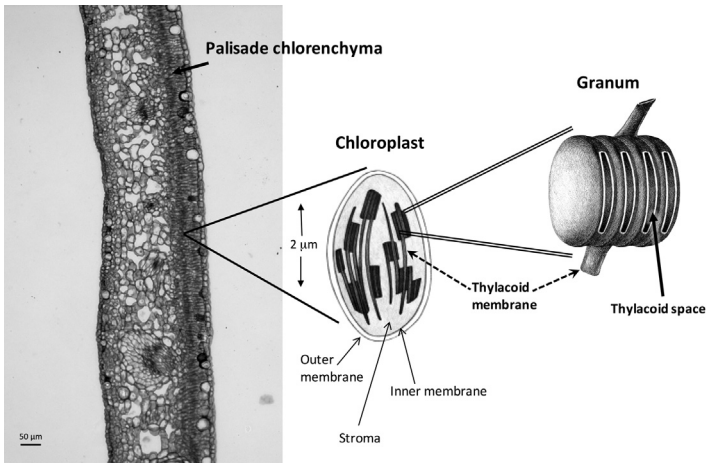

FIGURE 3.2

Leaf structure: chlorenchyma, chloroplasts, and granum the leaf picture is courtesy of the LANIVEG (Laboratorio Nacional de Caracterización Vegetal-UAQ-UdG, Mexico).

majority of plants, allowing atmospheric CO_2 diffusion toward the interior of the chlorenchyma cells, where the chloroplasts transform light energy, necessary for carbon fixation in photosynthesis.

3.2.2 Chloroplasts

Each mesophyll cell contains about 20–50 chloroplasts. Chloroplasts are intracellular organelles present in plant cells. The internal structure of the chloroplast has some similarity to the mitochondria because it is surrounded by a permeable outer membrane and another selectively permeable inner membrane. Both chloroplasts and mitochondria evolved from bacteria that were phagocytosed by an ancestral eukaryotic cell, leading to an extremely important historic process known as endosymbiosis. Chloroplasts could be considered semiautonomous organelles because they contain DNA, RNA, and 70S ribosomes, and are able to synthesize proteins. In addition, chloroplasts reproduce by binary division, and therefore, transmit genetic information. Inside the chloroplasts there is a space enclosed by the inner membrane, called the stroma, and this membrane extends in an arrangement that forms a set of numerous flat disk-like sacs, called thylakoids (Fig. 3.2). A stack of these forms a unit called granum (plural: grana). Grana are connected with an extension called stroma lamellae. The absorption of light and all the light-dependent reactions occur in the membranes of the thylakoids, where the pigments necessary for photochemical processes are embedded. The energy transformed by these reactions (in the form of ATP and NADPH) is released in the stroma where the CO_2 fixation reactions occur. Therefore, plants need to couple the functioning of chloroplasts with the entry of CO_2 through the stomata to reach the stroma. The stomata use some photosynthetic energy from their own

chloroplasts to direct the opening and closing of the pore, allowing the control of water loss through evapotranspiration while favoring the entry of CO_2 into the leaf. This event is known as gas exchange.

3.3 LIGHT ABSORPTION

The very first step in photosynthesis is the absorption of sunlight by pigments; where every different pigment or substance possesses a unique absorption spectrum of light. The absorption spectrum of a substance is the range of frequencies of electromagnetic radiation readily absorbed by such a substance; and pigments are molecules that are good absorbers of electromagnetic radiation in the visible range.

Visible light is the electromagnetic radiation ranging from 400 to 700 nm. The ability of a chemical to absorb light depends on the arrangement of electrons around its nucleus. When a photon of light is absorbed by a molecule, an electron is pushed to a higher level of energy. The molecule that absorbs the photon will be an energy-rich molecule as it passes from a basal state to an excited state. When this excited electron returns to the basal state, the energy absorbed is lost as light or as heat. The light produced is referred to as "fluorescence." The event of electron excitation and fluorescence is a very rapid process

3.3.1 Pigments and Photosynthesis

The main pigment that absorbs light in plants is chlorophyll, but other secondary pigments, such as carotenoids, can also trap light. Chlorophyll is a chromo-protein found in all green plants. It contains four pyrrole rings, a magnesium molecule, and a long isoprenoid chain called phytol. Typically, most plant cells contain two types of chlorophyll: chlorophyll a and chlorophyll b (Chl a, Chl b); whereas brown algae, diatoms, and dinoflagellates contain chlorophyll c, which is slightly different in structure from types "a" and "b," although the function of chlorophyll c in algae is similar to the function of chlorophyll b in plants. A fourth type exists, chlorophyll d, which is present in red algae. Depending on their light wavelength absorption peak (nm), different Chl a molecules can be distinguished as: Chl a-670, Chl a-680, Chl a-695, or Chl a-700.

Most plants contain almost twice as much chlorophyll "b" as chlorophyll "a." These two kinds of chlorophyll slightly differ in their structure, however, both Chl a and Chl b chlorophylls absorb preferentially violet-blue and red light. Chl a is the main photosynthetic pigment because it is the only one that can directly transfer an electron to an electron transport chain in the process of converting light energy into chemical energy, whereas Chl b acts as an accessory or secondary light-absorbing pigment. That is, Chl b absorption is complementary to the light absorption of Chl a. This is because Chl b can absorb more photons that Chl a is able to capture.

The absorption of light is due to the double bonds of the pyrrole rings. The phytol molecule is hydrophobic and serves to fix the chlorophyll molecule and orient it in the lipid part of the thylakoid membrane.

When a chlorophyll molecule is isolated and excited with light in a test tube, the absorbed energy is released very quickly as fluorescence and heat. However, when an intact spinach molecule of chlorophyll is excited with visible light, no fluorescence is observed, but a high-energy electron is expelled from the excited chlorophyll molecule and jumps toward the first member of a chain of electron transporters. It is in the process of electron transport where energy is transformed in the form of ATP and NADPH.

In addition to chlorophyll, the thylakoid membrane contains other secondary pigments that absorb light. These are referred to as "accessory pigments" and include carotenoids such as β-carotene and xanthophyll. In fact, from all pigments that can be found in plants, there are only two general types that are used by photosynthesis: chlorophylls and carotenoids.

Carotenoids are able to capture sunlight at wavelengths that are not efficiently absorbed by chlorophyll a or b. In this way, Chl b and carotenoids greatly increase the sum of photons that can be harvested by plants. However, in the case of carotenoids, their main function is as an antioxidant molecule that protects chlorophyll from photooxidative damage.

3.3.2 Photosystems

Photosystems are the functional units for photosynthesis, defined by a particular pigment organization and association patterns, whose work is the absorption and transfer of light energy, which implies transfer of electrons. Physically, photosystems are found in the thylakoid membranes. There are two kinds of photosystems: photosystem I (PSI) and photosystem II (PSII)

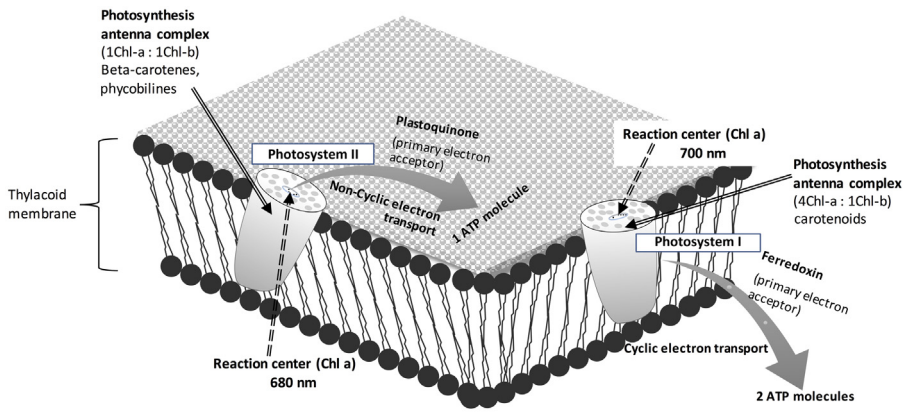

FIGURE 3.3
Photosystems I and II in the thylakoid membranes.

(Fig. 3.3). PSII acts first during the light transformation process in photosynthesis, but it was named PSII because it was discovered second.

Each photosystem consists of two closely linked components: the first is the antenna complex formed by hundreds of pigment molecules that capture photons and transfer the harvested light energy to the second component named the reaction center, which possesses Chl a molecules in a matrix of protein. When excitation energy reaches chlorophyll a at the reaction center, electron transfer is initiated through an electron transport chain.

PSI is located at the outer surface of the thylakoid membrane, and contains chlorophyll b; chlorophyll a (in the forms: a-670, a-680, a-695, a-700), and carotenoids; and one particular chlorophyll a-700 form (named Chl a-P700) is the active reaction center. PSII is located at the inner surface of the thylakoid membrane, and contains chlorophyll b; chlorophyll a (forms a-660, a-670, a-680, a-695, a-700), phycobillins, and xanthophylls; and a Chl a-P680 form is the active reaction center.

3.4 LIGHT-DEPENDENT REACTION IN PHOTOSYNTHESIS

The location of photosystems in the thylakoid membranes allows electrons to move by an electron transport chain, generating a pH gradient across the thylakoid membrane where ATP synthase is present. As a result, phosphorylation reactions are activated, producing photosynthetic ATP and reduced coenzyme NADPH as well. This photochemical process is named the Hill reaction (Fig. 3.4), and its final products (ATP and $NADPH^{+}$) are the metabolic energy necessary to incorporate atmospheric carbon (CO_2) into organic compounds derived from the Calvin cycle.

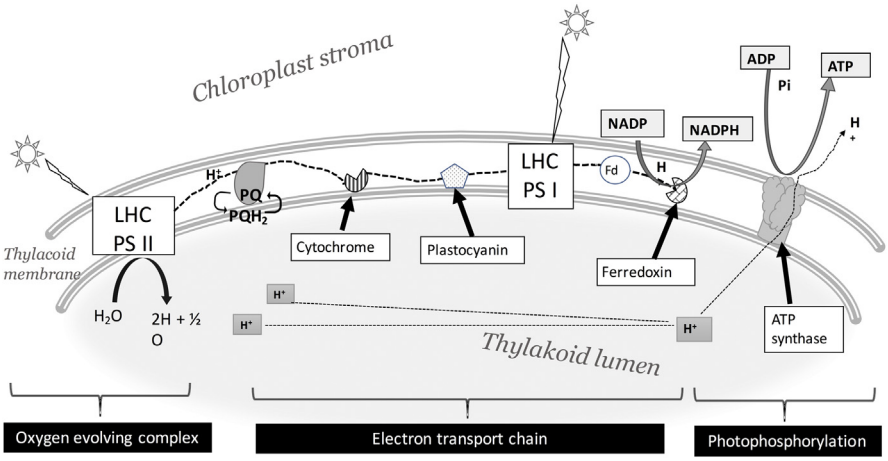

FIGURE 3.4

Light-dependent reaction in photosynthesis (Hill reaction).

At the first event in the Hill reaction, when chlorophyll excitation occurs, two water molecules are oxidized by four successive charge separation reactions by PSII to produce one molecule of O_2 (diatomic oxygen) and four hydrogen ions. The produced electrons are transferred to a redox active tyrosine residue which then reduces the oxidized chlorophyll a (called P680) which serves as the electron donor driven by the light in the reaction center of PSII. That photosensitive receiver is in effect restored and is then capable of repeating the absorption of another photon and the release of another photo-dissociated electron. The oxidation of water is catalyzed in PSII by an active redox structure containing four manganese ions and one calcium ion. This oxygen-avoiding complex binds to two molecules of water and contains the four oxidizing equivalents that are used to drive the oxidation reaction of water. PSII is the only biological multisubunit enzyme known to carry out this oxidation of water. The released hydrogen ions contribute to the transmembrane chemiosmotic potential leading to ATP synthesis in a process called photophosphorylation.

3.4.1 Cyclic and Noncyclic Photophosphorylation

Photophosphorylation is the conversion of ADP to ATP using the energy of sunlight by activation of PSII. This involves the splitting of the water molecule in oxygen and hydrogen protons (H^+), a process known as photolysis. Subsequently, a continuous unidirectional flow of electron from water to PSI is performed (Fig. 3.5). Electrons move spontaneously from donor to acceptor through an electron transport chain, and ATP is made by the action of the enzyme ATP synthase. An electron transport chain consists of a series of redox reactions which sequentially proceed to transfer electrons from a high-energy molecule (the donor) to a lower energy molecule (the acceptor). During the function of the electron transport chain, a transmembrane electrochemical potential gradient is produced by the flow of protons from the stroma to the thylakoid space and this proton gradient is established as the power for ATP synthase activity and thus ATP is produced by phosphorylation.

3.4.2 Cyclic Photophosphorylation

This process begins in PSI as follows: the electron passes from chlorophyll P680 to ferredoxin (Fd, the primary acceptor), then to pheophytin (Ph), then to complex b_6-f, and then to plastocyanin (Pc), before returning to chlorophyll P680, and then the cycle is repeated. During the transport chain operation, a pumping of H^+ ions is produced across the thylakoid membrane and a concentration gradient is established and can be used to power ATP synthase to produce ATP (Fig. 3.5). This pathway produces neither O_2 nor NADPH.

3.4.3 Noncyclic Photophosphorylation

Noncyclic photophosphorylation involves the two chlorophyll photosystems: PSII and PSI. They work sequentially to produce ATP, NADPH, and O_2, the

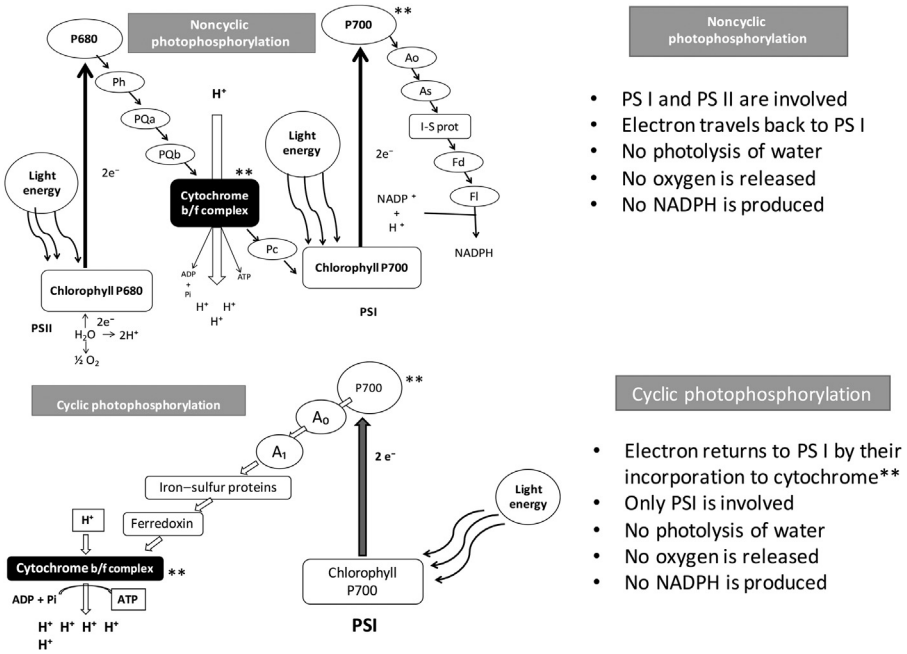

FIGURE 3.5
Cyclic and noncyclic photophosphorylation.

latter as a byproduct. Firstly, two water molecules are broken down into four protons (H^+) and one molecule of O_2, releasing four electrons (a process called photolysis). The electrons from the water molecule continue the process through the electron transfer chain. After leaving PSII, the primary acceptor in this electron transfer chain is a quinone molecule named plastoquinone, which is a strong electron donor that transfers the excited electron to the complex b_6-f and this passes the electron to a copper-containing protein called plastocyanin and then the electron continues to PSI. The complex b_6-f is also a proton pump that due to electron passage through the electron transfer chain, simultaneously protons are pumped from the chloroplast stroma to the thylakoid space and contribute to the proton concentration gradient that powers ATP synthase to produce ATP. PSI receives an electron from Pc to occupy the vacant space generated once the electron from chlorophyll P700 is ejected by photon incidence. PSI transfers the high-energy electron to Fd (an iron–sulfur protein) and then to $NADP^+$ to form NADPH, which accumulates in the stroma. NADPH formation is catalyzed by the membrane-bound NADP reductase. When the chloroplast is low in ATP, and the NADPH concentration is high enough for the Calvin–Benson cycle, the plant may shift from noncyclic to cyclic electron flow in order to satisfy the ATP requirements (Fig. 3.5).

With a continuous electron flux between both types of phosphorylation (Fig. 3.5), plants can convert ADP to ATP and obtain a provision of NADPH,

in order to power the synthesis of energy storage molecules in the next photosynthesis phase, the Calvin–Benson cycle.

3.5 CARBON DIOXIDE FIXATION (PHOTOSYNTHESIS C3)

Plants obtain carbon, their main macronutrient, from atmospheric CO_2 by a metabolic pathway known as the Calvin–Benson cycle (Fig. 3.6), also called the cycle of light-independent reactions of photosynthesis. The chemical reactions are carried out in the stroma that converts carbon dioxide into glyceraldehyde 3-phosphate (G3P), consuming the ATP and NADPH produced during the photophosphorylation process. The Calvin–Benson cycle is composed of three phases: carbon fixation, reduction reactions, and ribulose 1,5-bisphosphate (RuBP) regeneration.

Carbon fixation is the starting phase of two steps in the Calvin–Benson cycle and it consists in the enzymatic addition of a carbon atom from a CO_2 molecule to a five-carbon molecule named RuBP. The resulting six-carbon molecule is unstable and immediately splits into two molecules, a phosphorylated three-carbon molecule (3-phosphoglycerate, abbreviated as 3-PGA). The enzyme performing this reaction is called ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). The second phase of the Calvin–Benson cycle (reduction phase) also consists of two steps. Firstly, the phosphorylation of 3-PGA with photosynthetic ATP by the enzyme phosphoglycerate kinase to produce 1,3-bisphosphoglycerate (1,3-BPGA) and ADP. Then, the reduction of 1,3-BPGA with photosynthetic NADPH by the enzyme G3P dehydrogenase to produce G3P and $NADP^+$. The third phase of the Calvin–Benson cycle is the

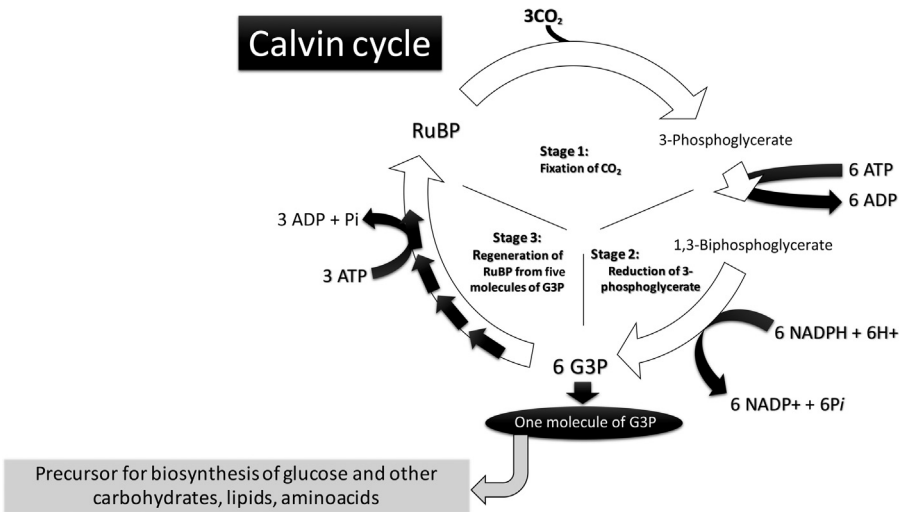

FIGURE 3.6

Carbon dioxide fixation (Calvin–Benson cycle).

regeneration of three RuBP molecules from five G3P and three ATP molecules. The reduction phase produces six G3P molecules, of which, five are used to regenerate three RuBP molecules, leaving a net gain of one G3P molecule per three CO_2 molecules entering the cycle (Fig. 3.6). The direct product of the Calvin cycle is G3P that accumulates in the chloroplast stroma. Each gained G3P molecule withdraws from the Calvin–Benson cycle to be converted by enzymes of the glycolysis in the reversal reactions to glucose-1-phosphate and then to starch, or it can be exported from the chloroplast to the cytoplasm of the cell where it can be transformed into fructose-6-phosphate and glucose-1-phosphate and then into sucrose, which can be translocated to other parts of the plant. Therefore, G3P, as a prime end product of photosynthesis, is the source of carbohydrates that plants require for both cell maintenance and cell growth (Fig. 3.6).

3.6 PHOTORESPIRATION (ALSO CALLED PHOTOSYNTHESIS C2)

The fundamental basis of photorespiration was found in the dual nature of the enzymatic activity of Rubisco, because it has the ability to catalyze both the carboxylation (Calvin–Benson cycle) and oxygenation of ribulose 1,5-biphosphate. That is, when molecular oxygen is the substrate for Rubisco in the Calvin–Benson cycle, the products are one molecule of 3-phosphoglycerate and another of 2-phosphoglycerate, whereas the carboxylase activity yields two molecules of 3-PGA. In other words, photorespiration is the result of Rubisco oxygenase activity that implies O_2 consumption, spent photosynthetic ATP, and converts phosphoglycolate to 3-PGA, releasing CO_2. There are some common environmental conditions that promote Rubisco to react as oxygenase, such as elevated internal temperatures (32°C), or low CO_2 concentrations inside photosynthetic cells in relation to higher levels of O_2 accumulated in the stroma. This can happen when the stomata are closed or not fully open, and even more, oxygen accumulation is accelerated by high irradiation levels that maintain a high water photolysis by PSII. This results in a decrease of up to 25% of the carbon that is normally fixed during photosynthetic carbon assimilation, because a molecule of ribulose 1,5-bisphosphate is lost for the Calvin–Benson cycle, as well as part of the ATP generated by photosystems. Therefore, photorespiration strongly reduces the photosynthetic capacity of plants, affecting also the normal functions of subcellular organelles, since it induces a complex series of reactions that take place across three separate subcellular compartments: chloroplasts, peroxisomes, and mitochondria.

It is known that high irradiation levels can saturate the photosystems, leading to the generation of reactive oxygen species (ROS) which cause oxidative damage to the chloroplast and the cell; and recent studies have demonstrated that photorespiration helps in some way to regulate such situations, because during photorespiration, O_2 and ATP are consumed, and this minimizes the

formation of ROS. In addition, if photorespiration consumes ATP, the saturation of electron acceptors in photosystems is avoided, since the electronic flow can continue because of the increase in ATP demand.

Even when plants are growing under generally favorable conditions, photorespiration often occurs during the hottest hours of the day, when stomata tend to close, avoiding high evapotranspiration rates; therefore, plants experience a reduction in their photosynthetic efficiency on a daily basis, and that is why some species have overcome these limitations by developing different strategies to increase the concentration of carbon dioxide around Rubisco. These include the C4 pathway of carbon fixation and the crassulacean acid metabolism (CAM).

3.7 PHOTOSYNTHESIS C4 AND CRASSULACEAN ACID METABOLISM

Photosynthesis in most of the world's flora is carried out using the metabolic pathway known as C3, which means that the first stable product derived from CO_2 fixation by Rubisco, is a three-carbon organic acid (3-PGA). However, since Rubisco oxygenase activity affects photosynthetic efficiency, some plants have developed a more efficient enzymatic alternative.

In plants that grow in very sunny and warm environments, the C3 pathway is preceded by an additional step, where CO_2 is attached into a four-carbon organic acid (oxaloacetate, OAA, an unstable acid that rapidly changes to malate) before it is incorporated into ribulose 1,5-diphosphate, so then the Calvin–Benson cycle can run without CO_2 limitations. This metabolic modification is named C4 because the first compound derived from carbon fixation is a four-carbon acid; and for this, the enzyme phosphoenolpyruvate carboxylase (PEP-C) is specifically participating to capture CO_2. This reaction, prior to the Calvin–Benson cycle, diminishes the risk of photorespiration, and therefore, overcomes the tendency of the enzyme Rubisco to wastefully fix oxygen rather than carbon dioxide as observed in C3 plants in warm climates.

Likewise, in dry environments, plants must deal with high evaporative water losses by closing their stomata in the hottest hours during the day, drastically decreasing gas exchange and CO_2 fixation. Therefore, some plants under arid environments changed their stomatal behavior pattern toward diurnal closure and nocturnal opening, when evapotranspiration is minimal. If CO_2 enters through open stomata at night, the Calvin–Benson cycle cannot be initiated because Rubisco needs photosynthetic ATP exclusively to catalyze the CO_2 binding reaction, and this is not possible because no light transformation reactions are active. Since PEP-C can catalyze the same binding reaction without the need for photosynthetic ATP, this enzyme is able to be active at night. Therefore, in this case, organic acids (malate) accumulate throughout the night, to be decarboxylated during the next day. In this way, there will be high levels of CO_2 inside the photosynthetic tissues to drive the Calvin–Benson

cycle minimizing photorespiration and avoiding stomatal aperture. This pattern of nocturnal acid accumulation was first described in several species of the crassulacean family, hence denominated as CAM. In summary, CAM plants made a temporal separation of CO_2 fixation, operating a C4 pathway at night but with a C3 pathway operating during the day.

3.7.1 C4 Photosynthetic Metabolism

The role of PEP-C in C4 plants is a biochemical advantage that evolved along with anatomical and functional changes in leaf anatomy, differentiating into two types of cells: (1) the bundle-sheath cells, which surround the vascular conduits and (2) the mesophyll cells, which are located along the leaf, between the two epidermal cells (Fig. 3.7). This different structural and metabolic framework (called the Kranz anatomy) allows for the compartmentalization of carboxylation and decarboxylation reactions that permit the accumulation of CO_2 around Rubisco, thereby enabling these plants to assimilate atmospheric carbon dioxide with very high efficiency.

In mesophyll cells, the three-carbon molecule (phosphoenolpyruvate) binds to CO_2 to make OAA, which is reduced to malate, a four-carbon molecule. Then malate enters bundle-sheath cells (Fig. 3.8), where it is decarboxylized to produce pyruvate, and CO_2 is released by the action of the malic enzyme. Pyruvate returns to the mesophyll cell, where two of the high-energy bonds in an ATP molecule are split to convert pyruvate back into PEP, thus completing the cycle. Meanwhile, the CO_2 released in bundle-sheath cells is fixed with ribulose 1,5-diphosphate (Rubisco), as in

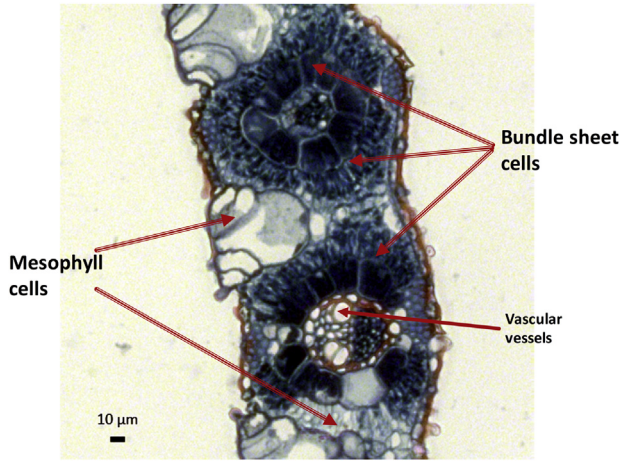

FIGURE 3.7

Transversal section of a leaf showing the Kranz anatomy of a C4 species (*Cyonodon dactilon*) the leaf picture is courtesy of the LANIVEG (Laboratorio Nacional de Caracterización Vegetal-UAQ-UdG, Mexico).

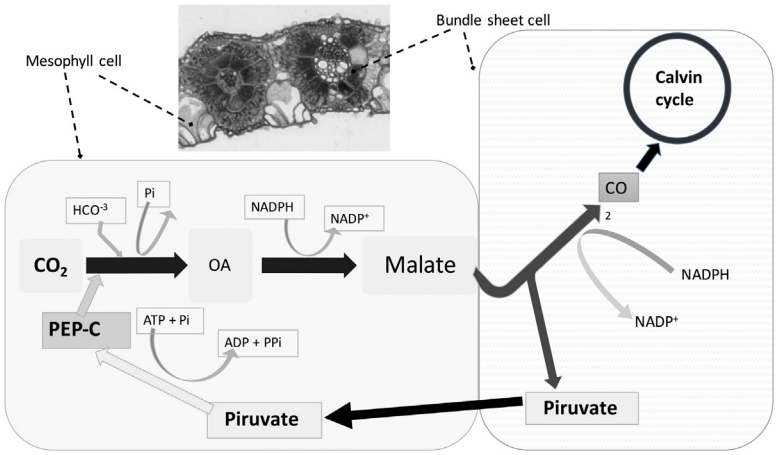

FIGURE 3.8
Spatial separation of carbon fixation from specialized cells in C4 plants.

the case of C3 plants. Pyruvate that is formed by the decarboxylation of malic enzyme is transported to the mesophilic cell and converted to PEP by the enzyme pyruvate phosphate dikinase. Hence, C4 metabolism separates carbon fixation reactions into two different kinds of cells: mesophyll and bundle-sheath cells.

It is worth mentioning that C4 metabolism is found at different intermediate levels; from an incipient cellular differentiation that mostly facilitates internal CO_2 recycling, or a scarce bundle-sheath differentiation in some plants, to a definitive Kranz anatomy in other plants.

In C4 photosynthesis, the energetic cost of forming glucose is almost twice that of C3 photosynthesis because to fix a molecule of CO_2 in C3 plants, three molecules of ATP are needed, in contrast with C4 plants that need five molecules of ATP. However, such a disadvantage is offset by bundle-sheath cells, where Rubisco is isolated from atmospheric oxygen and saturated with the CO_2 released by decarboxylation of malate. In addition, the enzyme PEP carboxylase in mesophilic cells has much more affinity for CO_2 and can fix CO_2 more efficiently. Thus, CO_2 is concentrated in the cells (bundle sheath) and the ribulose enzyme diphosphate carboxylase can also act efficiently. Therefore, although the fixation of CO_2 in C4 plants requires more energy, C4 plants grow faster than C3 plants and generate more biomass per leaf unit.

All C4 plants are tropical herbaceous plants, capable of tolerating high temperatures and irradiation levels, which, along with their rapid growth rates, can turn them into potential weeds. However, important high-yield crops such as maize, sugar cane, sorghum, amaranth, and some livestock grazing are also characterized with this photosynthetic pathway.

3.7.2 Crassulacean Acid Metabolism

CAM plants have evolved to avoid high evaporative water loss through the stomata, opening them in the night; therefore, CO_2 binding is nocturnal, and the resultant organic acids (malate) are accumulated in the photosynthetic cells throughout the night, to be decarboxylated in the next morning. CAM plants need succulent tissues in order to store water; therefore, they have large cells with particularly large vacuoles, where malate can also be stored at night. During the day, the stored malate at night leaves the vacuole to be decarboxylated and CO_2 is released and efficiently incorporated into the Calvin–Benson cycle, by the regular C3 path (Fig. 3.9).

The temporal separation of CO_2 fixation on a day/night basis, in addition to the higher energy cost of the C4 pattern, causes CAM plants to have lower growth rates than C3 plants. Even so, this photosynthetic adaptation allows plants to survive under such harsh and limiting conditions of aridity.

Since CAM metabolism does not require functional and anatomic specialization, it is possible to find facultative CAM plants that shift from C3 to CAM metabolism when water availability decreases. Most agave species or various species from Euphorbiaceae, Portulacaceae, Asteraceae, Vitaceae, Clusiaceae, Apocynaceae, and several other plant families are CAM facultative; whereas most Crassulaceae, Cactaceae, Orchidaceae, and Bromeliaceae species are obligate CAM (Table 3.1). There are also some extreme expressions of CAM metabolism in very dry deserts, where plants are able to maintain their stomata closed for several months, eliminating any possible water loss by evaporation but also disrupting carbon dioxide entry; therefore, they keep recycling CO_2 produced by cellular respiration, until water is available again. This is known as the CAM idling mode.

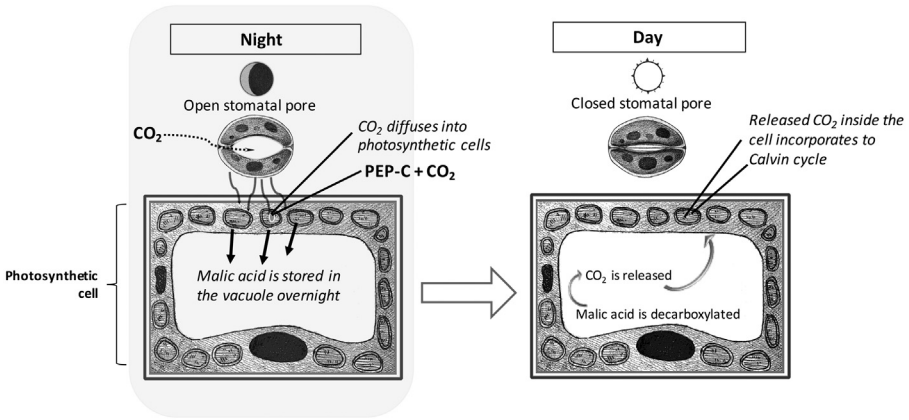

FIGURE 3.9
Temporal separation of carbon fixation in a single photosynthetic cell of a CAM plant.

Table 3.1 Some Important Plant Species and Their Photosynthetic Type

Photosynthetic Type	Common Name	Scientific Name	Importance
C3	Apple	*Malus pumila*	Horticulture
C3	Wheat	*Triticum aestivum*	Agriculture
C3	Pinyon pine	*Pinus cembroides*	Forestry, food supply
C3	Tomato	*Solanum lycopersicum*	Horticulture
C3	Mistletoe	*Phoradendron* sp.	Parasitic plant
C3: Almost 95% of the Total Flora in the World			
C4	Corn	*Zea mays*	Agriculture
C4	Sugar cane	*Saccharum spontaneum*	Agriculture
C4	Rice	*Oryza sativa*	Agriculture
C4	Buffel grass	*Cenchrus ciliaris*	Animal food, range management
C4	Invasive pink grass	*Rhynchelitrum repens*	Ornamental, invasive grass
C4: Most of the Tropical, Invasive Grasses (Poaceae Family)			
CAM	Pineapple	*Ananas comosus*	Horticulture
CAM	Tequila agave	*Agave tequilana*	Alcoholic beverage
CAM	Prickly pear	*Opuntia ficus indica*	Horticulture, invasive plant
CAM	Vanilla	*Vanilla planifolia*	Condiments
CAM: All Succulent Plants, Mainly in Arid Environments			

Moreover, it has been demonstrated that cacti, which are obligate CAM plants, grow with a C3 metabolism at their seedling stage, and at a certain moment they shift to a definitive and complete CAM path in a very juvenile stage. Such ability of CAM metabolism to change can be explained by the four phases of PEP-C and Rubisco activity (Fig. 3.10) in all CAM plants. Phase I is characterized by nocturnal PEP-C carbon fixation, whereas phase III is when Rubisco is active in the Calvin–Benson cycle under sunlight. At the very early hours of the morning (phase II) or in the afternoon (phase IV), both PEP-C and Rubisco are active, since stomata can be opened because temperatures are lower and water evaporation diminishes; therefore, CO_2 is eventually fixed by the two enzymatic modes. Thus, the plasticity of CAM metabolism depends on the extent to which phases II and IV are extended or contracted, in response to stomata opening.

It is also important to note that a type of CAM metabolism occurs in some submerged aquatic plants, where the primary barrier to CO_2 leakage is the extremely high diffusional resistance of water. CAM aquatic plants live in shallow temporary pools with extreme diel fluctuations in carbon availability,

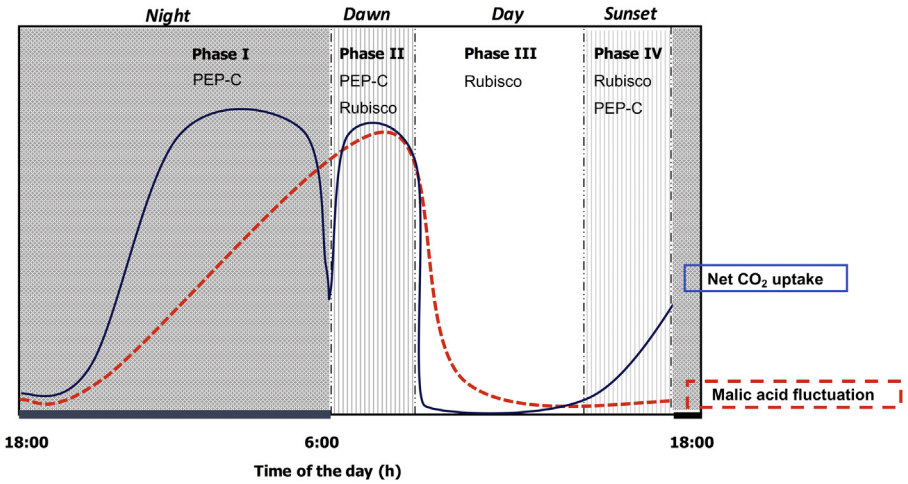

FIGURE 3.10
Phases of CAM carbon fixation pathway.

showing elevated nighttime CO_2 levels. PEP-C can catalyze carbon fixation independently to light or oxygen, and even under very low CO_2 concentrations in the tissues (contrary to Rubisco, that needs photosynthetic ATP to be activated and certain proportions of CO_2 to O_2); and besides, PEP-C is also able to recognize as a substrate HCO_3, which is the transformation of atmospheric CO_2 when it is hydrated.

3.8 PHOTOSYNTHETIC ADAPTATIONS TO DIFFERENT ENVIRONMENTAL CONDITIONS

Plants have certain requirements to keep the photosynthetic machinery in operation. Among the environmental factors limiting the photosynthetic rate, the essential ones are light incidence (quality, quantity, and intensity), CO_2, nutrients and water availability, and temperature.

3.8.1 Light Quality (Wavelength)

The color of light is a property of its wavelength, so that the energy level of different light colors can be variable. Blue light, with shorter wavelength and higher frequency, is about 1.8 times more energetic than the same number of red light photons.

Photosynthesis occurs in special structures or organelles called chloroplasts (see Section 3.2), located in green leaves and stems, and which contain pigments capable of intercepting light and converting the electromagnetic energy into chemical energy, necessary to perform the photosynthetic process. When these pigments (chlorophyll a, chlorophyll b, and some carotenoids), are irradiated with light containing all wavelengths of visible light (400–700 nm),

they absorb most of the red and blue portions of the spectrum and reflect the green portion (hence the human eye perceives them as green). When plants do not receive light within this spectrum, their pigments and photoreceptors are not capable of absorbing this energy. Plants under a dense canopy cover do not get the same quality of light as plants above the canopy, and therefore a higher incidence of far red light (710–850 nm) in this area is common, which inhibits morphogenetic processes, as well as the photosynthetic activity. Plants submerged in water also have restrictions in the amount and quality of light they receive.

3.8.2 Light Intensity (Brightness)

The intensity of light is defined as brilliance in the form of radiant energy, and is measured in watts, calories, luxes, or foot candles. The intensity of light affects the growth of plants, as it alters the rate of photosynthetic activity. The effect varies with different types of plants. Some species require high intensities to grow well, such as corn, wheat, potatoes, sugar cane, most pastures, and some fruit trees. These plants are commonly called "sun plants," and have developed adaptations to cope with high light intensities, such as thicker leaves with a greater concentration of chloroplasts per area unit, vertical leaves to absorb light on both sides, and a higher light saturation point to avoid photoinhibition.

Those species that do not grow well under high light intensities are called "shade plants," many grow adequately on the shady forest floor, and some are used as ornamental plants. Generally, these plants show thinner, but larger, leaves than sun plants, and with horizontal disposition, to maximize the area of light absorption. Some varieties of coffee, vanilla, wild chilies, and many ornamental plants require different amounts of shade.

Other species are indifferent and grow well under moderate light intensities, such as in the case of some flowers, like chrysanthemums.

The amount of shade in a particular point of the plant photosynthetic organs is generally quantified by the leaf area index (LAI), a nondimensional measurement of the amount of shade provided by the leaves and branches of the trees, shrubs, or herbs in the plant community or cultivars. An LAI with a value of 1 is equivalent to a monolayer of leaves covering the area. Generally, "sun plants" require LAI measurements between 0 and 0.75, and shade plants values greater than 1.

3.8.3 Duration of Light (Photoperiod)

The photosynthetic activity of plants is directly proportional to the duration of the day. Between the boundaries and all other factors being equal, leaves will photosynthesize as they receive more light and grow more actively. In the seasons where the days last longer (spring and summer), plants reach their maximum photosynthetic activity. In contrast, during winter, the photosynthetic activity is

reduced to its minimum or is absent, resulting in color change in the leaves, and the deciduousness or leaf fall in many species that inhabit temperate climates due to the decomposition of the chlorophyll. In this case, plants store photoassimilates produced during the summer.

3.8.4 Concentration of Carbon Dioxide

The concentration of CO_2 in the air around the leaves markedly affects photosynthesis. Normally, the average content in the atmosphere is 0.03% of CO_2 (O_2 is around 21%). Physiologists have found that by increasing the CO_2 concentration to 0.10% in a closed system, the photosynthetic rate of some crops such as wheat, rice, soy, and some vegetables and fruits, is doubled. The increase in the amount of CO_2 for the plants can be achieved by manipulating the planting density and height of the foliage, in order to increase the rate of CO_2 diffusion and, in turn, its concentration around the leaves. Applications of organic matter in the form of crop residues or green foliage to the soil tend to increase CO_2 levels in the soil surface atmosphere.

The rate of photosynthesis of different crops not only increases with increasing light intensity, but also increases of CO_2 concentrations, unless the stomata are closed due to water-deficient conditions.

Climate change studies predict future increases in CO_2 that must be considered for future location and migration of cultivars, and future distribution of plant communities. Apparently, C4 plants will be better adapted to enriched CO_2 conditions, but recent studies suggested also that CAM plants will increase their tolerance to this kind of environment.

3.8.5 Temperature

At low light intensities (20,000 lux or 1850 foot candles), temperature does not have a remarkable effect on the rate of photosynthesis, as light acts as a limiting factor. However, as a general rule, if light is not a limiting factor, the rate of photosynthetic activity doubles approximately every 10°C, which as a consequence increases the temperature in the environment of plants in temperate climates.

The effect of temperature is different in each species; plants adapted to tropical conditions require a higher temperature to reach the maximum rate of photosynthesis than those of cold regions. However, very high temperatures—above 40°C—affect the photosynthetic rate of most nonadapted plants, because the rate of transpiration exceeds the rate of water absorption, thereby reducing the internal water potential and decreasing the turgor pressure of the guard cells and the stoma is closed to prevent dehydration of plant tissue. In addition, excessively hot environments lead to a denaturation of the RuBP carboxylase enzyme, which binds the atmospheric CO_2 in the mesophyll of the leaves, causing photosynthesis to decrease or be nullified.

A reduced rate of photosynthesis, together with the increase in respiration rate at high temperatures, decreases the sugar content of fruits.

Similar to increasing CO_2 rates, temperature increases are expected globally as a result of climate change, therefore, some photosynthetic groups such as C4 and CAM plants will increase their potential distribution areas, while C3 plant distribution will be reduced. Important future agricultural and horticultural adaptation strategies must include photosynthetic adaptations to global increase in temperatures.

3.8.6 Availability of Water and Nutrients

Like carbon dioxide, water is used in the photosynthetic process. When the availability of water for the plant decreases, the exchange of CO_2 and O_2 is restricted, resulting in a dramatic reduction in CO_2 assimilation. In environments where water availability is the main limiting factor, plants increase their water-use efficiency (amount of carbon gain in photosynthesis per water vapor lost in the process) by manipulating stomatal closure. For instance, CAM plants open their stomata during the night to avoid losing great amounts of water during the hottest time of the day. Other plants, the deciduous species, lose their leaves during the dry season.

Nutrients also play an important role in photosynthetic activities. Although several nutrients are essential for plant performance, nitrogen is the main macronutrient involved in photosynthesis for plant growth and development. Nitrogen balance is crucial for carbohydrate synthesis, since photosynthesis depends on Rubisco, therefore the carbon:nitrogen ratio may limit or promote plant growth.

Plant C and N assimilation are correlated with each other: carbon allocation in organs is determined by plant allometric growth, but N is essential for C assimilation, and when photosynthesis is more active, the balance must be higher for carbon.

If the plant allocates resources for photosynthesis and hence vegetative and reproductive growth, the C:N ratio is low; in contrast, if the plant allocates its resources to defense or accumulation of reserves, the C:N ratio will be higher.

3.8.7 Photosynthetic Groups Around the World

Throughout geological history plant photosynthesis has been adapted to particular environments leading to differential geographical distribution patterns, especially with photosynthetic groups. C3 plants generally inhabit all the land emerged areas, represent almost 90% of terrestrial plants, and are predominant in temperate regions. However, C4 plants have a tropical distribution and are represented mainly by tropical grasses that include important grain cereals such as maize and rice. Interestingly, in some regions such as the Sonoran Desert between northwestern Mexico and southwestern United States, a seasonal distribution replacement exists among C3 and C4 ephemerals,

while C3 plants dominate in the winter months, summer replacement by C4 plants completely changes the vegetation structure in the desert.

CAM plants have a different geographical origin, related to tropical dry environments, and drought metabolic conditions in areas with high temperature. Therefore, it could be conceived as an adaptation to drought conditions that leads to a geographic distribution related to arid and semiarid environments.

Table 3.1 presents some characteristic species of each of the three metabolic routes (C3, C4, and CAM), and their agricultural or economic importance.

3.9 PHOTOSYNTHESIS IN FRUITS

At the time of flower and fruit development, carbon demands increase to such an extent that, in many cases, strategies of additional carbon acquisition by nonfoliar photosynthetic organs are needed. Photosynthetic capacity has been reported in reproductive organs such as calyx lobes, sepals, and anthers. Also, carbon fixation is possible in green or immature chlorophyllic organs (fruits or vegetables), where photosynthetic activity is very important, as it has been demonstrated with immature tomatoes, that represent around 15% of the total photosynthetic activity of the mother plant. Studies in several species indicate that up to 60% of total carbon requirement is used up in the course of fruiting. In young fruits of six species of platyopuntias, daily net CO_2 uptake measured accounted for an average of 10% of the daily dry mass gain.

Certain physiological and morphological characteristics in fruits are needed for their ability to photosynthesize; so, the presence of chloroplasts, chlorophyll, Rubisco, or PEP-C enzymes is essential, as well as functional stomata. Thus, photosynthesis in fruits can be interpreted as identical to the leaves. If fruits are green, they are able to capture energy that excites chlorophyll molecules for photosynthesis. This can be confirmed by chlorophyll fluorescence measurements, used as a parameter to evaluate quantum efficiency during light transformation reactions, because fluorescence signals indicate that the electron transport system is effective. The efficiency of PSII in green tomato fruit was observed with a mean value close to the average found in leaves of a wide range of C3 species.

Therefore, in relation to electron transport, fruits are able to carry out the regular photochemical process, so it is unlikely that photosynthesis is limited by electron transport.

Chlorophyll fluorescence measurements have shown that the electron transport system is working in tomato fruit until the late stages of maturation; and the total chlorophyll content of various fruit peels may vary from 7 μg g/FW for *Malus* to 42 for *Vitis*, 252 for *Cucumis*, 278 for *Capsicum*, and 850 μg g/FW for *Cucurbita*.

Other evidences of photosynthetic activity in fruits are documented in chloroplasts of *Arum italicum* berries at the green stage, where the thylakoid membrane hosts a very high amount of light-harvesting complex II (LHCII), and PSII (PSII), showing similar patterns to those of the leaf (monomers, dimers, LHCII−PSII supercomplexes). An early study using fluorescence measurements in mango fruit indicated high PSII efficiencies comparable to those typical for leaves.

In addition to light transformation by chlorophyll and photosystems in green fruits, carbon dioxide fixation is possible because stomata are present in the outer layer of many fruits. However, stomata in fruits usually represent only 1%−10% of the frequency in contrast to leaves of the same species. Depending on fruit size in oranges, the ratio of fruit to leaf stomatal densities varies between 10% and 30%; however, 20,000−30,000 stomata per fruit have been reported in avocado (*Persea americana* Mill.) cv. Fuerte.

Despite the stomatal density, their proper functioning is essential. Stomata in young fruits are as sensitive as in leaves; however, in mature Solanaceae, stomata of fruits like pepper (*Capsicum*), tomato, or eggplant, are absent from the epidermal layers. Therefore, fruit gas exchange may be attributed to a cuticle diffusive conductance in the fruit epidermis, or to a CO_2-concentrating mechanism, that may be similar to the CAM pathway.

It has been demonstrated that many fruits perform effective internal CO_2 recycling, like some measurements of gas exchange in green tomato fruit that revealed no net CO_2 fixation, although chlorophyll fluorescence was evident and very similar to those of the leaves. This clearly suggests CO_2 recycling in fruits; and although Rubisco activity in green fruits is similar to that recorded in leaf tissues, PEP-C activity is about twofold compared to Rubisco activity. Evidences of concentrating mechanisms to CO_2 assimilation were observed in preclimacteric avocado fruit cv. Fuerte, in which the enzyme PEP-C recaptures part of the respiratory CO_2 accumulated within the fruit. This conjecture resulted from the difference observed between CO_2 respired versus CO_2 fixed; the activity of PEP-C was 106 μmol CO^2/fruit/h, whereas the fruit respiration rate was only 40−60 μmol CO^2/fruit/h.

The activities of PEP carboxylase and other enzymes of C4 metabolism are generally much higher in fruiting structures than in the leaf. Fruit photosynthesis does not resemble any of the well-characterized categories of photosynthesis (C3, C4, or CAM). For this reason, some authors consider that fruits may have an intermediate status among C3, nonautotrophic tissue, and C4/CAM photosynthesis.

In addition, neither significant night/day acid fluctuations nor a consistent net assimilation of CO_2 have been clearly reported for fruits, even when fruits have a clear photochemical activity. Therefore, it is evident that fruit photosynthesis is still poorly understood.

Despite doubts over how photosynthesis occurs in fruits, it should be recalled that fruits have been physiologically defined as a reserve tissue, and considered a metabolic "sink" for photosynthetic products. The higher activity of PEP-C compared to Rubisco in fruits is very much related to this. Some studies show that PEP-C carboxylation is not directed to produce assimilates for fruit growth. Refixing respiratory CO_2 allows for the accumulation of malate, which acts as an osmolyte in maintaining positive cell turgor. This is a common condition in sink organs showing high PEP-C activity and very low photosynthetic efficiency (as happens in maize ears). Most fruits contain chlorophyll during the initial stages of development, which declines during fruit maturation and ripening. In some fruits, chlorophyll is totally absent at maturity (such as in tomato), but in other fruits it may totally disappear. However, once the ripening process starts, in several fruits, chlorophyll is degraded to nonphotosynthetic chromoplasts and the metabolic activity of these cellular organelles contributes to further synthesis of specific metabolites. For example, as tomato fruits ripen, their chloroplasts turn to chromoplasts, where lycopene, β-carotene, and other metabolites are synthesized and accumulated. Indeed, those compounds are very important for ripe fruit sensory, nutritional, and health attributes.

Studies on photosynthesis in fruits have revealed some aspects that represent important tools for postharvest management. Monitoring chlorophyll fluorescence images can be a feasible assay to predict postharvest damage, because in some fruits like lemons, photosynthesis is active throughout the postharvest ripening process, and chlorophyll fluorescence is a result of saturated photosystems, which is a common parameter to detect stress in leaves. Other studies have demonstrated that enhancing chloroplast development and function in tomato fruit, an important increase in sugar content and other specialized metabolites is induced, resulting in fruit quality improvement.

3.10 MEASUREMENTS OF PHOTOSYNTHETIC ACTIVITY

Photosynthesis is a complex process and, therefore, its measurement represents a challenge. Some methods have been developed and their use depends on whether photosynthesis can be measured in the laboratory or in the field. Laboratory methods are generally destructive, since the whole plant must be harvested to estimate the accumulation of dry matter in its tissues. Dry weight of tissues, where all water content has been removed, represents the cumulative photosynthetic activity during the plant's life. Other laboratory techniques include the measurement of pressure change of CO_2 or O_2 in isolated chambers with photosynthetic organisms; however, this technique is highly sensitive to environmental factors, such as temperature, and may be quite inaccurate. This method is mostly used with algae.

The most common currently used method is the gas-exchange method, because it gives short-term (instantaneous) measures, is nondestructive, and

FIGURE 3.11
Photosynthetic measurements conducted with a LICOR LI-6400 XT photosynthetic open system in a tropical acacia tree (*Acacia shaffneri*) and a parasitic tropical mistletoe (*Psytacanthus calyculatus*).

allows the measurement of CO_2 assimilation from individual leaves to canopy photosynthetic rates. In addition, there has been a great development of portable instruments, such as the LICOR LI-6400XT exhibited in Fig. 3.11. The gas-exchange systems estimate the rate of CO_2 assimilation by determining the change in CO_2 concentration in a chamber with an enclosed leaf. CO_2 concentration is measured with an infrared gas analyzer (IRGA), as CO_2 absorbs infrared radiation. Total net photosynthesis (A_n), is measured with the following equation: $(c_b - c_f)V/\Delta t$, which indicates the initial concentration of CO_2 (c_b) minus the final concentration (c_f) by the system volume (V), divided by the time between the start and the end of the measurement (Δt); although, the calculation may be modified depending on whether the system is closed or open. A closed system uses an absolute CO_2 concentration and measures its depletion, while water vapor increases. An open system has controlled air flow at a known rate, and some systems compensate for the CO_2 removed by the leaf by injecting a constant concentration of CO_2 to the chamber.

Since CO_2 consumption also implies water exchange and the activity of stomata, gas-exchange systems can also measure water vapor leaving and entering the chamber, and with this estimate leaf transpiration, as well as stomatal conductance (the rate of CO_2 entering or water vapor exiting through the stomata).

Another method to estimate photosynthetic activity is through fluorescence. When electrons return to the basal state, the energy absorbed can either emit heat, fluorescence, or enter the electron transport chain associated with photochemistry. Hence, the photochemical efficiency (P) can be estimated with the relationship between the minimum fluorescence (F_0) and the maximum (F_m), as $P = (F_m - F)/F_m$, where F is fluorescence in a steady-state environment. Photosynthesis estimation through fluorescence is recommended because it is

not sensitive to environmental factors, and it can be coupled with CO_2 exchange results to produce more robust interpretations.

The aforementioned methods are suitable to measure individual plant performance, but sometimes the aim is to estimate the performance of a large set of plants, such as crops. Gas-exchange systems allow the measurement of a set of plants because they can be used with any chamber size; thus, larger enclosures (enough to host a set of plants) can be built and adapted, such as chambers with an aluminum frame. These should be used with an open system looking to maintain a constant air flow rate and monitoring changes in temperature. It also uses IRGAs to measure CO_2 concentrations.

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CHAPTER 4

Respiratory Metabolism

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4.1 INTRODUCTION

The postharvest life and quality of a commodity are influenced by the rate at which the many interrelated metabolic reactions of a cell occur. Respiratory metabolism furnishes not only the energy required to drive all these metabolic reactions, but it also produces the raw material used as substrates by these reactions. In its simplest form, respiration reacts with stored substrates (usually a carbohydrate) and with O_2 to produce high-energy compounds (e.g., ATP) and CO_2. However, the production of carbon fragments used in subsequent synthetic reactions is also of major importance.

The rate of respiration changes throughout the life of the commodity. Metabolic activity is especially high during the initial growth of the commodity, during ripening of climacteric fruit, and during periods of wound healing. After an initial surge to repair wounds encountered during harvest, respiration usually declines in vegetative tissues and nonclimacteric fruit (Fig. 4.1). In contrast, the ripening of climacteric fruit is accompanied by a rapid rise in ethylene production and respiration. It is thought that the added energy derived from this rise in respiration is necessary to power the many metabolic processes (e.g., tissue softening, and synthesis of pigments and volatiles) that accompany ripening. However, nonclimacteric fruit undergo similar ripening changes without a concomitant rise in respiration, and climacteric fruit left attached to the parent plant ripen with a pronounced lower rate of respiration than detached fruit, even though the rise in ethylene production is similar in both cases. The climacteric rise in respiration during ripening may be more an artifact of harvested fruit ripening than an integral function of fruit ripening.

The four major pathways of respiratory metabolism are (1) glycolysis, (2) the citric acid cycle, (3) electron transport, and (4) the pentose–phosphate shunt.

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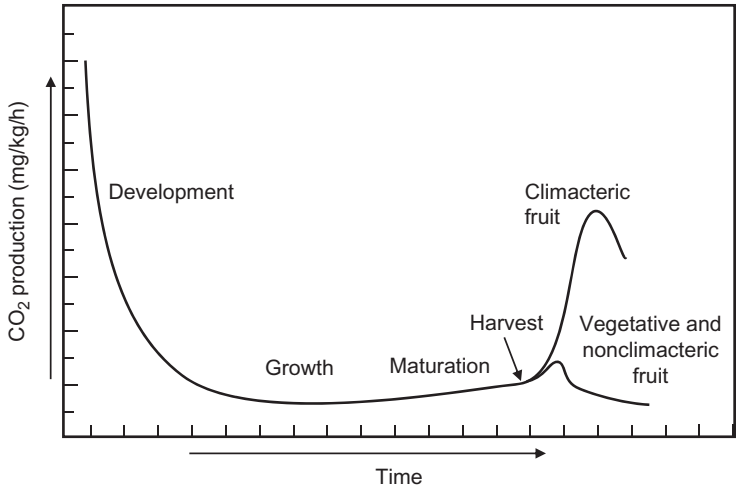

FIGURE 4.1
Change in respiration (CO_2 production) during the development, growth, maturation, and harvest of vegetative commodities and of climacteric and nonclimacteric fruit.

The pentose–phosphate shunt diverts material from glycolysis to the production of intermediates for specific synthetic reactions (Fig. 4.2). Unlike the many diverse and often unique reactions comprising secondary metabolism, these four pathways contain sets of reactions that are common to all vascular plants. The primary objective of postharvest research has been to maintain quality while extending the shelf-life of harvested horticultural commodities. These objectives have been best realized through control of the rate of respiratory metabolism.

4.2 CONTROL OF RESPIRATORY METABOLISM

Respiratory metabolism can be reduced and the shelf-life extended by lowering the temperature of harvested horticultural commodities. This has been the primary method used to retain quality since antiquity. Temperature is a measure of kinetic energy. Rapidly moving (or vibrating) molecules have higher energy; that is they are warmer than slower-moving molecules. Heat moves from warm to cold environments by conduction, convection, and radiation. Conduction moves heat from the interior of a warm commodity to its surface through intervening tissue. At the surface of the commodity, moving cold liquids (i.e., air or water) remove the heat by convection.

All bodies radiate energy. You can feel the energy being radiated by an extremely hot body like the sun or a fire. The rate of heat movement by radiation is proportional to the fourth power of the temperature difference between the radiating surface and a nearby cold surface. Therefore, the amount of energy lost by radiation approaches zero as the temperature of a commodity

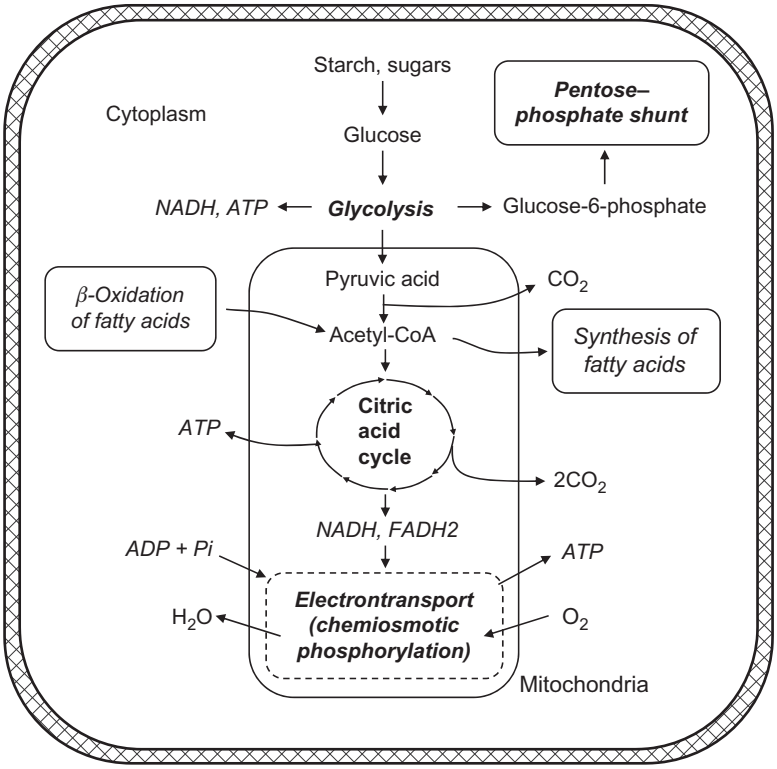

FIGURE 4.2

Overview of respiratory metabolism showing the relationship among the four major pathways (glycolysis, citric acid cycle, electron transport, and the pentose—phosphate shunt), where the reactions comprising these pathways are located in the cell, and some of the products derived from these pathways. The cellular components are not drawn to scale.

decreases to common storage temperatures. An exception is the heating of harvested commodities exposed to direct sunlight.

4.2.1 Temperature

The energy that must be removed from a harvested commodity is comprised of field heat (because of its warm temperature when harvested) and vital heat (due to ongoing respiration). This energy can be removed from the commodity by a cold medium circulating around the commodity (e.g., air in room or forced-air cooling, and water in hydrocooling), or by ice in contact with the commodity. Energy can also be removed by the evaporation of water from the surface of the commodity during vacuum cooling.

Each of these methods is particularly useful for specific crops. For example, forced air is used with commodities that do not tolerate being wetted (e.g., flowers, berries), hydrocooling is used with commodities that tolerate being wetted (e.g., sweetcorn, melons), and vacuum cooling is appropriate for

commodities with a large surface area (e.g., lettuce). Because of its ease of operation, forced air is the most commonly used method to remove field heat. To realize the maximum benefit of cooling, it should be done as soon as possible after harvest. Delaying cooling for even a few hours can drastically shorten the shelf-life of rapidly respiring crops like asparagus, broccoli, and strawberries.

As a rule of thumb, the rate of respiration is halved and the shelf-life doubled for every 10°C reduction in temperature. This level of reduction is referred to as having a respiratory quotient (RQ) (Q_{10}) of 2. However, Q_{10} values vary among specific commodities, during their development and storage, and for different temperature intervals. For example, harvested broccoli has a respiratory rate of about 20 mg CO_2/kg/h at 0°C and 80 at 10°C giving it a Q_{10} of 4 (80/20) between 0°C and 10°C, while the rate at 20°C is 300 mg/kg/h giving it a Q_{10} of 3.8 (300/80) between 10°C and 20°C. In contrast, harvested turnips have a Q_{10} of 2.1 between 0°C and 10°C and 1.5 between 10°C and 20°C. Most commodities that are natural storage organs (e.g., carrots, potatoes, turnips) have lower rates of respiration and lower Q_{10} values, while commodities that are rapidly developing after harvest (e.g., asparagus, broccoli) have higher rates of respiration and higher Q_{10} values. In both cases, lowering the commodity's temperature will maintain its quality and prolong its shelf-life.

4.2.2 Altered Gaseous Atmospheres

Respiratory metabolism can also be reduced by restricting the availability of oxygen to the tissue. Air contains 78% N_2, 21% O_2, 0.9% Ar, 0.04% CO_2, and 0.06% of an assortment of other gases. Water vapor is also part of the atmosphere. Most harvested commodities should be stored under high ($>90\%$) relative humidity (RH). A cubic meter (10^6 L) of 90% RH air at 0°C contains 4.52 g of water. That amount of water as a vapor occupies a volume of 5.6 L or 0.00056% of the total volume. Although water vapor is not a major component of the atmosphere, it exerts a significant effect on the rate of water loss from harvested commodities.

4.2.2.1 CONTROLLED AND MODIFIED ATMOSPHERE STORAGE

If the composition of the atmosphere around a commodity is actively regulated it is called controlled atmosphere (CA) storage, whereas if the concentration of the atmosphere is not actively controlled it is called modified atmosphere (MA) storage. If the package holding the commodity is designed to reduce gas exchange between the commodity and the surrounding atmosphere it is called modified atmosphere packaging (MAP). MAP relies on a delicate balance between the respiratory rate of the enclosed commodity and the diffusive properties of the package. Each must be within a narrow range for the desired atmosphere to be created and maintained within the package. The inherent variability in the respiratory rate of the commodity (e.g., resulting from differences in cultivar, growing conditions, maturity, ripening, and prior stress), and

the inability to maintain the temperature within a narrow range during transport and marketing have limited the use of MAP to a few products. Various attempts to design "smart" packages that overcome these problems have met with limited success because of their complexity and expense.

The biological activity of a gas is dependent on its concentration in the cytoplasm. Gas solubility is affected by the cell's temperature and solute concentration. The amount of gas in the cytoplasm is also governed by the partial pressure of the gas in contact with the liquid portion of the cell. For example, at atmospheric pressure (i.e., 101 kPa) the 21% concentration of O_2 in air has a partial pressure of 0.21 atmospheres or 21 kPa. The storage life of many commodities is lengthened when they are stored in an atmosphere containing 2% O_2 (i.e., O_2 at a partial pressure of 2 kPa). This atmosphere can be made by diluting 1 volume of air (21% O_2) with 9 volumes of nitrogen to create 10 volumes of 2% O_2.

4.2.2.2 LOWER PRESSURE STORAGE

A partial pressure of 2 kPa O_2 can also be created by lowering the pressure of the air surrounding the commodity to 0.1 atmospheres (10 kPa). This is termed low-pressure (LP) storage. An added advantage of LP storage is that the diffusion of gases is much higher at low pressures so the movement of gas into and out of the commodity is much faster and the likelihood of developing anaerobic conditions within the center of a bulky commodity in a low O_2 environment is greatly reduced. However, problems with excessive water loss may occur during LP storage since the diffusion of water vapor from the commodity would also be enhanced. The main impediment limiting the use of LP storage has been the expense of constructing containers that can withstand the vacuum necessary for LP to be effective. While vacuum chambers are commonly used for the vacuum cooling of lettuce, their cost is spread over the many loads they cool per day, and they can be moved to different production areas during the season, further amortizing their initial cost.

Increasing the concentration of CO_2 within the commodity also prolongs the storage life of some commodities by a combination of reducing respiration, reducing the biological activity of ethylene, and inhibiting microbial growth. However, not all commodities tolerate the often high CO_2 concentrations needed for these benefits to be realized.

4.3 MEASURING RESPIRATION

The many and varied reactions taking place in respiratory metabolism are often difficult to measure individually. However, since the rate of respiratory metabolism is tightly coupled to the rate of respiration (i.e., the production of high-energy intermediates like ATP and NADH from the oxidation of glucose) measuring the production of CO_2 or the consumption O_2 by the commodity will give a good approximation of the rate of other metabolic processes. Under the aerobic respiration of glucose, one molecule of O_2 is consumed for

every molecule of CO_2 produced. So either O_2 consumption or CO_2 production could be used to measure the rate of respiration. Since the background concentration of O_2 is around 21%, while that of CO_2 is around 0.04%, a small percentage error involved in measuring a change in O_2 concentration would be much greater than it would be in measuring a change in CO_2 concentration. For example, the rate of respiration of a commodity in a closed container that would lower the O_2 concentration from 21.0% to 20.9% (around a 0.5% reduction), would increase the concentration of CO_2 from 0.04% to 0.14% (around a 350% increase). Given the inherent variability in biological material, and errors associated with these types of measurements, a change of 0.1% against a background of 21% (a 0.5% change) would be much harder to detect as significant, than would a change of 0.1% against a background of 0.04% (a 350% change).

Measuring changes in dry weight (e.g., loss of substrate, like starch) or energy production (heat loss during the production of ATP) is appropriate in some situations. Dry weight loss can be found by periodically drying a sample of the commodity and calculating the rate of loss. Energy production can be found by enclosing a portion of tissue in a calorimeter and measuring the heat produced. However, unlike measuring gases evolved (CO_2, C_2H_4) or consumed (O_2) by the commodity, neither of these are nondestructive measurements. Dry weight loss can be calculated by multiplying the weight of CO_2 lost from the commodity by 0.68 (the ratio of the molecular weight of glucose (180) divided by the weight of the lost CO_2 ($6 \times 44 = 264$)).

The cost, robustness, ease of use, and rapidity of different measurement techniques often have a pronounced influence on which method is used. The predominant instrument used to measure respiration is the infrared CO_2 analyzer. Such analyzers range in cost from $400 for commercial units designed to control gas levels in greenhouses and office buildings to tens of thousands of dollars for laboratory dual-beam analytical instruments.

4.3.1 The Static System

The rate of CO_2 production and/or O_2 consumption can be measured using a static or flow-through setup (Fig. 4.3). In the static setup, a commodity of known weight is enclosed in a rigid container of known volume for a specified time. Respiration will consume O_2 in the container lowering its concentration, and will produce CO_2, raising its concentration. Samples of the gas in the container are taken at the beginning and end of the sampling period and analyzed. The change in concentration is multiplied by the volume of the container and the result is divided by the length of the sampling period and the fresh weight of the sample. It is important to know the exact volume of the container and to seal the container so no leaks occur.

For CO_2 the formula is: ((Final minus initial CO_2 level) $\times$ (0.01 if concentration is in percent) $\times$ (volume of the container))/((fresh weight of the commodity) $\times$ (length of the sampling period)) = mL CO_2/kg/h. For example,

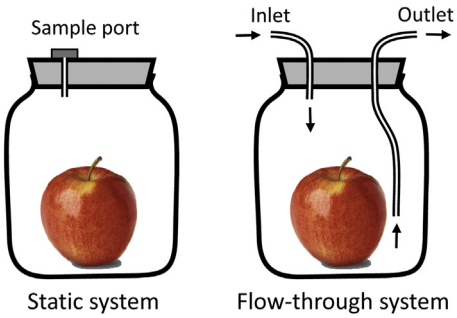

FIGURE 4.3

Setup for measuring respiration using a static or a flow-through system. In the static system, the commodity (e.g., apple) is sealed within a rigid container. Gas samples are periodically taken through the sample port. In the flow-through system there is a flow of gas at a known rate through the rigid container. Gas samples are taken from the inlet and outlet streams of gas after the system has come to equilibrium.

a 100 g apple at 20°C increased the CO_2 concentration in a closed 400 mL jar from 0.04% to 0.14% in 18 min. The CO_2 concentration increased 0.10%, so 0.4 mL of CO_2 was produced by the apple in the 400 mL container ($0.1 \times 0.01 \times 400 = 0.4$). The apple weighed 0.1 kg and the sampling period was 0.3 h, so dividing 0.04 mL by 0.1 kg and 0.3 h gives a production rate of 13.3 mL/kg/h at 20°C.

Since gases expand and contract with an increase or decrease in temperature, rates of respiration are often given in mg of CO_2 instead of in mL; as was done in the example given above for Q_{10} values. At 0°C, 10°C, and 20°C, 1 mL of CO_2 weighs 1.96, 1.89, and 1.83 mg, respectively. Applying this factor changes the rate of respiration from the apple at 20°C from 13.3 to 24.3 mg/kg/h. Similar changes would be needed to convert the consumption of O_2 in this closed system from milliliter to milligram. What would the O_2 concentration be in the gas sample? It would be 20.9%.

In both setups, the concentration of CO_2 should not be allowed to rise high enough, nor the concentration of O_2 to fall low enough, to significantly alter the rate of respiration. This is more of a concern with levels of CO_2, since CO_2 levels above 0.2% can alter rates of respiration, while levels of O_2 must fall below around 7% to have a significant effect on respiration (Fig. 4.4). In the static system, CO_2 accumulates linearly until it reaches a concentration that affects the rate of respiration ($\sim$ 0.2% in Fig. 4.4). At this point the rate of accumulation starts to deviate from linearity; it usually declines, but in some tissues the high CO_2 concentration causes damage that increases the rate of respiration.

In the previous calculation, no correction was made for the volume of the container taken up by the commodity. Apples have a density of about 0.9 g/mL, so the 100 g apple would have an external volume of 111 mL (100/0.9) and

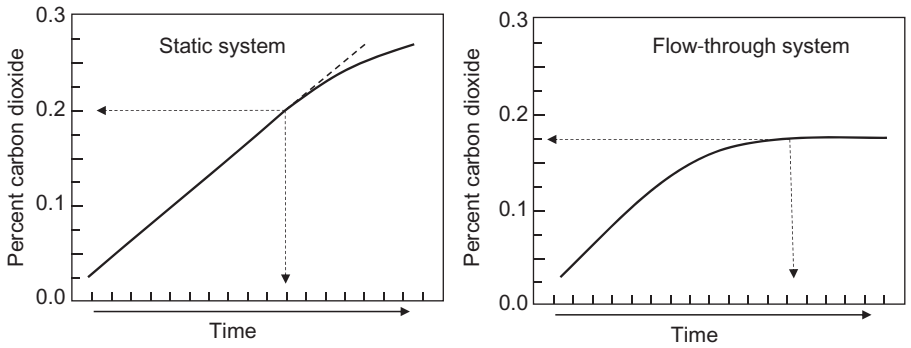

FIGURE 4.4

Graphs showing the concentration of CO_2 within a static and a flow-through system over time. The concentration increases linearly in the static system until it reaches a level that affects the rate of respiration. In a flow-through system, the concentration increases until equilibrium is reached between the amount of CO_2 produced by the commodity and the amount removed from the container in the flow of gas.

the void volume of the container would be 289 mL instead of 400 mL (400 − 111). Far less CO_2 (only 0.29 mL) would have been required to raise its concentration from 0.04% to 0.14% in the 289 mL volume compared to the 400 mL volume. The newly calculated production rate would be 9.6 mL CO_2/kg/h (17.6 mg/kg/h). However, this correction is not necessary because the very high solubility of CO_2 in water (1713, 1194, and 878 mL/L at 0, 10, and 20°C, respectively) means that the aqueous volume of the commodity can be ignored in these calculations. At 10°C 1194 mL of CO_2 will dissolve in water, above which is an atmosphere of pure CO_2 (100% CO_2 at 101 kPa). The amount of CO_2 dissolved will be proportional to the partial pressure of CO_2 over the liquid. That means that a molecule of CO_2 is equally likely to dissolve in the cell solution as to be partitioned into the surrounding gases. Only a minor error (<5%) will be introduced into the calculations by using the whole container volume; it is not necessary to subtract the volume of the commodity from the container volume.

The same caveat does not apply to O_2 because of its much lower solubility in water (49, 38, and 31 mL/L at 0, 10, and 20°C, respectively). When measuring O_2 consumption using the static system, the volume of the commodity should be subtracted from the container volume to get the appropriate void volume.

4.3.2 The Flow-Through System

While simple to set up and use, the static system does not lend itself for use in long-term studies or studies employing atmospheres of modified composition. In those cases, a flow-through system is preferred. The commodity is again enclosed in a rigid container, but instead of it being sealed shut, a flow of gas is directed through the container. The flow leaving the container will be diminished in O_2 and increased in CO_2 (and in other gases like ethylene produced by the commodity). After a period of time greater than three times the

volume of the container divided by the flow rate, an equilibrium will be reached in which the amount of CO_2 exiting the container will equal the amount of CO_2 produced by the commodity (this assumes complete mixing of gases within the container). For example, a 900 mL container holding 400 g of apples will have a void volume of 456 mL (900−(400/0.9)). At a flow rate of 100 mL/min, it would take 14 min for this setup to come to equilibrium ((456 mL × 3)/100 mL/min). At that time, samples could be taken from the inlet and outlet and analyzed. The difference in concentration times the flow rate will give the amount of CO_2 produced in the time interval. For example, there would be a 0.1% difference if the inlet and outlet levels were 0.04% and 0.14% CO_2, respectively. In an hour, the 100 mL/min flow would have carried away 6.0 mL of CO_2 ($0.1 \times 0.01 \times 100 \times 60$), and dividing that by 0.4 kg gives a production of 15 mL CO_2/kg/h.

Unlike in the static system, it is not critical to know the exact volume of the container or to seal it to prevent all leaks. A larger container will just take a longer time to come to equilibrium, and small leaks are not important if the internal gas is thoroughly mixed. However, it is crucial to know the exact flow rate.

4.4 RESPIRATORY QUOTIENT

The three main substrates for respiration in harvested horticultural commodities are glucose, organic acids, and fatty acids. Proteins (i.e., amino acids) are usually a very minor component of respiration in harvested horticultural commodities. In aerobic respiration, O_2 is the terminal electron acceptor and the substrates are oxidized to CO_2 and water. However, as pointed out earlier, respiration also produces many simple carbon compounds that participate in the subsequent reaction. RQ is the ratio of CO_2 produced to O_2 consumed. Carbohydrates have an RQ of 1.0, fatty acids have an RQ of 0.7, and organic acids have an RQ of 1.3. In anaerobic respiration CO_2 is produced but no O_2 is consumed so the RQ approaches infinity.

The complete oxidation of glucose ($C_6H_{12}O_6$) has an RQ of 1.0 ($6CO_2$ produced divided by $6O_2$ consumed).

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

Organic acids are synthesized by the partial oxidization of glucose (i.e., more O_2 was consumed than CO_2 produced during their synthesis), so they contain more O_2 than carbohydrates and therefore more CO_2 is produced per O_2 consumed during their subsequent complete oxidation (RQ > 1.0). The incomplete oxidation of glucose to form the organic acid malic acid ($C_4H_6O_5$) has an RQ of 0.66 ($2CO_2/3O_2$).

$$C_6H_{12}O_6 + 3\ O_2 \rightarrow C_4H_6O_5 + 2\ CO_2 + 3\ H_2O$$

The complete oxidation of malic acid ($C_4H_6O_5$) has an RQ of 1.33 ($4CO_2/3O_2$).

$$C_4H_6O_5 + 3\ O_2 \rightarrow 4\ CO_2 + 3\ H_2O$$

Therefore, the RQ for the complete oxidation of glucose through the intermediate of malic acid is still 1.0 ((0.66 + 1.33)/2).

In contrast, fatty acids are partially reduced during their synthesis, so they contain far less O_2 per carbon than carbohydrates, and therefore more CO_2 will be produced per O_2 consumed during their subsequent oxidation (RQ < 1.0). A stoichiometric balanced equation for the synthesis of the fatty acid palmitic acid ($C_{16}H_{32}O_2$) from glucose shows how little O_2 would have been consumed during its synthesis.

$$4\ C_6H_{12}O_6 + O_2 \rightarrow C_{16}H_{32}O_2 + 8\ CO_2 + 8\ H_2O$$

But this is an illusion since the synthesis of the 16-carbon palmitic acid requires 16 NADPH and eight 2-carbon acetate groups. The synthesis of fatty acids is very energy-intensive, and O_2 is consumed in other reactions that are needed to produce the energy needed to create the reducing power and transport intermediate substrates (e.g., pyruvate, acetyl-CoA) within the cell, and to drive subsequent synthetic reactions. The route from glucose through palmitic acid is very circuitous.

The complete oxidation of palmitic acid ($C_{16}H_{32}O_2$) has an RQ of 0.70 ($16CO_2/23O_2$).

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$

Because of their diverse structures and biosynthetic origin, no single RQ can be given for the oxidation of all proteins. However, the amino acids comprising proteins have the general structure $NH_2COOHCH_2$. Excluding the nitrogen, the formula becomes $C_2H_5O_2$ and the complete oxidation of a simple amino acid such as alanine has an RQ of 0.89 ($8CO_2/9O_2$).

$$4\ C_2H_5O_2 + 9\ O_2 \rightarrow 8\ CO_2 + 10\ H_2O$$

4.5 PRIMARY REACTION OF RESPIRATORY METABOLISM

The four primary pathways in respiratory metabolism are: (1) glycolysis, (2) citric acid cycle, (3) electron transport, and (4) pentose–phosphate shunt (Fig. 4.4).

4.5.1 Glycolysis

As its name implies, the set of 10 enzyme-coupled reactions in glycolysis splits or lyses the 6-carbon glucose molecule into two 3-carbon fragments. Glucose is a monosaccharide or simple sugar that usually occurs in small amounts in a cell. It occurs in much higher levels as a disaccharide such as sucrose, or in

polymerized forms such as starch. Glycolysis starts with an isomerase reaction rearranging the 6-carbon glucose molecule into a 6-carbon fructose molecule. Next, two reactions add phosphate from two ATPs to form 1,6-fructose diphosphate. This energized molecule is then split into two 3-carbon molecules that undergo an isomerization to form two 3-carbon molecules of pyruvate after five additional enzyme-coupled reactions. This essentially anaerobic series of reactions initially consumes two ATPs, but produces 4 ATPs and 2 NADHs. About 20% of the energy in glucose is captured in glycolysis. This pathway, which occurs in the cytoplasm, consumes no O_2 and produces no CO_2, but they do rely on NAD^+ being available.

4.5.2 Pentose–Phosphate Shunt

Most carbohydrates have an even number of carbons (the majority of carbohydrates are hexoses: 6-carbon sugars). However, both RNA and DNA have backbones made of pentoses (i.e., the 5-carbon sugars, ribose and deoxyribose). These 5-carbon sugars are synthesized from glucose-6-phosphate in the pentose–phosphate pathway. The many interrelated reactions also produce 4-carbon sugars, 7-carbon sugars, and NADPH. In the initial oxidative part of the pathway, two molecules of NADPH and one molecule of CO_2 are produced during the synthesis of ribulose-5-phosphate. In the final anabolic part of the pathway, other compounds are produced during rearrangements of the molecules. Most steps in these reactions take place in plastids.

4.5.3 Acetyl-Coenzyme A

Before entering the citric acid cycle, the product of glycolysis, the 3-carbon pyruvate, is decarboxylated (a CO_2 is removed and a NADH is produced) resulting in a 2-carbon acetate fragment (CH_3CO-) that is coupled to coenzyme A forming acetyl-CoA. This compound is composed of the nucleotide adenine linked to a ribose, which is linked through two phosphate atoms to pantothenic acid (vitamin B_5) which links to the acetyl fragment through a thioester bond. Because of the thioester bond and the two phosphate atoms, the resulting electron configuration in coenzyme A makes it relatively easy to add or remove the acetyl unit. Acetyl-CoA is used to transfer the 2-carbon acetate group in many reactions. It is used in the synthesis of fatty acids and is produced during β-oxidation of fatty acids. It is involved in the synthesis of the 5-carbon isoprenoid unit that is important in the synthesis of many molecules of secondary metabolism. It is produced during the oxidation of ethanol, ketones, amino acids, and of course pyruvate.

4.5.4 Synthesis of Fatty Acids

Lipids are composed of one or more fatty acids. Diglycerides are composed of two fatty acids linked by a molecule of glycerol, whereas triglycerides contain three fatty acids linked by glycerol. If all the bonds between the carbon atoms making up the fatty acid's backbone are single, the fatty acid is saturated,

whereas if some of them are double bonds the fatty acid is mono-, di-, or polyunsaturated. Saturated fatty acids have a higher melting point than unsaturated fatty acids and are linear in structure (Fig. 4.5). In contrast, double bonds produce a bend in the molecule so that it occupies a greater volume.

When incorporated in the diglyceride phospholipids that make up the bulk of cellular membranes, the degree of saturation of the component fatty acids exerts a significant effect on the membrane's fluidity. The fatty acid composition of the cell's membranes is modified during daily temperature fluctuations to maintain a constant fluidity in cellular membranes. Such changes can affect the tissue's chilling sensitivity by altering the membrane's phase transition temperature.

Fatty acids are synthesized in the cytosol and organelles by the sequential addition of the 2-carbon acetyl group from acetyl-CoA, so almost all fatty acids contain an even number of carbon atoms; 16 and 18 being the most common. Because of their chemical reactivity, most fatty acids are quickly processed after their synthesis to form monoglycerides, diglycerides, triglycerides, phospholipids (a significant component of cellular membranes), fats, and waxes (found in the cuticle). Because of their reduced state, fatty acids are an excellent store of energy. They are found as triglycerides (oils) in many seeds (e.g., cotton, rapeseed, safflower, and sunflower).

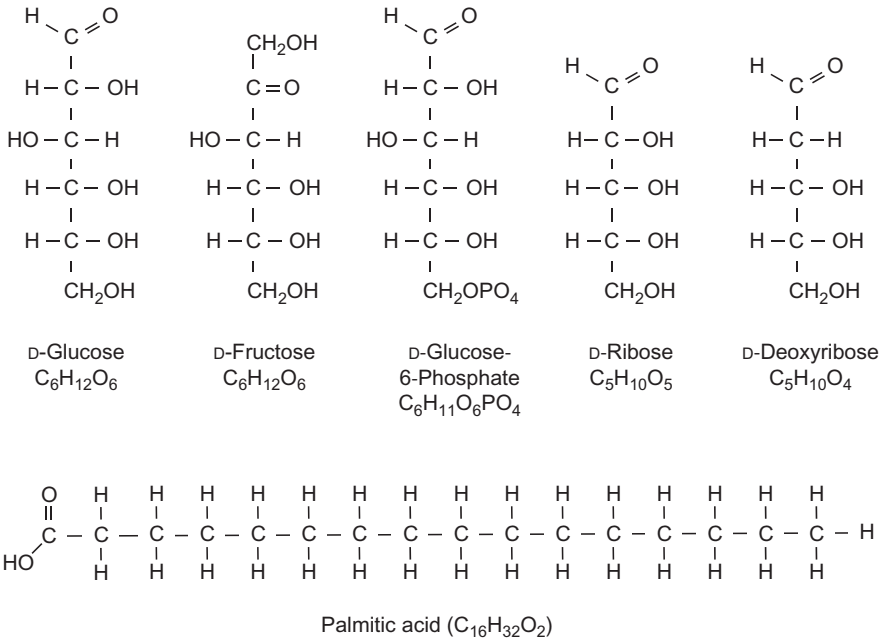

FIGURE 4.5
Linear structures of some important molecules found in respiratory metabolism.

Unlike the synthesis or organic acids (e.g., citric, malate) which are products of oxidative pathways (e.g., glycolysis and the citric acid cycle) fatty acids are synthesized by a reductive pathway. The reducing agent during fatty synthesis is NADPH (a reductase enzyme adds an H^+ from NADPH to a molecule forming $NADP^+$), while NAD^+ is the oxidizing agent (a dehydrogenase enzyme removes an H^+ from the molecule and adds it to NAD^+ forming NADH) in β-oxidation. In general, NADPH is consumed during biosynthetic reactions, whereas NADH is generated by energy-yielding reactions (e.g., the citric acid cycle).

4.5.5 Anaerobic Respiration

Without the regeneration of NAD^+ from NADH in electron transport (i.e., chemiosmotic phosphorylation) glycolysis would cease and there would be insufficient energy (e.g., ATP) to maintain the cell. This problem is overcome in anaerobic cells by the production of NAD^+ during the synthesis of lactic acid and/or ethanol from pyruvic acid (Fig. 4.6). In a nonacidic cell, lactate dehydrogenase uses NADH to convert pyruvic acid to lactic acid with the production of NAD^+. The accumulation of lactic acid acidifies the cell. In acidic cells, pyruvic decarboxylase is active and removes a CO_2 from pyruvic acid,

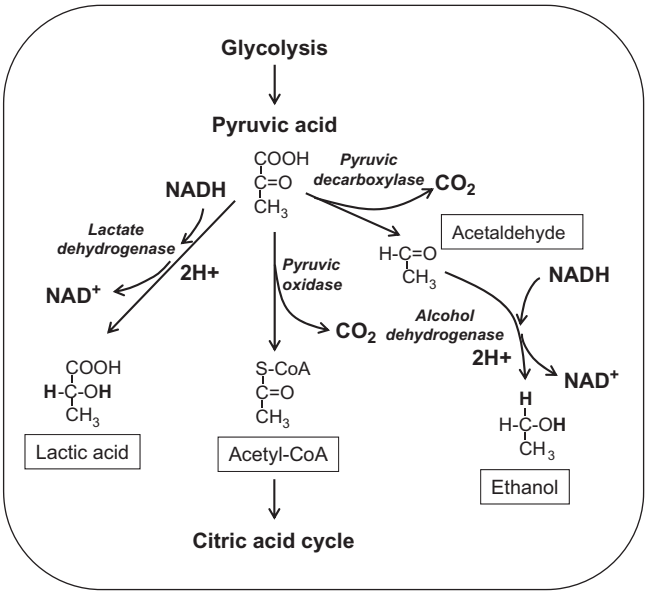

FIGURE 4.6

Fate of pyruvic acid under aerobic (acetyl-CoA produced) and anaerobic (lactic acid, and acetaldehyde and ethanol produced) respiration. Molecular structure and the regeneration of NAD^+ from NADH during anaerobic respiration. *Redrawn from Saltveit, M.E., 2016a. Respiratory metabolism. In: Gross, K.C., Wang, C.Y., Saltveit, M. (Eds.), Agricultural Handbook 66—The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. USDA Agricultural Research Service. Available from the National Technical Information Service, Springfield, VA, or at http://www.ba.ars.usda.gov/hb66/contents.html; Saltveit, M.E., 2016b. Respiratory metabolism. In: Sunil (Ed.), Postharvest Ripening Physiology of Crops. CRC Press. ISBN 9781498703802.*

converting it to acetaldehyde, which is quickly used by alcohol dehydrogenase to form ethanol with the production of NAD^+. These two anaerobic reactions allow glycolysis to proceed in the absence of O_2, however, glycolysis only captures about 20% of the energy in a glucose molecule and the accumulation of too much lactic acid or ethanol can be toxic to the cell.

4.5.6 Citric Acid Cycle

The starting point of the citric acid cycle (also called the Krebs cycle, or the tricarboxylic acid cycle) is the addition of the 2-carbon acetyl group from acetyl-CoA to the 4-carbon oxaloacetate to form the 6-carbon, 3-carboxyl molecule citric acid. This completes the cycle by regenerating citric acid from oxaloacetate, which is at the end of the cycle. The two carbons in the transferred acetate are eliminated as two CO_2 during the cycle's regeneration of oxaloacetate. During this cycle, one ATP, one FADH2, and three NADH are produced from each pyruvate. The diversion of cycle intermediates provides the substrates for many other synthetic reactions.

For example, acetyl-CoA is used in the synthesis of fatty acids, cuticular compounds, isoprenoids, carotenoids, sterols, terpenes, and aromatic amino acids. α-Ketoglutaric acid is used in the synthesis of glutamic acid, other amino acids, chlorophyll, cytochromes, and phytochrome. Oxaloacetic acid is used in the synthesis of aspartic acid, alkaloids, and nucleic acids. The organic acids citric, succinate, and malic are also byproducts of the cycle. Citric acid can accumulate in citrus fruit (e.g., orange, lemon) and can easily enter the cycle. Malic acid can accumulate (as in apple, i.e., *Malus domestica*) and can readily enter the cycle, or it can be converted to pyruvate. So far, all the carbons from glucose have been released as CO_2, but no O_2 has yet been consumed.

4.5.7 Chemiosmotic Phosphorylation (Aka Electron Transport)

The high-energy compounds produced by the citric acid cycle (NADH, FADH2) contain more energy than is needed for most cellular functions. Partitioning that energy into smaller packets (i.e., ATP) occurs in electron transport via chemiosmotic phosphorylation. The terminal acceptor of the high-energy electron is O_2, which accepts electrons and protons to become H_2O. While many enzymes are involved in this process, these enzymatic reactions do not produce ATP, but rather establish a proton gradient across the inner mitochondrial membrane. High-energy electrons from NADH and FADH2 flow through the electron transport chain and furnish the power to pump protons (H^+) from the matrix into the intermembrane space, establishing a proton gradient as evidenced by the development of an electrical potential across the membrane, and a difference in pH (around seven in the matrix and eight in the intermembrane space). ATP is produced from ADP and inorganic phosphate as the protons flow from the intermembrane space back to

the matrix through an ATP-ase complex. This chemiosmotic process is very similar to that used to produce ATP in the chloroplast during photosynthesis.

An alternate oxidase is induced in some plants during certain phases of development or during periods of stress. This cyanide-resistant respiratory pathway diverts electrons from the main electron transport chain, thereby reducing ATP formation and increasing the tissue's temperature because the energy that would have been captured in the formation of ATP is released as heat. While useful in assisting pollination in some thermogenic flowers, this seemingly wasteful process also helps regulate the rate of carbon flow through the citric acid cycle and limits the production of reactive oxygen species during stress.

4.6 FACTORS AFFECTING RESPIRATORY METABOLISM

Since respiratory metabolism furnishes both the energy and the substrates for a myriad of subsequent reactions, it is not surprising that a number of morphological and physiological changes are associated with changes in the rate and direction of respiratory metabolism. We have already discussed how external factors, such as temperature and atmospheric composition, can alter respiration. Internal factors, such as the specific commodity, the cultivar of the commodity, its stage of growth and development (e.g., cell division, cellular expansion, maturation, ripening, and senescence), and metabolic responses to stresses and injury can also have a pronounced effect on the rate of respiratory metabolism.

4.6.1 Maturity and Fruit Ripening

Young, rapidly growing tissues have a much higher rate of respiration than more mature tissues. Asparagus and broccoli are examples of commodities that have rapid rates of respiration and short shelf-lives. In contrast, commodities that are natural storage organs (e.g., bulb onions, carrots, potato, and winter squash) have lower rates of respiration and longer shelf-lives.

Fruits harvested before full maturity (e.g., cucumbers, string beans, and zucchini) usually have relatively high rates of respiration that slowly decline in storage. Mature fruit can exhibit two dissimilar patterns of respiration following harvest (Table 4.1). Some, called climacteric fruit (e.g., apples, avocados, bananas, some melons, pears, and tomatoes), have a pronounced increase in respiration during ripening after harvest, while others, called nonclimacteric fruit (e.g., citrus, strawberries), lack this rise and rather show a slow decline after recovering from the trauma of harvesting. However, the distinction between climacteric and nonclimacteric is not hard and fast, with varying intensities of the climacteric rise apparent in cultivars within a species.

Table 4.1 A List of Some Fruits That Are Classified as Having a Respiratory Climacteric Coincident With Ripening. Fruits That Do Not Exhibit a Rise in Respiration Coincident With Ripening Are Termed Nonclimacteric

Climacteric Fruits		Nonclimacteric Fruits	
Apple	Nectarine	Blueberry	Olive
Apricot	Papaya	Cacao	Orange
Avocado	Passion fruit	Cherry	Pepper
Banana	Peach	Cucumber	Pineapple
Breadfruit	Pear	Grape	Strawberry
Fig	Persimmon	Grapefruit	Tamarillo
Guava	Plum	Lemon	
Kiwifruit	Tomato	Lime	
Mango	Watermelon		
Muskmelon			

Climacteric fruit usually have extensive food reserves (e.g., starch) that are converted to sugars during ripening, while most nonclimacteric fruit lack theses extensive food reserves. Nonclimacteric fruit should be harvested at the peak of quality because very little improvement of quality is realized after harvest. In contrast, climacteric fruit can be harvested when mature, but far from acceptable levels of quality. For example, bananas and tomatoes can be harvested at the mature-green stage of development and ripened after transport to distant markets. The external application of ethylene is used in ripening rooms for both these fruit to hasten the color change, softening, and aroma development characteristic of high-quality ripe fruit.

Ethylene is a bioactive gas that is naturally produced by many plants to initiate and coordinate metabolic changes associated with responses to stress and developmental changes such as ripening. During ripening of climacteric fruit, a rapid rise in ethylene production and the resultant rise in internal ethylene concentrations within the commodity stimulates the many changes associated with ripening, including an increase in the rate of respiration. Exposure to ethylene also stimulates a rise in the respiration of vegetative tissue and nonclimacteric fruit, but the lack of a positive feedback of ethylene on ethylene synthesis in these commodities precludes the endogenous rise in ethylene that produces the rise in respiration in climacteric fruit. In fact, many nonclimacteric tissues exhibit a negative feedback wherein ethylene actually suppresses its further synthesis.

4.6.2 Biotic and Abiotic Stresses

Many biotic and abiotic stresses stimulate respiratory metabolism. Diseases (mainly caused by fungi) cause a rise in respiration as the plant mounts both a morphological and physiological response. Likewise, mechanical injury,

whether caused by chewing insects or the traumas of harvesting are met with both a morphological and physiological response. In lettuce, wounding stimulates respiratory metabolism that furnishes the energy and substrates necessary for the enhanced phenolic metabolism that is involved in wound repair and avoidance of further biotic injury. However, these same reactions foster the accumulation of phenolic compounds that can cause subsequent tissue browning.

While the plant has an impressive array of physiological and morphological responses to various stresses, the plant is often limited in what response is elicited by any specific stress. There appears to be a hierarchy in what response is elicited, with some responses taking precedence over others. This hierarchical response can be used to modify the plant's response to stress. For example, brief exposures to elevated temperatures elicit a heat-shock response in all living cells that entails the production of protective heat-shock proteins. When a heat shock is given before or soon after wounding (e.g., the production of fresh-cut lettuce) innocuous heat-shock proteins are produced instead of the proteins (i.e., enzymes) that contribute to tissue browning. The heat-shock treatment will be ineffective if the adverse effect (e.g., tissue browning) is the response of endogenous compounds (e.g., preformed phenolic compounds in apples and potatoes), and not the induced synthesis and accumulation of compounds. High-temperature treatments are used to disinfect harvested commodities of microorganism and insects by the lethal effect of the temperature on the pest.

4.6.3 Chilling and Freezing Temperatures

Temperature is the primary means to maintain quality after harvest, but not all harvest commodities respond similarly to low temperatures. Many commodities indigenous to tropical and subtropical regions (e.g., avocados, bananas, cucumbers, and tomatoes) suffer a physiological disorder called chilling injury when exposed to nonfreezing temperatures below about 10°C. The extent of injury depends on the temperature and duration of exposure. Some commodities are very sensitive (e.g., bananas) and suffer irreversible injury after a few hours, while other commodities can recover from a few days of chilling temperatures. This ability to recover is the basis of intermittent warming, where a chilling-sensitive commodity is held at a chilling temperature for a period of time shorter than that required to produce irreversible injury. The commodity is then warmed to a nonchilling temperature for a few days where it recovers before it is again returned to the chilling temperature. Cycling through these chilling and recovery phases can significantly extend the storage life. However, cooling and warming the commodity is expensive, and condensation of water on the cold commodity during warming can foster microbial growth.

Commodities are damaged if frozen. The solute concentration in plant cells lowers their freezing point (i.e., freezing-point depression) from a few tenths

of a degree (e.g., in tissue with few solutes like avocados and lettuce) to a few degrees (e.g., in tissue with high levels of solutes like artichokes, figs, and pomegranates). Formation of ice crystals in the low solute solution in the cell wall draws water out of the cell and causes damage by dehydrating the cell. Respiratory metabolism is disrupted as the solvent in which all the reactions occur (i.e., water) is removed from the cell and its contents become concentrated.

Just as too low a temperature can cause damage by chilling injury or freezing, too high a temperature beyond the physiological range can cause damage. Near the thermal death point, metabolism becomes disorderly and enzyme proteins are denatured. Many tissues can tolerate high temperatures for short periods of time (e.g., a few minutes), and this property is used to advantage in quarantine treatments that kill insects and surface fungi. Continued exposure to high temperature results in phytotoxic symptoms and then complete tissue collapse.

The deleterious responses to some stresses can be mitigated by prior conditioning treatments. While not as severe as a heat shock treatment, holding chilling-sensitive commodities near their chilling temperature can condition them to be more chilling tolerant. For example, holding grapefruit at 16°C for 7 days reduced their level of injury when exposed to chilling temperatures (i.e., temperatures below 10°C) during storage.

4.7 CONCLUSION

The quality and storage life of harvested horticultural commodities has been increased by the judicious use of temperature and altered atmospheres. Commodities developed by plant breeders with enhanced disease resistance, yield, and quality were the basic materials postharvest physiologists had to work with. Often the postharvest characteristics of a commodity were of minor interest in comparison to these other qualities. The commercial application of postharvest technology still relies heavily on maintaining the "cold chain" from harvest to the consumer, with CA, MA, and MAP being only significant for a few products. However, many postharvest problems could be reduced through plant breeding. In the past, naturally or induced mutations were incorporated in breeding programs to alter a deleterious postharvest characteristic. For example, sweetcorn has a naturally occurring recessive mutation that limits the conversion of sugar to starch. Yet this conversion still occurs after harvest and it was crucial to rapidly cool the corn and keep it on ice to limit starch formation during marketing. Additional mutations were incorporated in newer cultivars that essentially eliminated this problem. Now only normal-temperature precautions are needed to prevent sweetcorn from turning starchy during marketing. Advances in the ability to directly target genes involved in specific metabolic pathways now allow plant breeders to modify many traits that can adversely affect the postharvest life of many horticultural commodities.

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CHAPTER 5

Biology and Biochemistry of Ethylene

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5.1 INTRODUCTION

Among the plant hormones, ethylene is unique for several reasons, mainly linked to its simple two-carbon gas chemical nature: C_2H_4. Both its structure and chemical state imply that if on one hand ethylene is normally synthesized in its site of action, on the other it can also diffuse and accumulate in localized areas, acting as a long-distance signaling molecule.

The identification of ethylene as a plant hormone is preceded by a long and surprising story of agricultural and cultural practices involving the unaware utilization of this hormone already more than 2,000 years ago, when in ancient China incense smoke was used to accelerate ripening in pears. We now know that the combustion of incense, which is derived from plants, generates the small hydrocarbon ethylene and that plants produce this hormone to promote fruit ripening. Ancient Egyptians used to gash figs, as pointed out by some archeological finds, to induce ethylene production and trigger ripening, even in the absence of the insect pollinator.

The history of ethylene research starts in 1896, when, at the Botanical Institute of St. Petersburg, Dimitry Neljubow observed that the growth of his pea seedlings was affected by the gas lamps that illuminated the laboratory. Dark-grown seedlings displayed a triple response of reduced elongation and radial swelling of the hypocotyl, short roots, and enhanced apical hook formation,

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allowing Neljubow to identify ethylene as the causal agent, and to establish that plants are able to sense this compound and respond with a well-defined biological effect. A few years later, in 1934, Richard Gane added another milestone to the history of this fascinating gas. He quantified the ethylene produced in one month by 30 kg of ripening apples and demonstrated definitely that plants are able to synthetize ethylene. Although the development of new methods of detection in the 1950s allowed to improve the knowledge of ethylene physiology, the biosynthetic pathway of this hormone was revealed only two decades later, by Shan Fa Yang (see Section 5.2.1). With the advent of molecular biology techniques and the adoption of *Arabidopsis thaliana* as a model plant, the whole of the perception and signaling mechanisms were definitely disclosed.

Several studies carried out in the following years, not only in model species but also in crops, greatly improved the understanding of the biological effects of this hormone. Ethylene plays either pivotal or accessory roles in many diverse processes, ranging from seed germination, adventitious root formation, interactions with microbes, and pathogen-mediated cell death, to leaf and flower senescence and abscission, responses to stress, up to fruit ripening and senescence. One of the most difficult aspects of ethylene research deals with the high specificity of its action in many of its roles. For example, ethylene inhibits cell elongation in the dark, whereas it can promote it in light or it stimulates elongation in plants grown in low-phosphorous conditions, but has an inhibitory role in high phosphorous. Moreover, to further complicate its study, ethylene has extensive synergistic, additive, and/or antagonistic interactions with the other phytohormones.

Despite the wide involvement of this gaseous hormone in several processes of a plant's life, the most important commercial potential of ethylene research deals with fruit ripening and the postharvest physiology, especially for the so-called climacteric fruits. This type of fruits, including, for example, tomato, apple, pear, peach, and banana, displays a ripening-related burst of both respiration (oxygen consumption and carbon dioxide production) and ethylene production, termed the "ethylene/respiratory climacteric." Conversely, nonclimacteric fruits (such as, e.g., citrus, grape, and strawberry) do not show such a climacteric rise, also keeping carbon dioxide and ethylene production at basal levels during ripening. When mature climacteric fruit are treated with exogenous ethylene, an acceleration of ripening occurs, with an anticipated respiratory climacteric rise and the upregulation of ethylene-dependent genes, including those responsible for the biosynthesis of the hormone resulting in the autocatalytic production (see Section 5.2). This physiological feature leads to an irreversible activation and promotion of ripening. Nonclimacteric fruits do not require ethylene action for normal ripening to take place, however they still maintain the ability to respond to exogenous ethylene through the upregulation of a subset of ripening

processes. In fact, when treated with exogenous ethylene they display some physiological responses (such as a temporary increase in respiration, degradation of chlorophyll) but they do not produce autocatalytic ethylene, thus the physiological effects are limited and dependent on the presence of the gas in the environment.

During ripening, ethylene stimulates its own biosynthesis and induces the expression of many genes associated with the processes characterizing the ripening syndrome. Some of these genes are involved in the synthesis of pigments, aromas, and flavors, sugar production from starch, and cell wall changes associated with fruit softening. Consequently, optimal conditions in the preharvest phase and remarkably during the postharvest (transport and storage) have significant implications for human health and wellbeing, as they may strongly affect the final quality of the fresh fruits.

A considerable effort has been dedicated to studying the role of ethylene in fruit development and ripening, and a huge amount of research is still ongoing on this topic, with significant impacts on the global agricultural marketplace. Researchers have tried to modulate ethylene biosynthesis and perception/response either to delay or block ripening, thus improving fruits' tolerance to storage and long-distance transport, or to accelerate ripening to anticipate harvesting. Different approaches have been adopted, either through chemical tools or genetic engineering, but all of them depend on a successful research strategy and the achievement of new knowledge about the regulation of ethylene biosynthesis, perception, and signal transduction. A detailed understanding of these aspects may also allow the exploration of the natural variability to identify interesting genotypes to be used to constitute new superior varieties.

5.2 ETHYLENE BIOSYNTHESIS, PERCEPTION, AND SIGNAL TRANSDUCTION

5.2.1 The Ethylene Biosynthetic Pathway: Yang's Cycle

The biosynthesis of ethylene began to be elucidated relatively late, not only because of the lack of technical tools able to accurately quantify ethylene, but also due to both the instability and the low active levels of the key enzymes involved in the last steps of the pathway. The first "eureka moment" (late 1950s) was the discovery that ethylene synthesis requires oxygen, implying that at least one enzyme with oxidase activity was needed. Later, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) was identified, opening the way to the following studies. ACC is synthesized by the enzyme ACS (ACC synthase) from the amino acid methionine, which is first converted to S-adenosyl-methionine. ACS, therefore, catalyzes the conversion of S-adenosyl-methionine to ACC and methylthioadenosine. In the presence of oxygen, ACC

is converted to ethylene, CO_2, and HCN, thanks to the action of the enzyme ACC oxidase (ACO). Methylthioadenosine resulting from ACC synthesis is used to regenerate methionine following the steps described in the so-called Yang's cycle (Fig. 5.1).

The accumulation of ACS represents the rate-limiting step of ethylene biosynthesis and this feature is used by plants to finely tune the levels of the hormone. This enzyme acts as a dimer and is encoded by a multigene family with the different members differentially expressed in diverse cell types, tissues, and

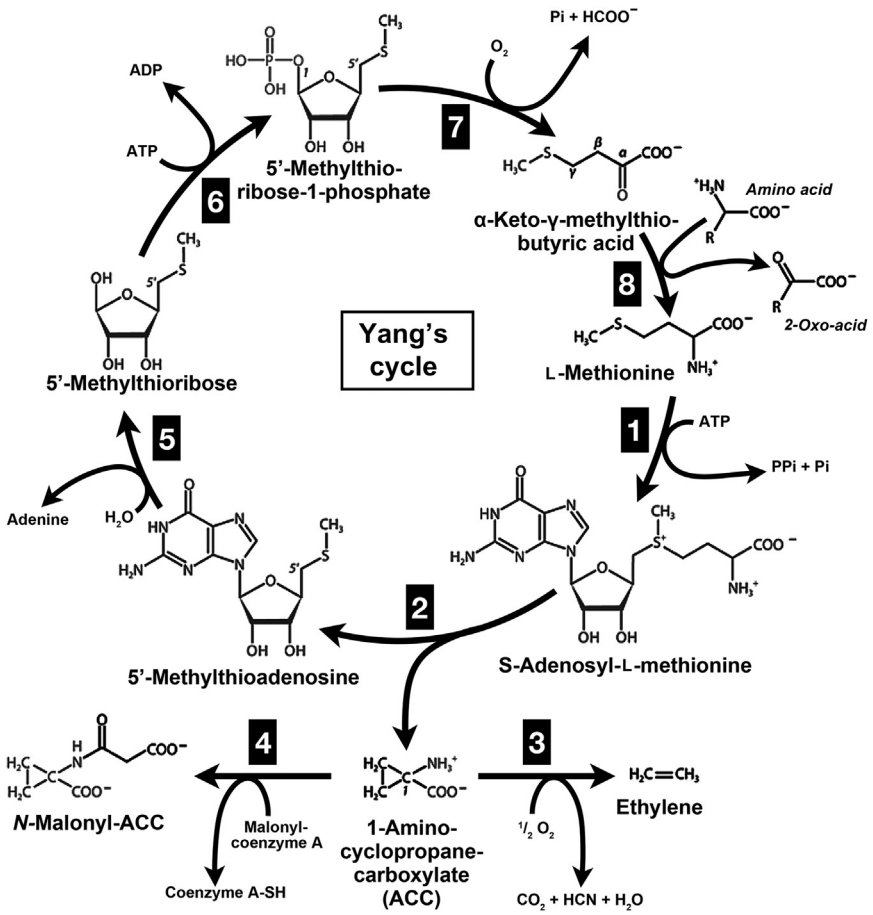

FIGURE 5.1

Yang's cycle of ethylene biosynthesis and regeneration of its precursors. The formation of S-adenosyl-methionine is catalyzed by S-adenosyl-methionine synthase (1) from methionine and ATP. Aminocyclopropane-carboxylate (ACC), the precursor of ethylene, is then produced by ACC synthase (ACS; 2) along with methylthioadenosine (MTA). ACC oxidase (ACO; 3) catalyzes the final step of ethylene biosynthesis, using ACC as a substrate and generating carbon dioxide and cyanuric acid as secondary products. MTA recycling allows a constant level of methionine, even under conditions of high ethylene production (steps 5–8) (5, MTA nucleosidase; 6, MTR kinase; 7, transaminase; 8, spontaneous reaction). ACC malonylation to malonyl-ACC deprives ethylene biosynthesis of ACC (ACC *N*-malonyl transferase; 4).

organs. Therefore, according to the ACS genes expressed and their levels of expression, their combination into a dimer may have different enzymatic activity and stability, thus determining the final levels of ethylene. This regulatory aspect becomes functionally relevant when the mode of ethylene biosynthesis shifts from autoinhibitory (the so-called "system 1" responsible for basal ethylene production before the climacteric burst) to autocatalytic ("system 2" responsible for climacteric ethylene production), thus allowing the climacteric burst. The change from system 1 to system 2 is allowed by the upregulation of specific ACS with a high catalytic activity and stability. Application of propylene (an analogue of ethylene) can trigger an increase in respiration in both climacteric and non-climacteric fruits but a propylene-mediated induction or rise in endogenous ethylene production occurs only in climacteric fruit, mainly due to the upregulation of the same ACS genes naturally induced during the system 1 to system 2 transition.

5.2.2 Ethylene Perception and Signal Transduction

The adoption of *Arabidopsis thaliana* as a model plant gave a significant acceleration to ethylene research, in particular regarding the studies on ethylene perception and signaling. The triple response represented a very rapid and easy way to screen for mutants with an impaired ethylene response, allowing the identification of the genes encoding its receptors several years before any plant genome was sequenced.

Ethylene is perceived by a family of receptors localized at the endoplasmic reticulum, with the ethylene binding site lying within the membrane and binding ethylene with the aid of a copper cofactor. Due to its chemical features, ethylene not only freely crosses cell membranes, but actually solubilizes and freely diffuses within, thus facilitating its binding to receptors. The first ethylene receptor, ETR1 (EThylene Resistant 1), was isolated and identified by Anthony Bleecker's group. The sequence of the corresponding gene is very similar to the two-component family of histidine protein kinase receptors that are highly prevalent in prokaryotes and rare in eukaryotes. ETR1 was the first protein unambiguously identified as a hormone receptor in plants. Its discovery was immediately followed by the identification of another receptor, ETR2, and the remaining three ethylene receptors in *Arabidopsis*.

The most important feature of the ethylene perception mechanism deals with the negative regulation exerted by the receptors, as confirmed by plants that are heterozygous for wild-type and dominant mutant alleles and show an ethylene-insensitive phenotype. In the absence of ethylene, wild-type receptors block the ethylene response and when bound to ethylene this block is released, thus triggering the response to the hormone. On the other hand, the mutated receptors keep the ethylene response blocked, even in the presence of the ligand.

Ethylene receptors can be divided into two families, according to their structural and functional characteristics. In *Arabidopsis*, subfamily I receptors (characterized by three membrane-spanning domains and intact HK domains) play a more prominent role in the ethylene response than those belonging to subfamily II (four membrane-spanning domains and degenerate HK domains), as demonstrated by loss-of-function mutants. Ethylene receptors function as dimers, but may also associate with additional proteins, such as the downstream components Constitutive Triple Response 1 (CTR1) and Ethylene INsensitive 2 (EIN2), or be affected by accessory mediators, such as Reversion To Ethylene sensitivity 1 (RTE1;Green-Ripe,GR, in tomato), the latter exerting a negative regulation on the perception of the hormone (Fig. 5.2).

The signaling pathway of ethylene downstream of the receptors has been elucidated and most of the components identified and assembled in the first draft of the ethylene signal transduction. The first two key elements are CTR1 (the name derives from the constitutive triple response of its mutant) and EIN2. The former is a negative regulator of the ethylene response and is active when bound to the free receptor. Its activity is due to an mitogen-activated protein kinase kinase kinase (MAPKKK) domain that is inactivated when ethylene binds its receptors. The following element, EIN2, is a positive regulator and its function was elucidated quite recently (2012) by three independent groups. EIN2 is normally phosphorylated by CTR1 in the absence of ethylene, to be then degraded through the proteasome. When CTR1 is inactivated by ethylene binding to receptors, EIN2 is stabilized and its C-terminal end cleaved, acting as a mobile signal to the nucleus (Fig. 5.3).

Although a link between the presence of the C-end of EIN2 in the nucleus and the following molecular events is still missing, the sequence of events leading to the final transcriptional response to ethylene is well established. In the presence of the hormone, EIN3 and EIL1 (EIN3-like 1) transcription factors are stable and positively regulate the expression of ethylene-related genes. In the

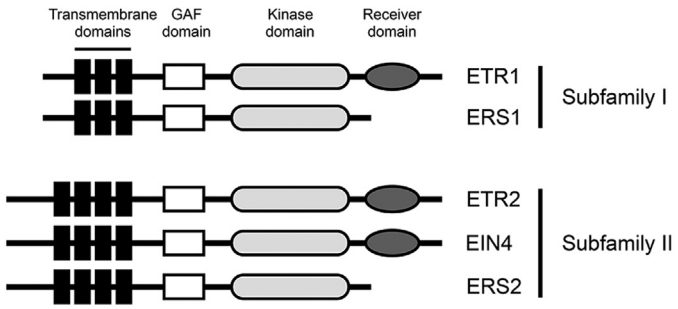

FIGURE 5.2

Ethylene receptors are encoded by a multigene family that can be grouped into two main subfamilies according to the number of hydrophobic domains at the N-terminal. Subfamily I includes EThylene Resistant 1 (ETR1) and Ethylene Response Sensor 1 (ERS1), while subfamily II comprises EThylene Resistant 2 (ETR2), Ethylene INsensitive 4 (EIN4), and Ethylene Response Sensor 2 (ERS2). The ERS receptors differ for the absence of the C-terminal receiver domain.

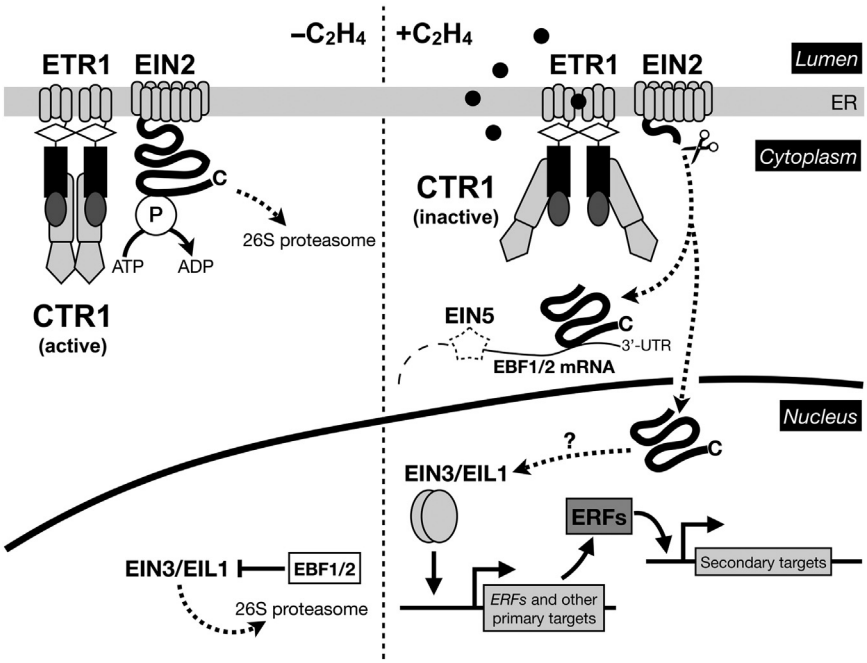

FIGURE 5.3

The ethylene signal transduction requires the involvement of several elements, among which CTR1, EIN2, EIN3, and the EIN-like (EIL) proteins are the most important. Constitutive triple response 1 (CTR1) is the first element downstream from the receptors, working as a negative effector that leads to proteasome-mediated degradation of EIN2. In the presence of ethylene, CTR1 is deactivated and the transduction pathway is unlocked, with the C-terminus of EIN2 that is cleaved, thus entering the nucleus to trigger ethylene response. EIN3 and EIL1 transcription factors are activated and bind the promoters of ethylene-responsive genes as a homodimer, thus promoting their transcription. Among these genes, the *ERFs* encode transcription factors that promote in turn the transcription of secondary target genes (described in Section 5.3), thus coordinating the downstream ethylene response. The levels of EIN3 are also regulated by 26S proteasome-mediated degradation due to targeting by specific F-boxes (EBF1/2) belonging to poly-ubiquitination complexes. The levels of EBF1/2 can in turn be regulated through posttranscriptional degradation (EIN5 ribonuclease) and translational inhibition (EIN2 C-end). *Modified from Ju, C., Yoon, G.M., Shemansky, J.M., Lin, D.Y, Ying, Z.I., Chang, J., et al., 2012. CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 109, 19486–19491. doi:10.1073/pnas.1214848109.*

absence of the hormone, both transcription factors can be recognized by two F-box proteins, EBF1 and EBF2, and proteolyzed. When ethylene levels increase, these two F-boxes cannot only be proteolyzed themselves, but the translation of their mRNAs can also be inhibited outside the nucleus thanks to the collaboration between the EIN2 C-end and the exoribonuclease EIN5.

The accumulation of stable EIN3 and EIL1 proteins initiates a transcriptional cascade that results in the activation and repression of hundreds of genes, thus determining a coordinated response to ethylene. The *ethylene response factors* (*ERFs*), belonging to the APETALA2/ethylene response element binding protein transcription factor family, represent the most important direct targets of EIN3/EIL1 transcription factors. This is suggested not only by the number

of genes included in this superfamily (147 in the model plant *Arabidopsis*), but also by the wide range of responses (detailed in Section 5.3) that are coordinated by these elements.

The complex regulatory network of ethylene signal transduction represents not only a well evolved mechanism, but also a way to interact with many other signaling pathways, involving either other hormones or exogenous signals. A detailed description of the ethylene biosynthetic route can be found in Chang and Williams (2012) and Wang et al. (2002), while a comprehensive evaluation of the mechanisms of ethylene perception and signal transduction is given by Ju and Chang (2015).

5.2.3 Ethylene Quantification

Ethylene's story is strictly linked to the technological progress made in the methods for its quantification. Advancements in ethylene research during the 1950s and in the following decades were possible thanks to the development of gas chromatographic (GC) techniques, which currently still represent the most common tool adopted by scientists to quantify ethylene production in plants. In practical terms, whole plants or, more often, their detached organs (i.e., the fruits) are normally incubated within sealed containers of suitable volume, in order to allow the volatile gas emitted by the sample to

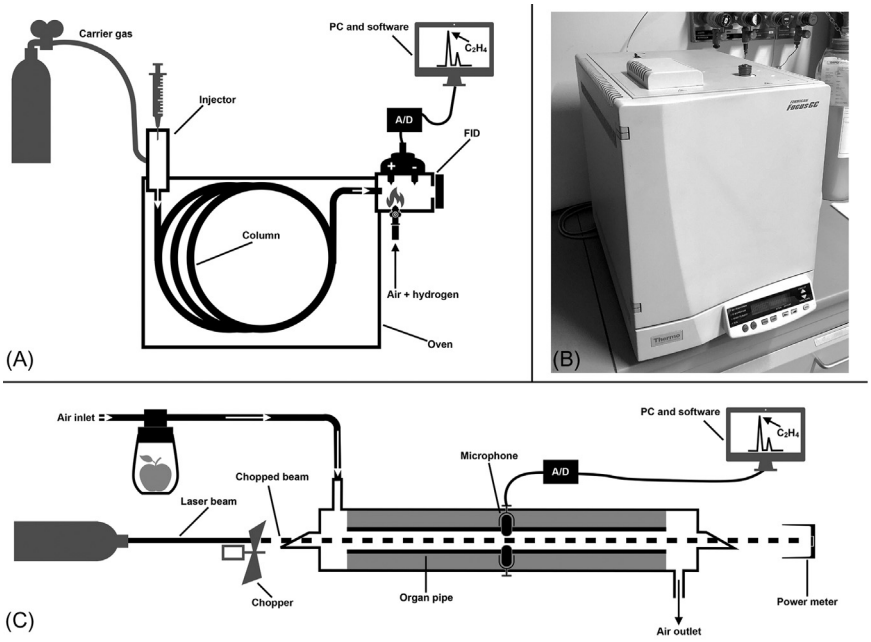

FIGURE 5.4

Methods for ethylene quantification. Schematic representation of the main elements of a gas chromatograph (GC) (A) and a common GC used in a laboratory (B). Schematic representation of the laser photoacoustic detection system (C). See text for details.

accumulate in the so-called "headspace." The headspace gas is then withdrawn with a syringe and injected into the GC.

A GC is mainly composed by three elements, whose temperature is strictly controlled to meet the quantification requirements: (1) injector; (2) column (within an oven); and (3) detector (Fig. 5.4).

The flow of the sample from the injector through the column is guaranteed by a carrier gas (i.e., air or an inert gas, such as helium). The different volatiles, among which ethylene, are separated within the column and finally elute at different times. The exact elution time of ethylene is established with a standard gas mixture of known composition. At the end of the column, ethylene is measured by a detector, usually a flame ionization detector (FID) powered by a mix of hydrogen/air. The ionization of ethylene generates an electric charge proportional to its amount, which is measured by the FID. The resulting electric signal can be then converted by suitable A/D interfaces and processed by a quantification software upon a calibration procedure.

The GC methods have allowed significant advances in ethylene research, although their detection limit is close to the ppm level. Further progress was made in the late 1980s, by adopting CO_2 laser-based photoacoustic (LPA) spectroscopy that has suitable features for the detection of trace gases in the ppb and sub-ppb concentration range. This methodology is based on the generation and detection of pressure waves (i.e., sound) inside a resonant cell, where the gas samples are placed or allowed to flow through. Samples are exposed to a modulated radiation, which can be absorbed at specific wavelengths ($\sim 10\,\mu m$ for ethylene), heat the sample and, thus, generate a sound signal. This sound is measured by highly sensitive microphones, inside the cell, and converted into an electric signal, which is filtered and detected by an amplifier (Fig. 5.4).

More recently, the light-emitting diode (LED) technology has allowed setting up of portable LPA devices that can be used even for measurements of ethylene production directly "in the field."

The significant evolution and current developments of ethylene measurement approaches in recent years are described in depth by Caprioli and Quercia (2014).

5.3 ETHYLENE PHYSIOLOGY AND CROSSTALK WITH OTHER HORMONES DURING POSTHARVEST

Ethylene is widely considered the dominating hormone responsible for the activation and acceleration of climacteric fruit ripening and plant senescence. Sound evidence, based on chemical and biotechnological manipulation experiments, has unequivocally proven the role of ethylene in determining the onset and progression of ripening in climacteric fruits, in inducing certain ripening-associated events in nonclimacteric fruits, as well as in accelerating overall senescence in plant tissues (reviewed by Seymour et al., 2013). The

action of ethylene is amplified following abiotic (mechanical damage, wounding, wilting, etc.) or biotic (attack by pathogens) stress conditions. Counteracting ethylene action and senescence is a central issue in the context of extending the shelf-life of commodities.

Ethylene action in pre- and postharvest ripening and senescence underlies the development of both positive and negative attributes, such as the loss of firmness, the synthesis of pigments and of aroma volatiles, as well as the stimulation of ethylene biosynthesis itself. All these events are enhanced by treatments with exogenous ethylene and are delayed or blocked by inhibiting ethylene perception or biosynthesis either chemically (e.g., by treatments by 1-MCP or silver ions, or AVG, respectively) or genetically, providing compelling evidence on the master regulatory role played by this hormone. Ethylene-induced loss of firmness is a primary aspect in postharvest life influencing decay and susceptibility to mechanical stress and pathogen infection. It is brought about by means of transcriptional activation of several genes encoding enzymes involved in cell wall remodeling (e.g., glucanases, EGAs; expansin, EXP; beta-galactosidase, GAL; pectin methylesterase, PME; pectate lyase, PL; polygalacturonase, PG; xyloglucan endotransglucosidase/hydrolases, XET) and of the regulatory factors (i.e. a Rab GTPase encoding gene) responsible for the correct secretion of cell wall enzymes to the apoplastic space. Ethylene also controls color change, through carotenoid biosynthesis (e.g., by enhanced transcription of phytoene synthase, PSY) and chlorophyll degradation, by inducing the overexpression of chlorophyllase encoding genes. The process of chlorophyll degradation, in particular, is responsible for the "degreening" process of florets (e.g., broccoli), fruits (e.g., citrus and bananas), and leafy vegetables (e.g., spinach, etc.), occurring during postharvest storage and representing a visible landmark of senescence.

The regulation of these events by ethylene is the final outcome of the interaction with a number of factors, among which plant growth regulators represent a key element. The concept that ethylene does not exert its ripening- and senescence-inductive action alone, but rather in the context of a finely tuned and balanced interplay with other hormones, has been put forward as soon as ethylene's implication in these processes was postulated. This hypothesis has progressively gained biochemical and, more recently, transcriptomic, proteomic, and biotechnological evidences in support (reviewed by Iqbal et al., 2017). Overall, considering the traditional categories of plant hormones, gibberellins (GAs), auxins (IAAs), and cytokinins (CKs) seem to exert in general an antagonistic role against ethylene's action, while abscisic acid (ABA) plays a synergistic role (Fig. 5.5).

There are common as well as divergent specific effects brought about by each hormonal category. However, a prudent approach must be always adopted before drawing general conclusions, since it has to be considered that, in some cases, opposite responses can be triggered by the same growth regulator depending on the concentration, the timing, and the mode of application and the developmental and physiological state of the plant produce under investigation.

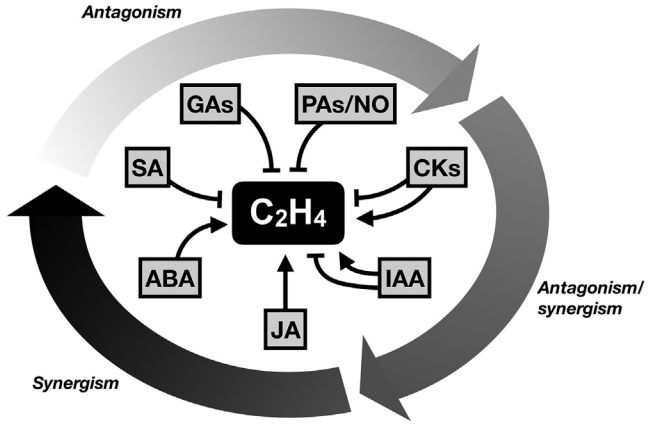

FIGURE 5.5

Synergistic and antagonistic relationships between ethylene and other hormones. Abscisic and jasmonic acid (ABA and JA) play a synergistic role together with ethylene in promoting ripening and senescence of fruits and vegetables, respectively. Nitric oxide (NO), poly-amines (PAs), gibberellins (GAs), and salicylic acid (SA) play a general antagonistic role. Auxins (IAAs) and cytokinins (CKs) may exert either antagonistic or synergistic actions, depending on the fruit/vegetable developmental stage (see text for details).

Auxins undergo a decrease in terms of free active levels, along with a corresponding increase in their inactive conjugated forms, before the onset and during ripening in both climacteric and nonclimacteric fruits as well as during senescence, supporting their antagonistic role with ethylene. The preharvest exogenous application of auxins (such as the synthetic auxins NAA or 2,4D) on immature fruits normally results in a delay in fruit ripening and senescence (e.g., in strawberries, grape, banana, tomato), manifested by a slower loss of firmness and chlorophyll content during postharvest, a slower accumulation of pigments, and delayed overall postharvest decay. Conversely, when sprayed after the onset of ripening, auxins may induce the enhancement of ripening in some systems, mostly climacteric fruits (peach, nectarine, and apple), through the stimulation of transcription of ACS-encoding genes and therefore of ethylene biosynthesis. An ethylene-counteractive role, similar to that played by auxins, can be also attributed to CKs, which are well known for their preventive role against yellowing of leaves in several plant species. Both pre- and postharvest exogenous applications of benzyl adenine (BA), zeatin, or forchlorfenuron (CPPU) result in the inhibition of ethylene-dependent chlorophyll degradation, a consequent delay in postharvest "degreening," loss of firmness, and overall decay, through the downregulation of chlorophyllase and cell wall enzymes, in both climacteric and nonclimacteric (e.g., strawberry, citrus) fruits as well as in broccoli florets and leafy vegetables. As for auxins, the preripening application of cytokinins reduces ethylene biosynthesis and sensitivity and the respiration climacteric rise while the application after the onset of ripening may result in an opposite effect. Even though these general conclusions can be considered overall valid, there are reports in the literature showing opposite effects. For example, in kiwifruit early (after bloom) preharvest treatments

with CK enhanced starch accumulation, followed by faster ripening. Treatments of broccoli florets with CKs lead to a decreased climacteric respiration, a delayed overall yellowing process, and longer shelf-life, while increasing levels of ethylene evolution, thus suggesting that some effects mediated by plant growth regulators may be evoked through differential effects on specific ripening-associated events. A similar picture can be drawn for GAs, also reported to counteract in some fruits the loss of firmness and the consequent spoilage due to pathogen attack during postharvest storage, and a stronger synergistic effect may occur when GAs and CKs are used in combination to delay chlorophyll breakdown and prolong shelf-life. Indeed, all these effects seem most frequently to involve the downregulation of ethylene biosynthesis and sensitivity and are counteracted by supplying exogenous ethylene, suggesting that all these growth regulators act in the context of a reciprocal balance coordinating their biosynthesis, metabolism, and perception/action. It must also be noted that use of early (at bloom) sprays with CKs + GAs to stimulate fruit development also results in some cases in fruit with longer shelf-life and delayed senescence. However, in this latter case, the antagonistic effect toward ethylene probably takes place through indirect crosstalk mediated by changes in metabolism, for example, manifested by an increased uptake of nutrients such as Ca^{2+} or sugars. An important antisenescence action, in clear antagonism with ethylene, is also played by new emerging growth regulators, among which polyamines (PAs) deserve mentioning. PAs cannot be regarded as bona fide hormones but can be nevertheless considered as general plant growth regulators. PAs (spermine, spermidine, and putrescine) display high levels in young tissues and progressively decrease in ripening fruits, reaching low levels in all senescent plant tissues. The use of pre- and postharvest PA sprays has been consistently and repeatedly shown to downregulate ethylene production and responses in a number of systems, enhancing fruit firmness by suppressing cell wall enzymes. Evidence is accumulating in support of a similar role for salycilic acid (SA), even though for SA action the data are scanty and, in some cases, apparently contradictory. Finally, among the small molecules with an ethylene antagonistic role, sound evidence is available for nitric oxide (NO), which downregulates ethylene biosynthesis by inhibiting the transcription and activity of its biosynthetic enzyme ACS and ACO through protein S-nitrosylation.

ABA can be considered a well-established ethylene allied in promoting ripening and senescence. ABA levels and transcript of genes involved in its biosynthesis (e.g., 9-cis-epoxycarotenoid dioxygenase) increase before ripening (and before the ethylene climacteric in climacteric fruits) in nonclimacteric (e.g., grape, strawberry) and climacteric (e.g., tomato) fruits. The inhibition of ABA biosynthesis or action, either chemically or through biotechnological manipulation, has been shown to result in a delay of the respiratory and ethylene climacteric, reduction of transcription of cell wall enzymes encoding genes, and lower accumulation of pigments, while exogenous ABA applications result in enhanced ethylene biosynthesis. Overall, these data clearly support a role for

ABA in the promotion of fruit ripening as well as of postharvest senescence, at least in some cases by means of a stimulatory effect on ethylene production. Similar experiments on climacteric (tomato) and nonclimacteric (grape) fruits have suggested a role consistent with that of ABA for brassinosteroids (BRs) and for jasmonic acid (JA), even though evidence in support is still limited. As a concluding remark, the progressive discovery of new elements in the cross-talk with ethylene in controlling ripening and senescence opens up novel opportunities for intervention in the context of improving the shelf-life of products. The new tools that may be developed will need to be increasingly eco-friendly and, besides the development of chemicals with low or no toxicity that can interfere with ethylene's action, such as the promising use of PAs, may include new molecular targets that could be tackled to interfere with ethylene-dependent responses through last-generation biotechnological and genetic approaches.

5.4 THE IMPACT OF POSTHARVEST CONDITIONS ON ETHYLENE PHYSIOLOGY

After harvest, horticultural commodities undergo several changes in terms of physicochemical properties, metabolism, and composition, leading to a more or less rapid evolution of ripening and senescence, as well as an increased susceptibility to postharvest pathogens, resulting in a reduced commercial- and shelf-life. Physical stress (wounding, bruising) and microbial infection not only cause direct losses, but also induce physiological responses, such as an increase in ethylene biosynthesis leading to an acceleration of ripening and of the onset of senescence, that can extend also to the surrounding commodities. Control of these factors, which can be obtained mostly by preventing mechanical damage, is a prerequisite for a successful storage. After detachment from the mother plant, fresh fruits and vegetables are still alive and, due to the high water content, are metabolically active and react to different stimuli present in the environment. Since high metabolism results in high rates of deterioration and shelf-life shortening, several strategies and techniques can be applied in order to slow down or delay ripening/senescence-related metabolic processes. The main goal of the storage protocols is to affect and control the following factors: (1) respiration; (2) biosynthesis and action of ethylene; (3) changes in composition and structure; (4) water loss (transpiration); (5) physiological disorders; and (6) pathogen infections.

Even though the goals of postharvest techniques may vary in relation to the crop and the final destination of the produce, a common feature is the need for reducing activities and processes related to primary metabolism. In addition to respiration, specific protocols or conditions applied after harvest are also effective, directly or indirectly, in altering ethylene physiology with marked effects on the ethylene-dependent processes typically characterizing the ripening of the climacteric fruit (see Section 5.3) and the senescence of

organs such as leaf vegetables with significant commercial benefits through the extension of shelf-life and the reduction of postharvest wastage.

Several postharvest treatments can be applied in order to delay ripening and senescence, some of which are innovative and still under evaluation of their efficacy. The most commonly applied storage techniques are: (1) low temperature; (2) controlled (CA) and/or modified atmosphere (MA), and (3) ethylene antagonists. CA and MA, as well as the application of ethylene antagonists, are used in combination with low-temperature regimes, so effects are additive.

5.4.1 Temperature

Lowering the temperature of produce soon after harvest and maintaining the cold chain throughout the postharvest phase is considered the main pillar of storage technology for appropriate handling of horticultural produce. According to Adel Kader, "*temperature management is the most effective tool for extending the shelf-life of fresh horticultural commodities*." The optimal temperature for storage varies according to the produce (origin, plant organ, developmental stage, etc.) and must be applied according to these two main principles: (1) low temperatures slow down metabolism and decrease the rate of compositional changes; and (2) temperatures below a specific threshold (different in relation to the fruit produce) and/or a prolonged cold storage induce chilling injuries. Refrigeration has a profound impact on the overall metabolism of plants and plant organs. In order to measure the changes for a biological system as affected by temperature, the Q_{10} temperature coefficient is used. Q_{10} indicates the rate of reactions in a biological system as a result of a temperature increase of 10°C. For most crops, within a temperature range of 5°C–25°C, Q_{10} values associated with respiration are around 2.0–2.5. This means that by lowering temperature from 15°C to 5°C the respiration is decreased by a factor of 2, with obvious benefits in terms of shelf-life, considering that the respiration rate of fresh fruits and vegetables is often used as a predictor of the effect of temperature on the overall metabolism.

In addition to respiration, low temperature applied in storage facilities markedly affected ethylene physiology. Since the discovery of ethylene as a natural compound produced by plant tissues and organs and, later, the biochemical and molecular characterization of the main steps of the hormone biosynthesis, the effects of cold storage on ethylene production of harvested hort produce have been described, in particular regarding the postharvest behavior of climacteric fruit. A steady decrease in the rate of ethylene biosynthesis is observed by lowering temperatures. This is the result of a downregulation of ACS and ACO gene expression as observed in both tropical (e.g., banana) and temperate (e.g., apple, peach) fruit species. In tomato fruit stored at 3°C, alteration of ethylene production correlates with the altered expression of specific ACC synthase (*ACS2*, *ACS4*) and ACC oxidase (*ACO1*) genes, involved in the onset of the climacteric rise. In addition to gene expression, low temperatures are also effective in altering enzyme activities: ACS activity

in particular shows a sigmoidal pattern in a range of temperature between 5°C and 35°C. Also, ethylene perception and the signal transduction pathway is affected by cold storage. In tomato fruit the expression of the receptor genes *LeETR1*, *NR*, *LeETR4*, and that of the signal cascade, *LeCTR1*, *LeEIL3*, *LeEIL4*, and *LeERF3*, is altered by chilling. Interestingly, also in nonclimacteric fruit (e.g., grapefruit) the expression of ethylene receptor genes is affected by low-temperature storage and ethylene appears to be implicated in the transcriptional regulation of ERFs under cold storage.

Storing fruit under cold conditions may result in the onset of chilling injuries (CIs). For example, in tomatoes, prolonged exposure to low temperature (about 7−13°C) induces the appearance of injury symptoms such as aroma loss, blotchy ripening, excessive softening, pitting, susceptibility to decay, electrolyte leakage, and failure to ripen. In peaches, internal breakdown, flesh browning, and bleeding and mealiness are associated with storage in the range of 2−8°C. The role of ethylene in the onset and development of chilling injuries is controversial and appears to be variable in different species. In many cold-sensitive fruits, low temperature stimulates ethylene production and this is considered as one of the inducing factors leading to the onset of CIs. This has been confirmed by the fact that in fruit with suppressed ethylene production (transgenic lines) or treated with ethylene antagonists (e.g., 1-MCP, see below), CIs are reduced. However, in some other fruits (e.g., peaches) the development of CIs may be due to the reduction or inhibition of ethylene production and strategies applied to increase ethylene production before or during cold storage are effective in lowering the incidence of CIs.

An interesting aspect concerning storage of fruits at low temperature is that in some cultivars of apples, pears, and kiwifruit, ethylene production is stimulated after more or less prolonged chilling exposure, when fruits are moved to room temperature (20°C). In these fruits, rapid ripening- associated processes, such as loss of flesh firmness, are coupled with higher levels of ethylene production, as a result of an upregulation of both ACS and ACO gene expression and enzyme activities. If for some fruit species this physiological behavior has a positive impact on quality (without chilling winter pears do not develop the best eating quality during shelf-life), for some other crops it represents a negative aspect that could be prevented by storing fruit in a controlled atmosphere.

5.4.2 Atmosphere Composition

In addition to temperature, atmosphere composition plays a key role in affecting the postharvest life of fruit produce: this is in particular related to the concentrations of oxygen, carbon dioxide, and ethylene. The control of these parameters represents the basis of the storage technique called controlled atmosphere (CA). In addition to CA, an expensive system widely applied only for specific fruits such as apples, winter pears, kiwifruits, and a few others, is MA and in particular modified atmosphere packaging (MAP), which represents an appropriate solution for prolonging the storage and shelf-life of different

hort commodities. Differently from CA, where gas concentrations are strictly monitored and controlled, in MA and MAP the gas balance is achieved by the respiratory activity of the product.

The reduction of oxygen level and the increase in carbon dioxide concentration (associated with low temperature) lead to a reduction in respiration as well as ethylene biosynthesis and action, and subsequently to better maintenance of commercial-quality parameters of the produce. It has been recognized that ACS is the major site at which elevated CO_2 and reduced O_2 atmospheres inhibit C_2H_4 biosynthesis in ripening apple and peach fruit. Also, the conversion of ACC to ethylene is affected, as observed in pears where changes in ACO mRNA levels and protein accumulation occur during CA storage conditions. The extremely low oxygen concentrations applied in the advanced CA protocols (e.g., initial low oxygen stress (ILOS) and dynamic controlled atmosphere (DCA)) have a pronounced effect on the overall metabolism and reduce the expression of a number of genes including those responsible for the ethylene biosynthesis as recently observed in Granny Smith apples stored at 0.4 and 0.8 kPa oxygen. In addition to the biosynthetic pathway, elements of the signal transduction pathways are also affected by hypoxia. As observed in model species (*Arabidopsis*), ethylene-responsive factors (ERFs) seem to be involved in oxygen-sensing mechanisms also in apple fruit tissues.

The inhibition of ethylene and ethylene-dependent processes (including autocatalytic synthesis) by CO_2 has been associated with the competition of the gas with ethylene at the receptor-binding site. Reduced ACS mRNA accumulation occurs in tomato and peach fruit exposed to high CO_2 levels while ACO members appear to respond differently to such conditions. The mechanisms by which CO_2 alters ethylene physiology are still to be fully discovered.

A key factor that has to be considered in storage facilities is the elimination of the ethylene produced by commodities and product. Some fruit species (e.g., kiwifruit) are extremely sensitive to even very low ethylene concentrations in the atmosphere. The use of adequate ventilation and ethylene removal systems (through chemical absorbers or catalytic conversion) is a requirement for a successful prolonged storage. For MAP, the removal of undesirable ethylene greatly improves the benefits of this technique and adsorbers (activated carbons) or oxidizers (potassium permanganate) are widely used in the so-called active packaging technology (see also Section 5.4.3).

5.4.3 Other Postharvest Treatments

In addition or as an alternative to refrigeration with or without CA/MA, other physical, chemical, and biological treatments may be applied to maintain fresh-like quality, preserve the nutritional/nutraceutical value and meet the safety standards of fruits. Heat treatments have been proposed as an alternative to chemical treatments to control fruit decay and/or for killing insects (pesticide quarantine treatment) but also for reducing the impact of CIs during the following refrigerated storage. In general, heat treatments are of

short duration (from a few seconds to several minutes/hours) and performed in hot water, hot air, or vapor heat at temperatures in the range of 40°C–55°C, depending on the commodity and the method. In general, heat treatments induce a delay of ripening in climacteric fruit due a reduction of ethylene production as ACS and ACO are among the enzymes affected by the treatment. However, depending on the treatment parameters (temperature and time), opposite effects can also be observed: in tomato fruit, for example, hot-water treatments performed before refrigerated storage may induce CI tolerance, and this effect seems to be related to the restoration of ethylene biosynthesis and signaling.

With the aim of controlling pathogens and reducing postharvest decay, advanced technologies such as edible films or coatings and antimicrobial packaging are increasingly used. Edible films or coatings are thin layers of material from different sources (natural, synthetic) applied to the surface of fresh produce providing an additional natural barrier. This reduces transpiration and, acting as a gas barrier, a modified atmosphere is established around the fruit surface with similar effects as those described in Section 5.4.2. In antimicrobial packaging, active ingredients are added to the packing system/material, resulting in the prevention of microbial growth. Some natural compounds, such as essential oils, are used in the substitution of chemical substances in general not permitted for edible produce. Recent advancements incorporate edible coating or nanoemulsions with essential oils and, besides controlling spoilage, these technical solutions result in altering ripening physiology with a decrease in respiration and ethylene production as observed in both whole fruit and fresh-cut produce.

Ultraviolet light, and in particular UV-C (far-UV) at low doses, is effective in inducing resistance against pathogens in harvest produce and is also effective for surface decontamination in fresh-cut produce. In addition, the application of UV light delays tomato fruit ripening and this effect seems to be due to the activation of ERFs that could act as regulators of metabolic pathways during ripening.

5.4.4 Specific Inhibitors of Ethylene Biosynthesis and Perception

Specific inhibitors of ethylene biosynthesis or antagonists of its action can also be effective in improving the storage performance of horticultural crops. For edible produce, the use of some of them is impossible, difficult, too expensive, or forbidden by law as, in the EU, for almost all postharvest chemicals on fruit and vegetables. This is the case of the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG), known commercially as ReTain, effective in inhibiting ACS enzymatic activity and, as a consequence, reducing ethylene production. Only preharvest applications of ReTain are allowed and performed in the fruit industry, in particular on pears, apples, and peaches, leading in general to a delay of postharvest ripening due to reduced production of

ethylene. Preharvest ReTain applications on apples are also effective in limiting losses due to the reduced mature fruit drop. Considering the inhibitors of ethylene action, since the early 1970s silver thiosulfate (STS) has been widely used to enhance the longevity of flowers and display life of potted plants that are ethylene-sensitive. However, for its toxicity, STS cannot be used on edible fruits and vegetables. A great advancement in optimizing the storage protocols of several horticultural crops has been represented by the discovery by Eduard Sisler of 1-methylcyclopropene (1-MCP), a cyclic olefin extremely effective in competing with ethylene at the level of receptors. This compound, now successfully used worldwide for ethylene-sensitive horticulture produce, including edible and ornamental crops, is commercially applied as a stable powder that easily releases a gas when dissolved in water. It is effective at very low concentrations (0.1−1 μL/L) and for short exposures (a few hours can be sufficient) and, as a gas, it can be easily used in storage rooms. The main effects of 1-MCP treatments are those of reducing ethylene production and respiration rate, thus controlling the quality parameters dependent on ethylene (loss of firmness, in particular). Several apple varieties benefit from the application of 1-MCP, which can be effective also on other fruit produce (pears, bananas, plums, tomatoes, etc.). Together with these positive effects (prolonging the commercial life), some negative effects result from treatment with the ethylene antagonist. This is the case for some apple varieties that do not fully develop the typical aroma. A huge scientific and technical literature demonstrates that 1-MCP effects are variable in relation to the fruit species and variety and several factors (concentration, duration, and temperature of the treatment, developmental stage of the fruit, posttreatment storage conditions) may affect the final result of the application. Even though the effects of 1-MCP treatments are markedly present in climacteric fruit, under specific conditions positive effects of 1-MCP can also be observed for maintaining quality and/or affecting some physiological processes in nonclimacteric fruit.

5.5 BIOTECHNOLOGICAL APPROACHES

The ever-increasing identification of the molecular factors recruited by ethylene during postharvest ripening and senescence has provided a number of possible targets for biotechnological interventions to enhance the shelf-life of fruits and vegetables. Historically, biotechnological approaches have followed the progressive discovery of the main players of the "ethylene scene." The first attempts to hasten shelf-life were mostly aimed at controlling specific ethylene-dependent responses, among which enhancing firmness maintenance in fruits through the manipulation of genes encoding cell wall enzymes clearly appeared as one of the most important targets to improve postharvest storage life. The biotechnological downregulation of expression of cell wall encoding genes either by RNA antisense technology and, more recently, by RNA interference (RNAi), proved to be at least in part successful in conferring better maintenance of fruit firmness for a certain number of enzymes, among which PGs, PLs, and PMEs should be listed, while it did not do so for other enzymes such

as, for example, EGases. Examples of successful manipulation of PLs and PMEs, resulting in an increased shelf-life and subsequent reduced postharvest spoilage, are available for both climacteric (tomato) and nonclimacteric (strawberry) fruit models. It must be noted that the lack of effects of several cases of manipulations of cell wall enzymes encoding genes could be ascribed to the fact that cell wall rearrangements taking place during ripening and influencing postharvest storability are the result of complex sequentially coordinated action of several enzymes. This in many cases can make the manipulation of single enzymes overall ineffective on fruit firmness.

As far as climacteric fruits are concerned, soon after the identification of the genes encoding the enzymes involved in the last two steps of ethylene biosynthesis (ACS and ACO), a logical further development for biotechnological control of postharvest life was the application of gene-silencing techniques for the reduction of endogenous ethylene release. This approach moved the target to an upstream level, where the rationale was counteracting the action of the master hormonal regulator of ripening and senescence, thus delaying all downstream events. Tomatoes transformed with antisense ACS and ACO genes, obtained through pioneering studies, have provided the proof of concept and paved the way to obtain fruits with very low ethylene biosynthesis and significantly delayed overall ripening and senescence. Since then, the same approach has been applied with success on a number of climacteric fruits, ranging from melon to apple, as well as on flowers (e.g., carnation) and florets (e.g., broccoli) with evident inhibitory effects on postharvest senescence and with increased shelf-life. Importantly, the biotechnological control of ethylene biosynthesis can be overcome by exogenous application of ethylene, thus giving the opportunity of removing the endogenous block and re-establishing normal ripening when required. Additional approaches downregulating ethylene biosynthesis have exploited the use of transgenes responsible for decreased levels of the ethylene precursors SAM and ACC through their enhanced metabolism. Such applications have included the overexpression of bacteriophage T3 SAM hydrolase and bacterial ACC deaminase encoding genes and resulted in overall lower endogenous ethylene release and longer shelf-life. Also, the overexpression of SAM decarboxylase, controlling the diversion of decarboxylated SAM toward the biosynthesis of PAs, has been successfully used for improving tomato fruit firmness, quality, and postharvest life, while unexpectedly leading to increased ethylene evolution, possibly through interference exerted by PAs on ethylene signaling.

After the discovery of the molecular components of ethylene perception and signaling, further biotechnological developments have included the exploitation of such elements in a number of systems. Due to the nature of their mode of action as negative regulators of ethylene responses, the overexpression of mutated versions of the ethylene receptors (e.g., the *Arabidopsis etr1-1* or the mutated receptor of the *Never Ripe* tomato mutant, *Nr*) has been used to confer dominant insensitivity to the hormone, thus making the transformed fruit or plant completely or partially insensitive to ethylene in a constitutive

manner. This approach leads to an intrinsic inability of the plant to respond to endogenous ethylene but also to exogenously applied ethylene. A consequence of such a constitutive ethylene insensitivity is definitely a rather significant increase in shelf-life and much slower decay. However, this also implies the impossibility of restoring a normal ripening process by providing exogenous ethylene and therefore of obtaining fruits of acceptable quality for the market. Similar nearly overlapping results, with the same pros and cons, have been obtained by inhibiting the expression of ERF and EIN encoding genes, thus acting on components of the signaling pathway downstream of ethylene perception, by antisense approaches. Biotechnological control of ethylene biosynthesis and signaling has proven to be of interest for the improvement of the overall postharvest quality of fruits and vegetables, however future studies should be devoted to set suitable tools for the conditional control of such transgenes in order to make it possible to re-establish a normal fruit-ripening syndrome when desired. Also, new approaches besides the use of antisense, cosuppression, or RNAi-mediated silencing technologies, may be exploited for improved efficiency and better spatiotemporal control and may include the use of artificial miRNAs and CRISPR/Cas9 techniques to open new opportunities for highly targeted gene manipulations.

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CHAPTER 6

Morphology and Anatomy

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6.1 INTRODUCTION

In biology, morphology is the branch that deals with the form of living organisms. For plants, plant morphology or phytomorphology is the study of the physical form and external structure of plants, whereas plant anatomy is the study of the internal plant structure, mostly at the cellular/microscopic level. This chapter deals with vascular plants, also known as higher plants or trichophytes, which are defined as those that have ducts for circulation of fluids, such as xylem and phloem. The main focus will be the morphology and anatomy of fruits and vegetables due to the important role that external and internal plant physical characteristics play during the different stages of the life of a given plant organ. In terms of gas exchange, the extent of interaction between a horticultural produce and its surrounding environment may be determined by the shape of the product (surface area/volume ratio) and by the thickness and nature of its epidermal tissue. An example is how fast a produce loses water by transpiration. The velocity of gas exchange may depend on the size of the intercellular spaces inside the fundamental tissue of the product. Tolerance to bruising during harvesting, packing, or other handling operations is dependent on the nature of the cells that make up the fundamental tissue. Therefore, knowledge on the morphology and anatomy of a certain commodity is essential for establishing the proper postharvest handling requirements of horticultural crops.

6.2 STRUCTURAL ORGANIZATION OF PLANTS (ROOT AND SHOOT SYSTEMS)

A whole plant is composed of two main organ systems: the shoot and the root. The shoot system is the aerial part, whereas the root system is the

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underground part. Each organ system is composed of various specialized organs. Stem, leaf, flower, and fruit are organs that make up the shoot system, whereas the roots and their parts make up the root system. Each organ is composed of three types of tissue system: the epidermal, the ground, and the vascular tissue systems. Furthermore, each organ can be composed of various other tissues. These may include parenchyma, collenchyma, or sclerenchyma tissues. These tissues are distinguished by the specific characteristics of their cells. Plant cells are formed at the meristems, which are found in regions of the plant where growth can take place, forming new tissue due to these actively dividing cells, named meristematic cells. These cells give rise to plant organs and keep the plant growing. The stem system supports the aerial part of the plant connecting the leaves and the roots. The leaves are the primary site of photosynthesis (capturing energy from sunlight and using it to make organic materials). Flowers and fruits are reproductive organs. Flowers attract pollinators and fruits attract seed dispersers. Roots anchor the plants into the soil, absorb water and minerals, and may store some nutrients.

6.3 FRUIT AND VEGETABLE ANATOMY

Plants tissues are organized into three types of tissue systems: dermal tissue, vascular tissue, and ground tissue.

6.3.1 Dermal System

How fast a horticultural commodity loses water by transpiration, how susceptible a produce is to the attack of pathogens, and how resistant the produce is to the penetration of chemicals depend on the characteristics of its dermal system. The dermal tissue system is the outermost covering of the entire plant and may comprise different elements depending on the specific part of the plant. Such elements include the epidermis, cuticular membrane, trichomes, stomata, and lenticels. The dermal tissue protects the internal tissues of the plant or part of the plant, and that is why the dermal tissue is also called the protective tissue. The protection is diverse and since the dermal tissue is in direct contact with the environment it confronts external physical, chemical, and biological threats, in addition of carrying out a dynamic interaction with its surrounding neighborhood. The dermal system protects against pathogen attacks, mechanical injuries, temperature stress, penetration of chemicals, loss of moisture by produce, among others. However, the dermal tissue facilitates gas exchange, allowing the entrance of oxygen to the inner cells, while at the same time allowing carbon dioxide to be released outward.

6.3.1.1 EPIDERMAL CELLS

The epidermis is usually single layered and is the outermost cellular layer of the plant body, made up of elongated and tightly arranged cells named epidermal cells. These epidermal cells are of the parenchymatous type (described

below). Although compactly arranged, these epidermal cells possess points of interruption to facilitate gas and moisture exchange.

6.3.1.2 THE CUTICULAR MEMBRANE

The cuticular membrane or plant cuticle is an extracellular composite structure made up of cutin and waxes. It is generally described as an extracellular thick waxy layer that covers the outside part of the epidermis. The cutin is a covalently linked macromolecular scaffold composed mostly of C16 and C18 fatty hydroxyacids esterified to each other and to glycerol. On the other hand, the cuticular waxes are a variety of organic solvent-soluble lipids mostly derived from very-long-chain fatty acids (C20−C34), including alkanes, ketones, aldehydes, alcohols, and esters. The cuticular membrane is characterized by its resistance to water loss by transpiration, and its limitation to pathogen invasion and chemical penetration. Based on histochemical staining, the cuticle structure is often divided into two sections: the "cuticular layer" and "cuticular proper." The former is a cutin-rich portion with embedded polysaccharides, whereas the latter is a wax-rich overlying layer. The wax in the "cuticular proper" can be found as intracuticular wax (within the cuticular matrix) or as epicuticular wax forming a film (epicuticular wax film) or in the form of crystals (epicuticular wax crystals). The glossy appearance in many leaves and fruits is due to the epiculicular film, whereas the dull appearance in broccoli leaves is due to epicuticular wax crystals. Cuticle resistance to water loss is mainly attributed to wax rather than to cutin. Furthermore, this resistance appears to be primarily determined by the particular mixture of intracuticular and epicuticular waxes and how they are organized rather than their amount. On the other hand, cutin seems to be more important than waxes as a pathogen barrier, at least in tomato fruit.

6.3.1.3 THE TRICHOMES

The trichomes are single-celled or multicellular appendages found on stems and some fruits such as peach and kiwifruit. The multicellular trichomes may be branched or unbranched and help prevent water loss.

6.3.1.4 THE STOMATA

The stomata are present in stems of herbaceous plants, in low amounts on fruit surface also in the upper epidermis of leaves, and in high amounts in the lower epidermis of leaves. Stomatal densities differ greatly between the different species and plant organs. In leaves of purple passion fruit about 100 stomata/mm^2 of the leaf surface can be found, whereas in the fruit surface only about 12 stomata/mm^2 were found. Stomata are made up of two specialized guard cells that give form to the stomatal pore, which regulates the exchange of carbon dioxide, oxygen, and water vapor, concretely between the outside atmosphere and the substomatal cavities inside the leaf (Fig. 6.1). The opening and closure of the stomatal pore is promoted by the

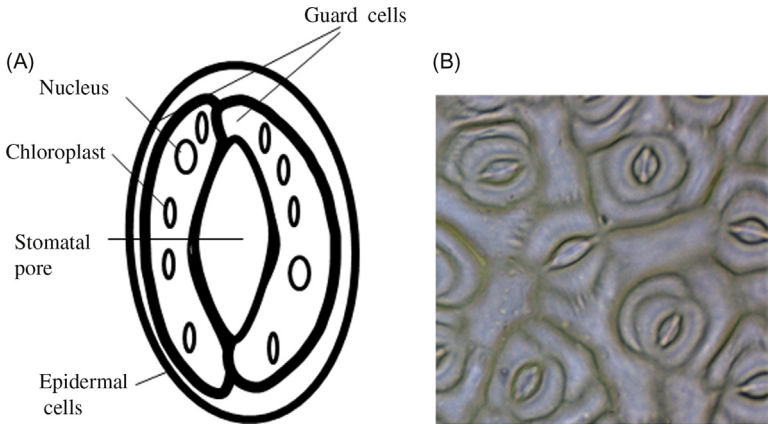

FIGURE 6.1
Parts of stomata (A) and stomata in Noni plant leaf (adaxial side) by optical microscopy (40 ×) (B).

inlet and outlet of water in guard cells. The inlet of water causes both guard cells to swell and acquire a kidney-like shape in opposition to each other, thus a pore is established between both guard cells. However, the outlet of water causes guard cells to constrict—the change in shape is reversed, and the stomatal pore is closed.

6.3.1.5 THE LENTICELS

The lenticels found on the epidermis of different plant organs (stem, petiole, fruits) made up of parenchymatous cells are pores that always remain open, in contrast to stomata, which regulate their extent of opening. Lenticels are visible on fruit surfaces, such as mango, apple, and avocado. Lenticels permit the exchange of gases between the environment and the internal tissue spaces of the organs (stems and some fruits) (Fig. 6.2). They permit the entrance of oxygen and simultaneously the output of carbon dioxide and water vapor. In apple fruit, lenticels account for up to 21% of the transpiration.

6.3.2 Ground System

The ground system accounts for the principal edible portion of fruits and vegetables whose cells carry on the main metabolic processes in order to accumulate diverse substances as nutrients to the plant by itself or to seed dispersers in the case of fruits. Also, this system may provide mechanical support for the organs they conform. This tissue system is made up of three different types of simple tissues: parenchyma, collenchyma, and sclerenchyma tissues, which can be described in terms of their cellular characteristics.

6.3.2.1 PARENCHYMA TISSUE

The parenchyma is composed of living cells with primary thin walls forming a tissue that may be compact or have extensive air-filled intercellular spaces.

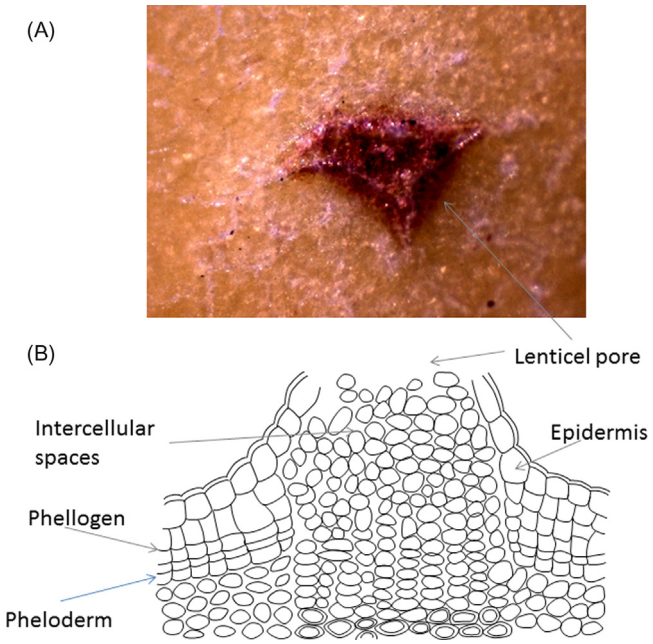

FIGURE 6.2
Lenticel pore on apple surface (A) and parts of lenticels (B).

These spaces can be as low as 1%, such as in potato, or vary from 15% to 25% as seen in fruits, for example, apples can reach up to 25%. Parenchyma cells make up the fleshy part of fruits, roots, tubers, and leaves, and form the most common and most abundant plant tissue. Various metabolic processes occur in the parenchyma tissues including the synthesis of hormones, enzymes, pigments, essential oils, toxic substances, etc. One of the most important of these processes to both plants and animals, including humans, is photosynthesis, which produces basic food substances for all living organisms. Another equally important metabolic process is respiration, which provides the energy utilized by the plant to carry out its various activities. Some other important processes include the conversion of glucose to starch, the form in which it is stored in parenchyma tissues, and the reverse process of digestion of starch, which makes glucose available for use by the plant. Parenchyma tissue is found in the leaf mesophylls, which are the tissues located between the upper and lower leaf epidermis, consisting of the palisade layer and the spongy parenchyma. Most leaf mesophyll cells contain chloroplasts (mainly the palisade layer cells), and, as mentioned above, carry on the process of photosynthesis. Parenchyma is also found in the cortex that is the outermost layer of the stem or root in the plant, and in the pith, which is a tissue in the stems of vascular plants, composed of soft, spongy parenchyma cells, and may store nutrients throughout the plant.

6.3.2.2 COLLENCHYMA TISSUE

Collenchyma tissue is composed by elongated living cells of uneven primary thick walls, which possess hemicellulose, cellulose, and pectic materials. It provides support, structure, mechanical strength, and flexibility to the petiole, leaf veins, and stem of young plants, allowing for easy bending without breakage. The stretchy properties of the strands of celery are due to collenchyma tissue. Collenchyma tissue is found immediately under the epidermis, young stems, petioles, and leaf veins. Also, it has been seen in avocado fruit hypodermis. Collenchyma cells may or may not contain a few chloroplasts, and may perform photosynthesis and store food.

6.3.2.3 SCLERENCHYMA TISSUE

Sclerenchyma tissue, when mature, is composed of dead cells that have heavily thickened walls containing lignin and a high cellulose content (60%–80%), and serves the function of providing structural support in plants. Sclerenchyma cells possess two types of cell walls: primary and secondary walls. The secondary wall is very thick and highly lignified (15%–35%) and imparts a great rigidity and hardness to the cell and tissue. There are two main types of sclerenchyma cells: fibers and sclereids. Fibers are very elongated cells that can be found in stems, roots, and vascular bundles in leaves. Fibers impart fibrousness as in the case of asparagus. Sclereids are found in different shapes (spherical, oval, or cylindrical) and are present in various plant tissues such as the periderm, cortex, pith, xylem, phloem, leaves, and fruits. The hardness of the shell of nuts, the coat of many seeds, and the stone of drupes (cherries and plums) is due to this type of cell. Sclereids may impart a grainy texture to some fruits when found scattered in their paranchymatous tissue, that is, in pears and quinces.

6.3.3 Vascular System

Vascular tissue transports food, water, hormones, and minerals within the plant, and includes the xylem, phloem, parenchyma, and cambium cells. The xylem distributes water and minerals throughout the plant, whereas the phloem conducts the products of photosynthesis from the leaves to all over the plant (Fig. 6.3).

6.3.3.1 XYLEM

The xylem provides mechanical strength to plant parts, and functions as a conducting tissue for water and minerals from the roots to the stem and leaves. It is composed of four different element types, namely, tracheids, vessels, xylem fibers, and xylem parenchyma. Tracheids are very elongated dead cells with thick and lignified walls and tapering ends. The vessel is a tubular structure composed of many dead lignified cells called vessel members interconnected through perforations in their common walls.

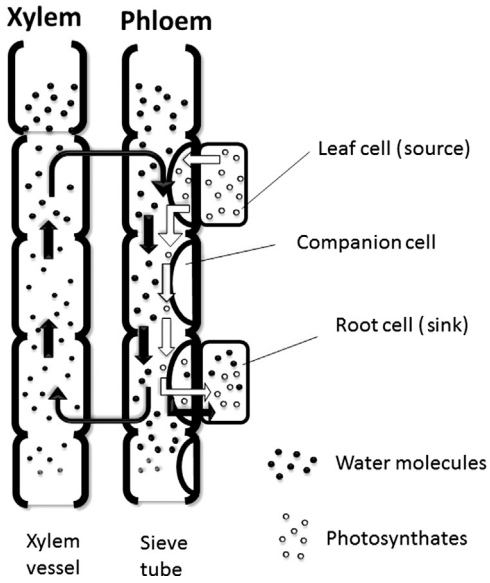

FIGURE 6.3
The vascular system.

6.3.3.2 *PHLOEM*

The phloem transports food materials derived from photosynthesis, usually from the leaves to other parts of the plant. The sieve tube elements are also long tubular structures, arranged longitudinally and associated with the companion cells. Their end walls are perforated in a sieve-like manner to form sieve plates. Companion cells are specialized cells (parenchyma), which are closely associated with sieve tube elements and help in maintaining the pressure gradient in the sieve tubes. The functions of sieve tubes are controlled by the nucleus of companion cells.

6.4 THE ROOT

Some examples of edible horticultural roots include carrots, beet, radish, turnip, jicama, and sweet potato. The root is the first organ appearing in sprouting embryos. The seed coat is broken and the root starts to grow downward into the ground, forming tube-like structures. The root lacks buds, leaves, and stomata. Based on the root shape, there are basically two types: taproot and fibrous. Taproots are made up of a large central root with shorter lateral or branching roots, whereas, in comparison, fibrous roots are shorter, smaller and hairy, growing more shallow. Both taproots and fibrous roots are called tuberous when they become swollen due to the accumulation of reserves. Root functions can be divided into primary and secondary. The primary functions of roots include: anchoring the entire plant, supplying the stem with water and minerals absorbed from the soil, and synthesizing plant

hormones. Among the secondary functions that can be performed by the roots of certain plants are those of storing food materials mainly sugars, starch, and water as in the case of beetroot, sweet potato, jicama, turnip, radish, and carrot (Fig. 6.4), that swell and become fleshy. The plant stores these substances in the roots with the purpose of utilizing them later for their physiological processes. However, due to these reserves the tuberous roots have been cultivated and used as horticultural edible produce. Furthermore, some of them accumulate pigments, such is the case of beetroot and carrot which are regarded as rich sources of betalains and carotenoids, respectively. Sweet potato may also be used for vegetative reproduction. Roots can be divided into several regions: (1) root cap, (2) meristematic tissue, (3) root elongation, (4) region of root hairs, and (5) region of maturation. Root cap is a thimble-like structure that gives protection to the root tip and is found in the deepest part of the primary root. The meristematic tissue region is immediately above the root cap and is composed of small, thin-walled undifferentiated cells dividing actively. The root elongation region undergoes rapid lengthening due to cell enlargement, allowing the root to get deeper into the soil. The root hairs region, also called the root absorption region, absorbs water and minerals from the soil and is formed by thread-like cells called root hairs. Ultimately, the maturation region, which normally is the major part of the root, is made up of enlarged and differentiated cells that become the different root tissues, for instance, cortex, xylem, and phloem. Lateral roots may also develop from this region. From the outermost to the innermost, the tissues of the root are the epidermis, the cortex, and the vascular cylinder. Morphologically, most root vegetables are products with low surface-to-volume ratio, and generally possess a crispy texture (such as carrot and radish). Root vegetables are sufficiently firm in texture to withstand moderately rough postharvest operations, such as hydrocooling and mechanized sorting.

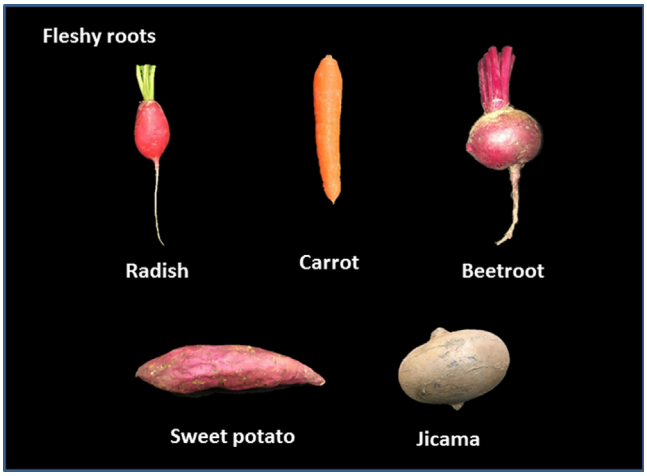

FIGURE 6.4
Some fleshy roots.

6.5 THE STEM

Some of the commercial edible horticultural stem commodities include potato, onion, ginger, turmeric, kohlrabi, asparagus, cactus stems (nopales), barrel cactus, and sugarcane (Fig. 6.5). The stem develops from the embryo plumule in a germinating seed and is established as a primary axis that grows in the opposite direction to the seed radicle, and although a part of it can be subterranean, becomes the aerial part of the plant. The stem possesses buds, nudes, internodes, and leaves, and lacks absorbent hairs and root cap. Some plants have stems that lack leaves, such as cactus stems. The buds which develop into flowers are called floral buds, whereas the buds which develop into branches are called vegetative buds. The latter can be apical when located at the apex of the stem, axillary when located in the axils of leaves, accessory when located on the sides or above the axillary buds, or adventitious when located at areas other than the nodes. The function of the stem is basically the

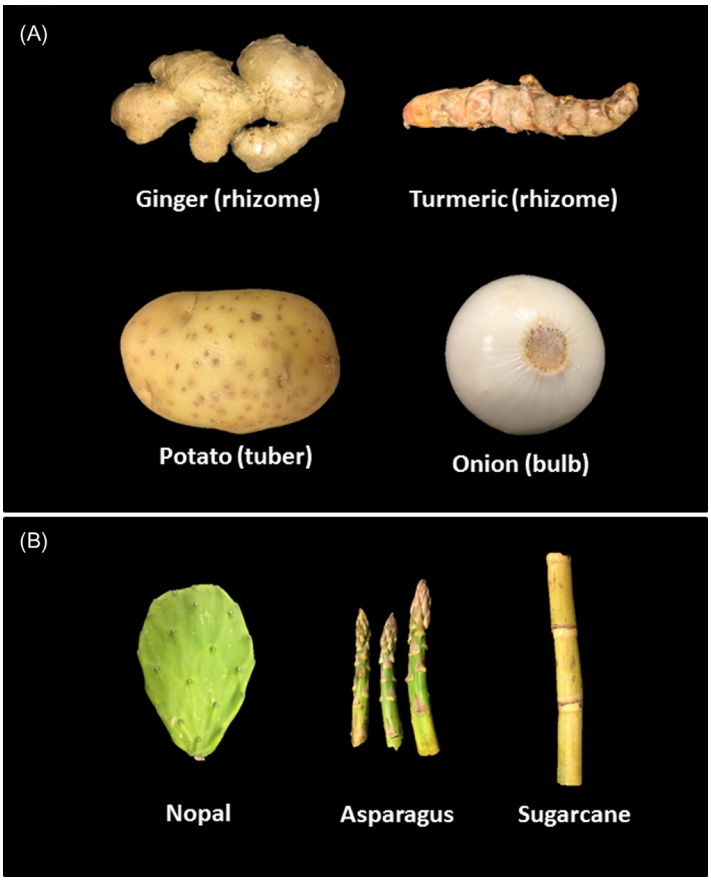

FIGURE 6.5
(A) Subterranean stems and (B) aerial stems.

conduction of water, minerals, and photosynthates, in addition to providing support to other parts of the plant. Based on the shape, stems can be classified into cylindrical (such as the case of sugarcane), racket-shaped (such as the case of the prickly pear cactus stem of the *Opuntia* genus, in which the stem is articulated by several green fleshy racket-shaped pads, also called cladodes), spherical (such as the case of the globulous plant of the barrel cactus that is common in arid regions), conical, triangular, quadrangular, polygonal, and acute-angular. Based on consistency, stems can be classified into herbaceous, semiwoody, woody, and fleshy or succulent. This latter class is characterized by its capacity to accumulate a large reserve of nutritive substances and water, and because of that some stems become edible tissue, such as prickly pear cladodes, barrel cactus, and potatoes. The prickly pear cladodes of young plants are tender and juicy. The stem of barrel cactus is eaten as a dessert when combined with sucrose, whereas potato is able to store a great amount of starch. Based on the environment in which the plants live, their stems can be classified as aerial, aquatics, or subterraneous. Among the aerial types are the subclasses named erected, creeping, or climber stems. The aquatics stems belongs to the plants that live in rivers or lakes, whereas the subterraneous stems are those growing underground and are subclassified as rhizomes, tubers, or bulbs. Rhizomes grow and extend horizontally under the soil surface and can be confused with roots, however, rhizomes possess buds from which branches, leaves, and flowers may emerge upward, and also adventitious roots downward. Rhizomes are cylindrical-shaped and are able to store reserve substances. Tubers are subterranean stems that possess evident buds from which may emerge aerial branches. Tubers accumulate nutritive substances, mainly starch, and develop spherical to ovoid shapes, such as potatoes. The bulbs are round-shaped subterranean stems that possess floral and lateral buds established over a basal plate or disc from which adventitious roots grow downward. Onion is an edible example of this type of stems whose fleshy concentric layers store reserve substances (Fig. 6.6).

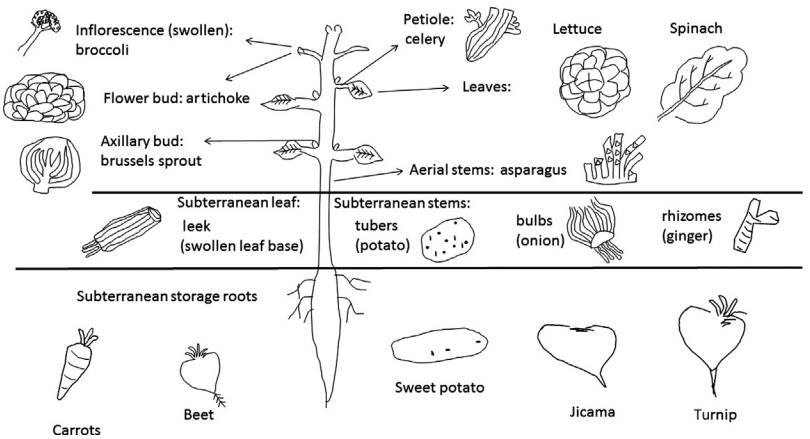

FIGURE 6.6
Derivation of vegetables.

6.6 THE LEAF

Among the most common horticultural leafy edible crops are lettuce, spinach, leek, celery, endives, and chards (Fig. 6.7). The first leaves originate at seed germination from plumule, and the following leaves to appear come from terminal and axillary buds of stems and branches. Leaves become lateral laminar-shaped structures attached on the stem or its branches at the end of the petiole and may bear two lateral small leaf-like structures called stipules (Fig. 6.8). Leaves bear a bud in their axil named the axillary bud, which later may develop into a branch. The leaf blade or lamina is the green expanded part of the leaf. There is usually a middle prominent vein, called the midrib, and lateral veins connected to the midrib which provide rigidity to the leaf blade and act as a channel for the transport of water, minerals, and food materials. The primary function of leaves is to capture sunlight for the manufacturing of food reserves by photosynthesis. Leaves are also very important in exchanging gases with the environment in order to satisfy the respiration and photosynthesis requirements, in addition to exchange of other gases such as ethylene and water vapor. During gas exchange, the leaf opens its tiny pores, named stomata, located mainly on the lower epidermis. When stomata are

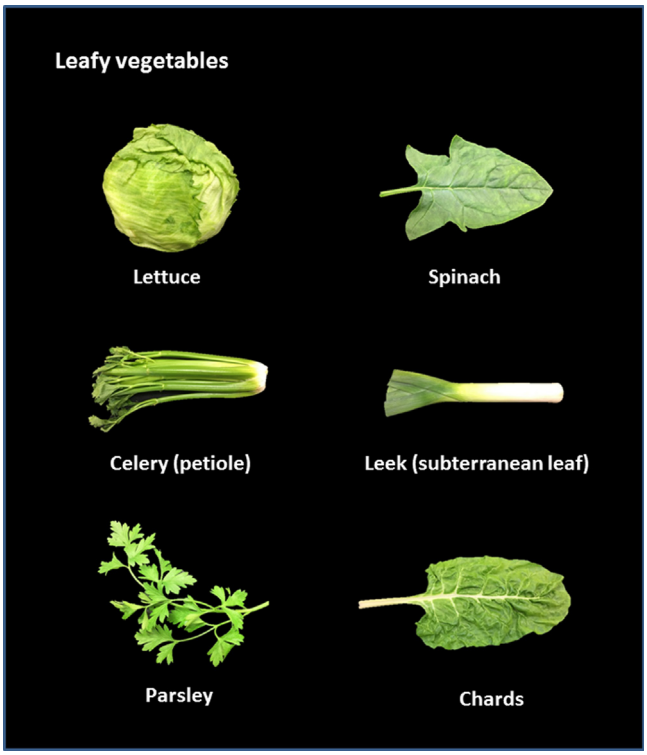

FIGURE 6.7
Some leafy vegetables.

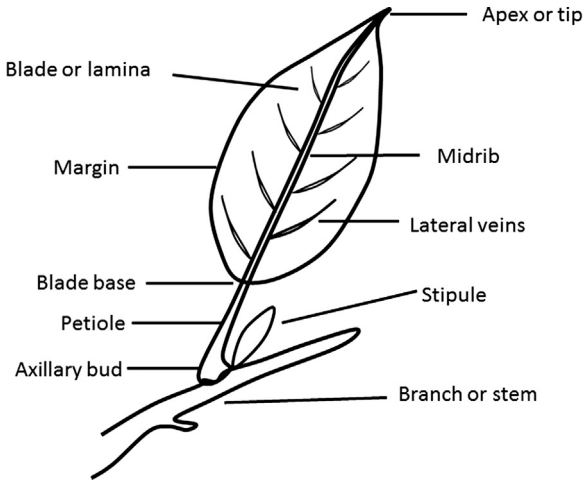

FIGURE 6.8
Parts of the leaf.

open, carbon dioxide enters into the leaf, and simultaneously oxygen and water vapor escape into the atmosphere. The phenomenon of water vapor exchange carried out by plants is called transpiration. The water that is lost by the plant due to transpiration should be rapidly recovered by the roots in order to avoid wilting.

The high surface-to-volume ratio of leaves due to their laminar morphology allows them to be very efficient organs for sunlight capture, plant temperature regulation, and gas exchange. Leaves easily lose water due to their laminar shape and the high stomata density (number of stomata/mm^2). However, once the leaves have been detached from the plant, they continue transpiring and no more water can be recovered from the soil. Hence, the loss of water from the leaves after harvest must be reduced in order to avoid rapid wilting of the produce. Accordingly, postharvest handling techniques acquire great relevance, and both the temperature and the relative humidity of storage must be optimal for the optimum preservation of leafy vegetables. Likewise, the high surface area-to-volume ratio of leaves, such as in lettuce and cabbage, permits an efficient precooling of the produce by using the method of vaccum-cooling.

6.7 THE FRUIT

Botanically speaking the fruit of a flowering plant (angiosperm) is a reproductive organ that can be edible or inedible. However, for the purpose of this textbook fruit has been described in terms of being an edible organ. The fruit is a mature ovary or mature ovaries of one or several flowers, developed after fertilization of flowering plants. After fertilization, all floral organs naturally detach, except the ovary that persists, develops, and undergoes transformations

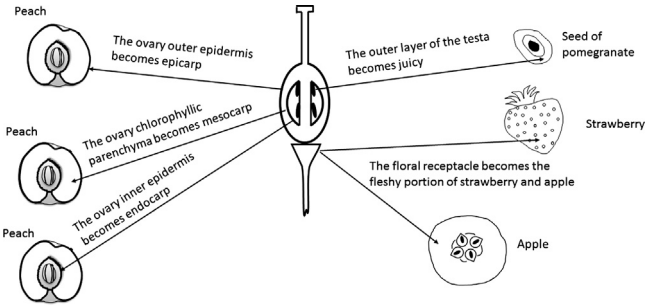

FIGURE 6.9
Derivation of fruits.

to becoming a fruit. In some cases, floral structures united with the ovary also develop, in which case it is referred to as accessory fruit. Strawberry is a fruit that has most of its edible part as a product of the development of the floral receptacle (Fig. 6.9). Furthermore, a fruit may be formed without fertilization of the ovary and if this is the case then it is called parthenocarpic fruit.

6.7.1 Parts of the Fruit

Generally, the fruit consists of two main parts: pericarp and seed. The pericarp tissue is often the edible part of the fruit and generally develops from the ovary wall (Fig. 6.9).

6.7.1.1 THE PERICARP

The pericarp may be dry or fleshy and is composed of three layers not easily distinguishable in dry fruits, but if the pericarp is thick and fleshy these layers may be easily differentiated into epicarp (outermost layer), mesocarp (middle layer), and endocarp (inner layer surrounding the seeds) (Fig. 6.10).

The Epicarp

The epicarp, also called the exocarp, forms the generally tough outer skin of the fruit. The epicarp is also called flavedo in the case of citrus fruits.

The Mesocarp

The mesocarp, which is found between the epicarp and the endocarp, is the fleshy middle layer of the pericarp. It is usually the edible part of the fruit, and is eaten along with the epicarp when the latter is rather soft, for example, peach, plum, guava. The mesocarp is known as albedo in the case of citrus fruits, where the epicarp or flavedo along with the mesocarp or albedo constitute the peel that is generally the nonedible part of the citrus fruit.

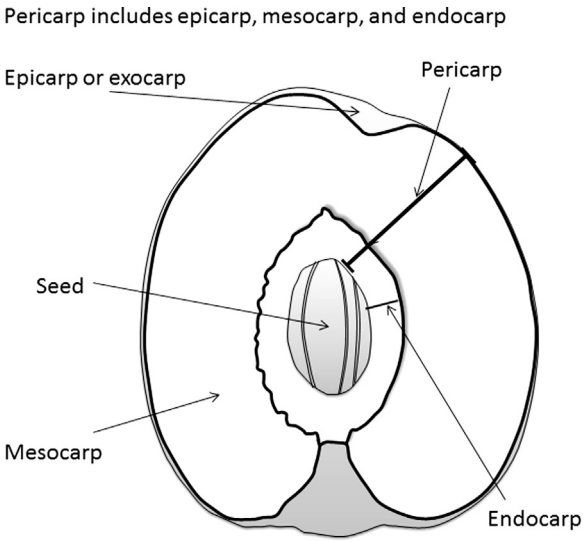

FIGURE 6.10
Parts of fruits.

The Endocarp

The endocarp is the innermost layer of the pericarp, which directly surrounds the seeds. This may be very hard and nonedible as in drupes (also called stone fruits) such as peaches, plums, and cherries, or may be membranous and edible as in the case of citrus fruit where the endocarp is separated into segments filled with juicy vesicles. In some cases, such as lychee, longan, and pomegranate, the edible portion of the fruit is not derived from the pericarp but the aril, which is the fleshy cover of some seeds, usually arising from the funiculus.

6.7.1.2 THE SEED

Examples of edible horticultural seeds are nuts whose stony endocarp must be broken to release the inner edible content, whereas the rest of the pericarp is inedible. Once fertilized the ovule develops into seeds whose general structure is made up of a seed coat and an embryo. The seed coat has two layers, the outer testa and the inner tegmen, whereas the embryo is made up of radicle and plumule. The ovules from the ovary become seeds, whereas the ovule wall becomes the seed coat.

6.7.2 Classification of Fruits

Botanically, fruits are classified in accordance with the number of ovaries and flowers involved in their development into three major categories: simple, aggregate, and multiple or composite (Table 6.1). Simple fruits are derived from one flower of a simple or compound ovary. A simple ovary is made up of a simple carpel, whereas a compound ovary is made up of two or more

Table 6.1 Classification of Edible Fruits

Simple fruits		
Simple monocarpus *(it develops from a simple ovary)*		
Dry	**Legume**	Dry- and pod-shaped fruit that possesses multiple seeds. Its immature pericarp is edible but withers and turns inedible and exhibits ventral and dorsal dehiscence at maturity, e.g., pea, green beans (Fig. 6.11)
	Caryopsis	Monospermy dry fruit whose very thin and small pericarp is fused with the seed, e.g., sweet corn (Fig. 6.11)
Fleshy	**Drupes**	Mesocarp is fleshy . The endocarp is a hard and woody layer that covers the seed, e.g., peaches, plums, walnuts, apricots, almonds
Simple syncarpous **(it develops from a compound ovary)**		
Dry	**Balausta**	Dry-coriaceous pericarp wrapping a cavity divided by false membranous walls with inserted seeds and juicy testa, e. g., pomegranate
Fleshy	**Berries**	Fleshy fruit of tiny fleshy endocarp whose frequently numerous seeds are found centered or dispersed all over the mesocarp, e.g., tomatoes, grapes, guavas
	Pepos	Fruit of hard epicarp, fleshy, and well-developed mesocarp. The fleshy and juicy endocarp is usually confused with the mesocarp, e.g., cucumbers, cantaloupes, watermelons, squash.
	Pomes	Fruits whose seeds are enclosed by a membranous endocarp. The central portion of the fruit, named the core, develops from the carpel and is clearly differentiated from the surrounding fleshy portion that develops from the floral receptacle, e.g., apple, pear, quince
	Hesperidium	Thick and soft epicarp that possesses essence excretory glands. Mesocarp is thin, soft, and white that can be spongy or compacted. Edible and juicy endocarp is divided into segments, e.g., all citrus
Aggregate fruits **(it develops from numerous separated ovaries of a single flower)**		
Poly-aquene		Fruit formed by several single-seeded fruitlets named aquenes is characterized by possessing a dry pericarp that is easily loosen from seed, e.g. strawberry
Poly-drupe		Fruit formed by several fruitlets that are drupes attached to a dry receptacle, e. g., raspberries, blackberries
Multiple fruits (also named composite fruits or infructescences. **It develops from clusters of many separate flowers of individual ovaries).**		
Sicono		Fruit composed by the fusion of numerous small aquenes surrounded by a fleshy-developed receptacle. It possesses a small pore at bottom end, e.g. figs
Sorosis		Infructescence formed by a moderately fleshy axis surrounded by the numerous and compacted fruitlets that come from the inflorescence. Bracts and calyces may be notorious, e.g., pineapples, cherimoyas

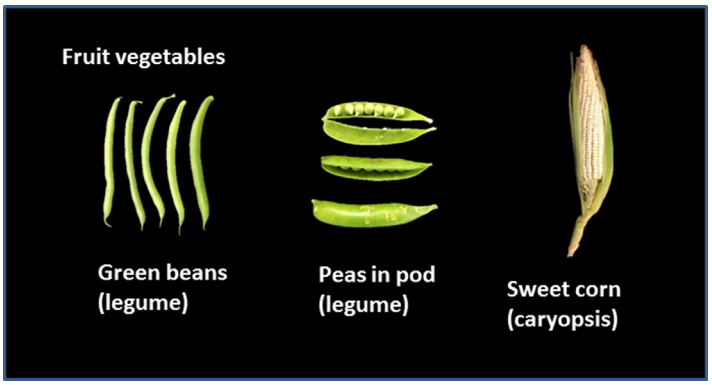

FIGURE 6.11
Some fruit vegetables.

fused carpels. Simple fruits of a compound ovary are also called syncarpous or fused fruits. Simple fruits can be either dry or fleshy. Dry fruits may be either dehiscent or indehiscent. Dehiscent fruits are those fruits whose structure is split in order to release their mature seeds, a phenomenon named dehiscence, which is not observed in indehiscent fruits. Simple fleshy fruits include the berries (such as grapes and tomatoes), drupes (such as peaches, cherries, apricots, olives, and plums), pomes (such as apples and pears), pepos (such as cucumbers and pumpkins), and hesperidium (such as oranges and lemons). Simple dry dehiscent fruits include the legumes (such as peas and peanuts). Simple dry indehiscent fruits include the achenes (such as caraway and sunflower) and balausta (such as pomegranates). Aggregate fruits develop from a single flower of numerous ovaries. Aggregate fruits include poly-achenes (such as strawberries) and poly-drupes (such as raspberries). Multiple fruits or infructescences develop from inflorescences which are clusters of many separate flowers of individual ovaries, for example, sicono (such as fig) and sorosis (such as pineapple and cherimoya). Berries develop from compound ovaries, and possess a fleshy endocarp, and usually contain many seeds.

6.8 SUMMARY AND CONCLUSIONS

This chapter describes the structural organization of flowering plants based on morphological and anatomical aspects focusing on horticultural crops marketed for edible purposes. Since the relationship between produce and environment is dynamic and depends on many factors, the specific characteristics for the dermal, ground, and vascular systems are addressed. Anatomical characteristics of produce, such as the number of stomata on epidermis, type of surface, thickness and chemical composition of wax and cuticle, tissue underlying the skin and the structure, each play an important role in determining the extent of the produce–environment interaction, and these features vary greatly among fruits. The different parts of a plant, such as the roots, stems,

leaves, and fruits are presented separately and commercial examples of products are given. This chapter includes a classification of fruits based upon fruit ontogenesis for which the flower characteristics are of main concern. The great diversity in morphology and anatomy of horticultural commodities complicates their classification in order to integrate groups in a convenient manner to facilitate their postharvest handling. However, same plant parts or organs from different species may present similar anatomical and physiological characteristics and therefore similar postharvest handling conditions, that is, many leafy vegetables such as lettuce, cabbage, and spinach can be subjected to similar postharvest handling techniques such as precooling and storage conditions (i.e., low temperature and high relative humidity). Similarly, for root vegetables (such as carrot, radish, and beetroot) or stem vegetables (asparagus). The group of fruits and fruit vegetables show more diversity in postharvest requirements than vegetables, due to differences beyond the morphological and anatomical characteristics. Aspects such as physiological behavior, climate/region of origin, and stage of development at harvest are more determinants to grouping fruits in groups of similar storage conditions.

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CHAPTER 7

Ripening and Senescence

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7.1 INTRODUCTION

Fruit ripening is probably one of the more complex developmental processes by which a plant organ suffers profound physiological and biochemical transformations. During early phases of growth and development, fleshy fruits are green, accumulate water and nutrients, and are covered by thick epidermal layers providing protection for seed. After fruit development has been completed, ripening evolves a series of transformations characterized by changes in color, texture, aroma, nutrients, etc. making the fruit attractive for predators to facilitate seed dispersal, and also nutritious for human consumption. Distinct structural, physiological, and biochemical mechanisms may operate during ripening in the different types of fruits but, in general, they evolve a series of sensory and nutritional changes that have been conserved throughout evolution and domestication in many species and humans have adapted for consumption in the diet.

For many years, ripening and senescence were considered as a series of degradative processes culminating with metabolic disruption and cellular disintegration. Despite natural ripening involving catabolic pathways and loss of cellular compartmentalization, it is generally believed that ripening is an active and genetically regulated program by which fruit prepares the biochemical and molecular transformations required during the last stages of development to culminate with senescence. Many of these changes comprise the traits of fruit quality and, therefore, understanding the biochemical and genetic basis regulating these processes have been the subject of intensive research. Moreover, the control of fruit ripening is essential to maintain quality and to reduce the losses during the postharvest shelf-life. Tomato fruit has become the model system to understand the basis of ripening and quality, mainly due to the following features: (1) well-characterized ripening program regulated by

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ethylene, (2) collection of mutants affected in ripening and other fruit properties, (3) short life cycle, and (4) genetic transformation and genomic resources (Klee and Giovannoni, 2011). In fruits of other agronomically important crops in which solid information of the ripening physiology is available, the advent of new large-scale "omic" approaches is now improving our understanding of the biochemical and molecular events regulating ripening. The objective of this chapter is to critically summarize the current understanding of regulation of fruit ripening and the mechanisms accompanying more relevant ripening-related processes, with emphasis on key pathways and their impact on fruit quality.

7.2 REGULATION OF FRUIT RIPENING

7.2.1 Climacteric and Nonclimacteric Ripening

The plant hormone ethylene influences many developmental processes in plants, including ripening and senescence, and responses to biotic and abiotic stresses. The involvement of ethylene in fruit ripening has long been known and there are many ancestral postharvest fruit manipulations that are now recognized as being mediated by ethylene (Bapat et al., 2010; Grierson, 2013). Based on the respiration rate and ethylene production, fruit ripening has been classified into two types: climacteric and nonclimacteric. Climacteric fruits are characterized by an increase in the rate of respiration and ethylene production at early stages of ripening (Fig. 7.1). This climacteric behavior is invariably

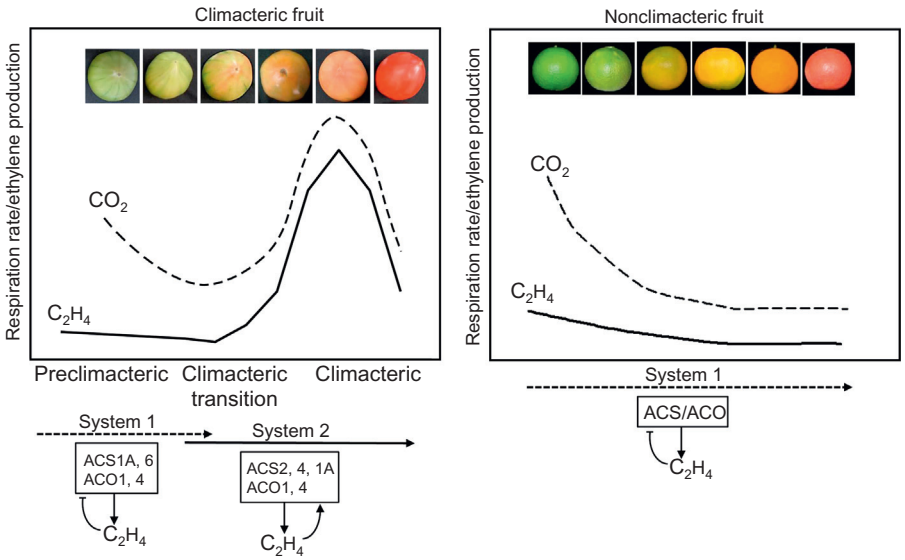

FIGURE 7.1

Generalized pattern of respiration rate and ethylene production during development and ripening in a climacteric (tomato) and a nonclimacteric (mandarin) fruit, and esquematic model of regulation of ethylene production during the transition from development to ripening in both fruits.

associated with an autocatalytic control of ethylene production. Within this category are banana, apple, pear, tomato, avocado, melon, peach, kiwi, etc. By contrast, nonclimacteric fruits are those in which no increase or reduction in the respiration rate and ethylene production takes place during the whole ripening period (Fig. 7.1), and includes fruit such as strawberry and other berries, citrus fruits, grapes, and cherry. Then, the increase in respiration appears not to be a strict requirement for fruit ripening as nonclimacteric fruits suffer ripening changes similar to those of climacteric without the burst in respiration. However, in general, elevated respiration rates accelerate fruit ripening and shorted postharvest life, in both type of fruits.

A second difference between climacteric and nonclimacteric ripening is the response to exogenous ethylene. In climacteric fruits, ethylene accelerates the time to reach the maximum respiration rate, without modifying the magnitude. In nonclimacteric fruits, ethylene increases the respiratory rate in a concentration-dependent manner. Once ethylene is removed, respiration declines to basal levels in nonclimacteric fruits since they lack autocatalytic ethylene production, but in climacteric fruit, once the autocatalysis is initiated, respiration follows at normal rates (Toivonen, 2016). These effects of exogenous ethylene illustrate the action of endogenously produced ethylene during maturation and are commercially relevant because ethylene may be a pollutant during the postharvest life of the fruit.

The onset of climacteric respiration is not always coordinated with the increase in ethylene production, it depends on the fruit species, and the maximum of each process may not take place simultaneously. The biochemical basis of this relationship is not fully understood but physiological and biotechnological evidences indicate that ethylene is the trigger factor for the increase in respiration rate, and then, the climacteric respiration can now be considered as an ethylene-regulated event (Grierson, 2013).

The classical concept of climacteric ripening considered that ethylene is not involved in the control of ripening in nonclimacteric fruits. This assumption is an oversimplification, since despite the lack of climacteric ethylene many ripening processes in nonclimacteric fruits are responsive to ethylene and natural ripening also requires low basal ethylene levels to proceed. Thus, ethylene sensitivity appears to be a fruit-specific feature independent of their climacteric and nonclimacteric behavior and is of special relevance during the handling and management of the fruit during the whole postharvest chain (Bapat et al., 2010; Toivonen, 2016; Table 7.1). Therefore, ethylene is a crucial regulator of ripening in climacteric fruits but it also plays a role in nonclimacteric fruits, probably by different metabolic networks in which changes in tissue sensitivity to the gas or interactions with other factors may be important. Then, other ripening models (referred to as pseudo-climacteric) in addition to the classical climacteric and nonclimacteric fruit may be considered, comprising fruits with different ethylene requirements and sensitivity to small amounts of the gas (Grierson, 2013).

Table 7.1 Comparison of Ethylene Production and Sensitivity in Selected Climacteric and Nonclimacteric Fruits

Commodity	Ethylene Production	Ethylene Sensitivity
Climacteric		
Apple	VH	H
Apricot	H	H
Avocado	H	H
Banana	M	H
Cherimoya	VH	H
Kiwi fruit	L	H
Mango	M	H
Melon	M	H
Nectarine	H	M
Papaya	H	H
Peach	M	H
Pear	H	H
Persimmon	L	H
Plum—prune	M	H
Tomato, mature green	VL	H
Tomato, ripen	H	L
Watermelon	VL	H
Nonclimacteric		
Berries	L	L
Cherry	VL	L
Citrus fruit	VL	M
Grapes	VL	L
Pineapple	L	L
Pomegranate	L	L

VL = very low, L = low; M = moderate, H = high, VH = very high.

7.2.2 Ethylene Biosynthesis and Perception

Although the involvement of ethylene in fruit ripening has long been recognized, direct evidences of the essential role of ethylene in climacteric fruits ripening come from fruits with reduced synthesis and perception of the gas that did not ripen properly and display long postharvest shelf-life (Klee and Giovannoni, 2011). To explain the differences in ethylene production and responses between climacteric and nonclimacteric fruits, McMurchie et al. (1972) postulated the occurrence of two systems of ethylene production: System 1 would be responsible for the basal levels of ethylene during normal growth and also in responses to stress conditions in both climacteric and nonclimacteric fruit and vegetative tissue, and it would be negatively regulated by ethylene. System 2 is exclusively present in climacteric fruits and is responsible for the autocatalytic ethylene. Ethylene production in nonclimacteric fruits is restricted to System 1, but climacteric fruits have the capability to shift to

System 2 at the onset of ripening, and then regulate the massive increase in ethylene production.

The pathway of ethylene biosynthesis in plants is now well established and its regulation during fruit ripening, especially in tomato, has been exhaustively studied. Ethylene is formed from *S*-adenosyl-L-methionine (SAM) via two steps: the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS), and the oxidation of ACC to ethylene by ACC oxidase (ACO), which also generates carbon dioxide and hydrogen cyanide. Transcriptional regulation of ACS is one of the key regulatory points of ethylene biosynthesis, as ACS is the rate-limiting step of the pathway. ACS is encoded by a multigene family, with at least 14 members in the tomato genome, whose expression is differentially regulated in the different tissues by developmental and environmental stimuli. ACO is an ascorbate-dependent dioxygenase, encoded by a small gene family (six in tomato) and in most physiological circumstances is not the rate-limiting step of ethylene production (Barry and Giovannoni, 2007; Gapper et al., 2013).

Transcriptional analysis of ACS and ACO gene members during tomato fruit development and ripening revealed a complex interplay that may well explain the changes in ethylene production (Fig. 7.1). Low levels of ethylene production during early stages of tomato fruit development (preclimacteric green fruit) appear to be sustained by the expression of *LeACS1a* and *LeACS6* genes (System 1), both being negatively regulated by ethylene. The transition to the climacteric phase is determined by an increase in the expression of *LeACS2* and *LeACS4* that are stimulated by ethylene and thus responsible for the autocatalytic ethylene production (System 2). Two *ACO* genes (*LeACO1* and *LeACO4*) are expressed at the preclimacteric stage but at the onset of ripening are upregulated in parallel with the climacteric rise of ethylene production (Liu et al., 2015). This pattern of changes indicates a coordinated expression of specific *ACS* and *ACO* gene members in a specific and temporal manner regulating the transition from low (System 1) to high and autocatalytic (System 2) ethylene production during fruit ripening. Expression of other ACS and ACO genes may contribute to the fine-tuning of regulation of ethylene production at specific stages, and in response to other situations. Moreover, other posttranscriptional factors such as ACS phosphorylation, or protein stability, may also be important contributors to the control of ethylene production. Nonclimacteric fruits are able to produce ethylene in response to different environmental cues but the major difference with climacteric fruits is the lack of ACS and ACO genes regulated by ethylene in an autocatalytic manner (Gapper et al., 2013).

Ethylene receptors are functionally related to bacterial histidine-kinases, located in the endoplasmic reticulum and comprise two families of proteins: receptors most homologous to histidine-kinases (subfamily I) and receptors lacking the kinase domain (subfamily II) (Klee and Giovannoni, 2011). Six

ethylene receptors, three of each subfamily, have been identifying in tomato; but the number of ethylene receptors differs for each plant species. The onset of tomato fruit ripening is associated with an upregulation of the *LeETR4*, *LeETR6* receptor genes, whereas other components remain constitutively expressed. The model postulated for the mode of action of ethylene established that receptors are negative regulators of ethylene signaling. In the absence of ethylene, the responses are repressed and the binding of ethylene to the receptor alleviates the repression and consequently allows the response to ethylene. This model predicts that elevated expression of a receptor gene and accumulation of the corresponding protein would inhibit ethylene responses and, in contrast, reduced ethylene receptor activates the responses to the gas. The different patterns of expression of the ethylene receptor genes are compatible with the involvement of LeETR4, LeETR6 in the response to ethylene at the onset of ripening. Moreover, LeETR4 and LeETR6 proteins are degraded in response to ethylene during accelerated fruit ripening. Experiments with transgenic plants in which ethylene receptors have been altered indicated functional compensation between some of the ethylene receptors. Moreover, receptors suffer phosphorylation and proteasome degradation, indicating that a complex metabolic network with multiple protein interactions operates in the signaling of ethylene during fruit ripening (Klee and Giovannoni, 2011). The expression of ethylene receptor genes has been determined during ripening of several climacteric (apple, pear, peach, melon, kiwi) and nonclimacteric fruits (grapes, citrus, strawberry) and, in general, does not always follow a pattern of changes parallel with the development of ripening. Thus, expression of the ethylene receptor genes does not necessarily reflect a direct relationship with the role of ethylene in fruit ripening (Grierson, 2013).

Signaling of ethylene implies a series of proteins acting downstream of the ethylene receptors. The Green Ripe (GR) protein was identified in a nonripe green tomato mutant and biochemical and genetic evidences indicate that it may interact with ethylene receptor proteins affecting the binding activity. This interaction represents additional components affecting the responses to ethylene. Ethylene receptors directly interact with the so-called constitutive triple response (CTR1) elements that are also negative regulators of ethylene signaling. Several elements have been deciphered thanks to studies in tomato, such as the demonstration of LeCTR1 function which comes from transgenic plants in which silencing of this gene accelerated tomato fruit ripening. Downstream CTR1, an Ethylene-Insensitive2 (EIN2) positively regulate ethylene signaling and mediated the interaction with the transcription factors EIN3/EIL (EIN-like). Although expression of LeEIN2 is ethylene-independent, its suppression inhibited fruit ripening and reduced ethylene sensitivity. EIL represent a family of genes that in tomato display differential expression during ripening and some appear to be related to the regulation of *ACS* and other ripening-related genes. Finally, these signaling pathways culminate with the transcriptional

regulation of a large family of ethylene transcription factors that regulate the expression of ethylene-responsive genes. This cascade of ethylene perception and signaling illustrates the complexity of the transduction from the ripening stimuli to the responses, with the participation of different elements and multiple interactions (Grierson, 2013; Liu et al., 2015).

7.2.3 Transcriptional and Epigenetic Regulation

The collection of ripening-related mutations of tomato has been essential to identify regulatory elements involved in the developmental regulation of ripening and the action of ethylene. Fruits of the *rin* (*ripening-inhibitor*), *nor* (*non-ripening*) and *Cnr* (*colorless non-ripening*) mutants fail to ripen properly, do not produce climacteric ethylene, and do not ripen in response to the gas (Klee and Giovannoni, 2011). *RIN* has been of particular interest in breeding programs and modern cultivars with extended postharvest life are hybrids carrying the *rin* allele. The RIN gene is a transcription factor of the APETALA MADS-box family that appears not to be involved in the initiation of ripening. *RIN* was thought to play a central role in the regulation of ripening, controlling ethylene-dependent and -independent pathways. However, recent evidences indicate that *rin* is not a null mutation, rather it is a dominant mutation conferring repression of ripening-related genes (Ito et al., 2017). The CNR protein is a promoter-binding protein, located upstream *rin* or *nor*, interacting with the promotor of many key ripening-related genes and when mutated produces a blockage of the ripening process (Gapper et al., 2013). It should be noted that both RIN and CNR proteins are conserved in climacteric and nonclimacteric fruits and may then be master regulatory factors. The *Nor* mutation also has severe effects on ethylene-related ripening genes and appears to act independently of *rin*. Downstream of these transcription factors a number of transcriptional regulatory elements have been identified, being either negative like the tomato homebox protein 1 (LeHB-1), and tomato NO apical meristem transcription activator factor 1 (SINAC-1) or positive Tomato Agamous-like 1 (TAGL1) regulators, or the transcription factors FRUITFUL that, to different extents, are involved in the complex interplay regulating fruit ripening (Gapper et al., 2013; Liu et al., 2015).

In recent years epigenomic modifications are evidenced to play an important role in the control of fruit ripening. The first evidence of this implication derives from the identification of the *Cnr* mutation, which encodes a SQUAMOSA promoter binding protein with a high degree of hypermethylation of the cytosine residues in the promotor. This indicates that hypermethylation of key ripening genes may be associated with a nonripening phenotype (Giovannoni et al., 2017). Further evidences have demonstrated that treatment of immature tomato fruit with inhibitors of methylation promoted fruit ripening, and demethylation of the *Cnr* promoter induced red coloration and stimulated other ripening processes (Zhong et al., 2013). The emerging conclusion from these studies indicates that the transition to ripen is associated with a

progressive demethylation of the tomato genome and the timing of this process is correlated with the acquisition of the sensitivity to ethylene. Then, cytosine methylation in promoter regions of ripening-related or induced-ethylene genes would suppress the transcription and the ripening process. Therefore, epigenetic changes by the dynamics of methylation/demethylation in the promoter of specific genes also contributes to the coordination of the timing and development of fruit ripening.

7.2.4 Interaction With Plant Hormones

The existence of a hormonal interaction in the control of fruit ripening has long been suggested, and even ethylene action appears to be critical in climacteric fruit, however it is still unknown if there are other hormonal signals initiating the process. In nonclimacteric fruit the involvement of other hormones controlling the process has been also suspected (Kumar et al., 2014). Auxins have long been recognized to affect initiation and development of ripening in fruits of many species, and it is well documented that a reduction in indole-3-acetic acid (IAA) concentration takes place before the onset of ripening. Moreover, exogenous auxins also delay fruit ripening in grapes or strawberry, and inhibited the expression of some ripening- and ethylene-dependent genes in tomato fruit (Su et al., 2015). Moreover, altered expression of Auxins Response Factors (*SlSARF2* and *4*) disturbs tomato fruit ripening and suppressed the expression of regulatory genes, such as *NR*, *CNR*, or *NOR*. All these evidences indicated an interaction between auxins and ethylene in the control of ripening in both climacteric and nonclimacteric fruits, by mechanisms that are still poorly known (Liu et al., 2015).

Abscisic acid (ABA) has been demonstrated to stimulate fruit ripening in both climacteric and nonclimacteric fruits. Initial observations indicated that exogenous ABA induced ripening in the fruit of several crops. More recently, it has been observed that ABA stimulated accumulation of anthocyanins and reduced acidity in grapes. In other nonclimacteric fruits, such as orange or strawberry, it was also shown that ABA deficiency delayed fruit coloration. These observations indicate that ABA may be the trigger stimuli to initiate ripening of fruits in this ripening category (Klee and Giovannoni, 2011). In climacteric fruits, such as tomato, manipulation of ABA levels (by exogenous treatment or chemical and biotechnological inhibition) altered the rate of fruit ripening in an ethylene-dependent manner (Liu et al., 2015). A similar interaction has been also demonstrated in other fruits, such as peach and banana, and it was demonstrated that ABA may be a signal triggering ethylene biosynthesis in climacteric fruits, and could be a potential link between developmental (ABA produced by the seeds) or environmental factors (water shortage) and the inception of the ripening and senescence program. Other plant hormones, such as gibberellins, are recognized to delay fruit ripening in citrus or tomato. Since gibberellins are antagonists of ABA and ethylene, whether their involvement in ripening is a direct effect or mediated by the action of these hormones remains to be determined (Kumar et al., 2014).

7.3 ETHYLENE-DEPENDENT AND -INDEPENDENT FRUIT-RIPENING EVENTS

It is becoming clear that ethylene is involved in the ripening of climacteric fruits and also participates in that of nonclimacteric fruits, suggesting the regulation of several ripening-related events may have been conserved throughout evolution. However, it is also evident that not all the events occurring during the ripening program are completely dependent on ethylene or at least it appears that there are different thresholds of ethylene sensitivity. Early observations of fruits stored under a controlled atmosphere indicated the existence of ethylene-dependent and ethylene-independent processes during fruit ripening. Application of the ethylene action inhibitor 1-MCP (1-methylcyclopropene) and experiments with transgenic plants with attenuated ethylene production have been useful to delineate the involvement of ethylene in different ripening events (Pech et al., 2008; Watkins 2008). As mentioned previously, climacteric respiration has been demonstrated to be an ethylene-mediated response. Charentais melon with reduced ethylene production has altered rind yellowing, softening of the flesh, development of the peduncle abscission zone, aroma formation, and climacteric respiration, indicating that these processes are totally or partially ethylene-dependent. Other processes such as pulp coloration, accumulation of sugars, and loss of acidity were ethylene-independent. It has also been observed that although fruit softening is under ethylene control, there are some cell wall hydrolytic enzymes acting in an ethylene-independent manner (Pech et al., 2008). The emission of volatile compounds and aroma formation are also processes sharing ethylene-dependent and -independent components. Thus, in fruits of transgenic plants with reduced ethylene production or treated with 1-MCP, the emission of volatile organic compounds is compromised and loses part of their characteristic flavor (Rambla and Granell, 2013). It should be mentioned that fruits' responses to ethylene are specific, and the threshold of sensitivity to the hormone for each ripening event may change considerably among fruits of different species.

7.4 CHANGES IN FRUIT COLOR

The most visible sign of fruit ripening of many fruits is the development of color, which is mainly determined by the loss of chlorophylls and the accumulation of colored pigments. There are three main types of pigments in fruit: chlorophylls (Chl), carotenoids, and anthocyanins. Other pigments, such as betalains, quinones, phenalones, and phyrones are less common in fruits (Gross, 1987).

7.4.1 Chlorophylls

Two forms of chlorophyll exist in higher plants: Chl a and Chl b. These pigments are responsible for the green color of immature and mature-green fruits and tend to decrease during ripening, being, in general, absent in ripe fruits

(Gross, 1987). Chl originates from glutamic acid after a series of reactions that lead to the formation of a porphyrin ring bound to a phytol chain, which is an isoprenoid derivative synthesized by the reduction of geranylgeranyl pyrophosphate (GGPP). GGPP is also a common precursor of tocopherol, phylloquinone, and carotenoids.

During ripening Chl b is converted to Chl a by Chl b reductase (CBR) and 7-hydroxymethyl Chl a reductase (HCAR). Chl a degradation starts with the removal of magnesium to convert Chl a to pheophytin a (Phein a) by the magnesium-dechelatase. Phein a is then hydrolyzed by pheophytinase (PPH) to yield pheophorbide a (Pheide a) and phytol. Subsequently, Pheide a is cleaved by pheophorbide a oxygenase (PAO), resulting in the red Chl catabolite (RCC), which is then catalyzed by red Chl catabolite reductase (RCCR) to generate primary fluorescent Chl catabolite (pFCC). Finally, the pFCC is converted to nonfluorescent chlorophyll catabolites (NCCs) by nonenzymatic tautomerization (reviewed in Zhu et al., 2017).

During fruit ripening, the Chl content decreases in most fruits and is usually accompanied by a coordinate biosynthesis and accumulation of other pigments such as carotenoids. Nevertheless, there are some exceptions to this general behavior. Thus, some fruits retain Chl at ripe stages, like the skin of green apples (e.g., Granny Smith) and pears (e.g., Conference) and the pulp of green kiwifruit (Hayward). In addition, there are some mutants with impaired Chl degradation, known as stay-green, that also retain Chl at ripen stage. Examples are the orange mutant Navel Negra (*nan*) and the tomato green flesh (*gf*). Since carotenoid accumulation in these mutants is not impaired, fruit develop a brownish appearance as a consequence of the addition of the green color of Chl to the orange or red coloration, respectively, provided by carotenoids (Alós et al., 2008). Repression of expression and loss-of-function mutations in the *STAYGREEN* (*SGR*) gene has been associated with this phenotype.

7.4.2 Carotenoids

Carotenoids are a large family of isoprenoid compounds biosynthesized by tail to tail linkage of two GGPP molecules. Their basic structure is a C40 backbone skeleton from which all the individual variations are derived. This skeleton can be modified by cyclization at one or both ends of the molecule, by changes in the hydrogenation level, addition of oxygen-containing functional groups and by shortening or extension of the chain. In general, carotenoids can be divided into two groups: carotenes, which consist of hydrocarbon backbone, and xanthophylls, which contain oxygen atoms in their structure, with hydroxy and epoxy being the most common oxygenated groups (Britton,1998).

According to their total carotenoid content, fruits can be classified into four groups: low (between 0 and 1 μg/g FW), moderate (1−5 μg/g FW), high (5−20 μg/g FW), and very high (more than 20 μg/g FW) (Britton and Khachik, 2009). Menawhile, when considering the carotenoid profile of the fruits at the ripe stage, another classification has been made (Bramley, 2013) and consists

of eight groups: group I includes fruits with insignificant amounts of carotenoids; group II fruits with a chloroplastic-type carotenoid pattern, mainly lutein, β-carotene, violaxanthin, and neoxanthin; group III clusters fruits with large amounts of lycopene accompanied by partly saturated acyclic polyenes such as phytoene, phytofluene, or ζ-carotene; group IV fruits containing large amounts of β-carotene and its hydroxyl derivatives, β-cryptoxanthin and zeaxanthin; group V, fruits with moderate to large amounts of epoxides such as violaxanthin, anteraxanthin, or luteoxanthin; group VI, fruits containing unique carotenoids such as capsanthin; group VII, poly-*cis* carotenoids; group VIII fruits with apocarotenoids such as β-citraurin. Table 7.2 shows carotenoid content and composition in fruits belonging to these groups from the main crops in the world.

The carotenoid biosynthetic pathway has been extensively investigated over the years and key biosynthetic steps and their regulation are fairly well understood. In plant tissues, carotenoids are formed from the 2-methyl-erythritol-phosphate (MEP) pathway which generates GGPP that is then used to synthesize phytoene via phytoene synthase (PSY), the first committed step in carotenogenesis. Subsequently, a series of desaturation and isomerization reactions

Table 7.2 Carotenoid Content and Composition in Selected Highly Produced Fleshy Fruits Around the World

Commodity	Tissue	Total Carotenoid Content (mg/g FW)	Main Carotenoids
Tomato	Whole fruit	50–135	Lycopene, β-carotene, phytoene, phytofluene
Grape	Whole fruit	1–3	Lutein, β-carotene, violaxanthin
Watermelon (red)	Pulp	35–112	Lycopene, β-carotene, phytoene, phytofluene
Apple	Peel	10–25	Lutein, violaxanthin, luteoxanthin, neoxanthin
	Pulp	2–29	Lutein, violaxanthin, neoxanthin
Banana	Pulp	1–30	β-Carotene, α-carotene, lutein
Orange	Pulp	4–30	9-*cis*-Violaxanthin, 9-*cis*-antheraxanthin, β-cryptoxanthin
Mango	Pulp	12–100	Violaxanthin, β-carotene, luteoxanthin, auroxanthin
Mandarins	Pulp	20–34	β-Cryptoxanthin, violaxanthin
Melon (orange-fleshed)	Pulp	12–50	β-Carotene, ζ-carotene
Melon (white- and green-fleshed)	Pulp	0–10	Lutein, violaxanthin, luteoxanthin, β-carotene
Pear (white-fleshed)	Pulp	<2	Zeaxanthin, lutein
Pear (yellow-fleshed)	Pulp	5–11	Anteraxanthin, zeaxanthin, luteoxanthin, mutatoxanthin

catalyzed by phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), ζ-carotene isomerase (ZISO), and carotenoid isomerase (CRTISO), lead to the formation of lycopene, the red-colored carotenoid. Lycopene β-cyclase (LCYB) and lycopene ε-cyclase (LCYE) together synthesize α-carotene or, alternatively, a lycopene β-cyclase (LCYB) or a chromoplast-specific lycopene β-cyclase (CYCB; Alquézar et al., 2009) form β-carotene. Then, the hydroxylation of α-carotene and β-carotene by β- and ε-carotene hydroxylases (BCH, ECH and P450-type) generate lutein in the α-branch and zeaxanthin in the β-branch. The epoxidation and de-epoxidation of zeaxanthin by zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) constitute the so-called xanthophyll cycle. The conversion of violaxanthin into neoxanthin by neoxanthin synthase (NXS) concludes the core biosynthetic pathway (Giuliano, 2017).

During tomato fruit ripening, the increase in the carotenoid content, mainly lycopene, correlates with the induction of the expression of the fruit-specific isoform *PSY1*, accompanied by a downregulation of *LCYβ* and *LCYε*, which determine the striking accumulation of lycopene. Similarly, a relationship between the enhancement of *PSY* gene transcription and the increase in total carotenoid content during ripening has been described in other fruits (Lado et al., 2016). Consequently, expression of PSY has been considered the key regulatory step in many fruits. The cyclization of lycopene is the branching step of the pathway. At this point, lycopene β-cyclases and ε-cyclases are the enzymes catalyzing these reactions and cover a prominent role modulating carotenoid (Bramley, 2013). In fruits with a predominant composition of β-carotene, β,β- or β,ε-xanthophylls, transcriptional regulation of LCY during ripening has a remarkable influence on the carotenoid profile. In the peel of immature citrus fruits, the expression level of LCYε is high and tends to decrease during maturation, consistent with the predominance of β,ε-xanthophylls (mainly lutein) at unripe stages. Concomitantly, there is an induction of *LCYβ1* at the breaker stage, promoting the shift in the pathway from the β,ε-branch to the β,β-branch (Lado et al., 2016). In fruits with a predominant content of β,β-xanthophylls or other downstream products, such as C30 apocarotenoids and ketoxanthophylls, a crucial role for βCHX has been proposed. The biosynthesis of the ketoxanthophylls capsanthin and capsorubin in pepper fruit involves the participation of an exclusive enzyme from pepper, capsanthin-capsorubin synthase (CCS), which is functionally related to LCYB. Contrastingly, yellow and orange pepper varieties do not accumulate ketoxanthophylls because of the lack of *CCS* transcripts (CCS gene deletion) or a loss-of-function mutation in *CCS* (Lado et al., 2016).

The existence of several carotenoid biosynthetic gene isoforms allows a tissue-specific expression that appears to be a mechanism controlling the carotenoid profile in fruit tissues without affecting the composition in other organs (leaves, flowers, or roots). This tissue specialization was firstly identified in tomato fruit, where the absence of *PSY1* transcripts in green tissues was concomitant with a remarkable enzymatic activity, suggesting the existence of different *PSY* isoforms regulating carotenoid biosynthesis in green and colored

tissues. Tomato mutants with impaired *PSY1* gene expression showed a substantial reduction in carotenoid content in colored tissues without affecting pigment composition in the leaves. The *PSY2* isoform was preferentially expressed in vegetative tissues and *PSY3* was described as being upregulated in roots under stress conditions. This organ specificity was also described for *PSY* isoforms in melon, loquat, mandarin and sweet orange, and apple (Lado et al., 2016).

A similar isoform-specialization mechanism has been described for the step catalyzed by the LCYB in different fruits such as citrus, tomato, or papaya, illustrating the tissue compartmentalization in carotenoid biosynthesis. In green tissues (leaves or immature green fruits) this reaction is controlled by the *LCYβ1* gene, whereas in fruit tissues this activity is regulated during ripening by the chromoplast-specific isoform *CYCB* or *LCYβ2*. The existence of two different isoforms of *βCHX* (nonheme β-carotene hydroxylases) has also been reported in plants. In tomato and pepper, only one isoform, *CRT-b2* tomato and *CRT-b1* in pepper, are induced in flowers and fruit, respectively, indicating a specialized role regulating accumulation of β,β-xanthophylls in chromoplastic organs (Lado et al., 2016).

Carotenoids are accumulated in plastids and the development of sink structures for their sequestration in this organelle is important for the regulation of their accumulation and also affects their bioavailability. In fact, perturbations in carotenoid composition are strongly associated with changes in the type of plastid and with chromoplast-like structures arising prematurely during fruit development. The massive presence of phytoene and lycopene in tomato or in red grapefruit has been associated with the development of round plastoglobuli for phytoene accumulation as well as crystalloid structures accumulating lycopene. The same crystals are present in red papaya fruit, where lycopene is the main carotenoid (Lado et al., 2016). In contrast, in yellow-orange papaya, β-carotene and β-cryptoxanthin are accumulated in globular and tubular structures, whereas in mango β-carotene could be accumulated in both plastoglobuli and crystals depending on the *cis–trans* configuration. Interestingly, plastids of fruits with very low carotenoid content display a lack of defined substructures, whereas fruits with significant amounts of rare carotenoids developed plastids with special structures (Schweiggert and Carle, 2017).

7.4.3 Anthocyanins

Anthocyanins are the largest class of water-soluble vacuolar flavonoids. These compounds confer red, orange, violet, and blue coloration to fruits and flowers, depending on the pH. Chemically, anthocyanins are based on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C6–C3–C6) and with one or more sugar molecules bonded at different hydroxylated positions of the basic structure. The biosynthesis and regulation of anthocyanin accumulation in plants has been well characterized in several species. The initial precursor of the flavonoid biosynthetic pathway is

phenylalanine, from which different types of flavonoids are synthesized as a result of various enzymatic reactions. Hundreds of anthocyanins have been identified, most of them primarily based on six common anthocyanidins: cyanidine, pelargonidin, delphinidin, peonidin, petunidin, and malvidin. The large number of anthocyanindins arises from glycosilation, methylation, coumarylation, and a variety of other additions such as modification of acyl moieties in a species-specific manner. Most fruits contain a mixture of anthocyanins, from only a major pigment, as in passion fruit, to a complex pattern of more than 20 different anthocyanins, as found in grapes and most berry fruits. During ripening, anthocyanins are synthesized at an increasing rate, reaching a maximum at the ripened stage and concomitantly anthocyanidins and glysosilation patterns gain complexity (reviewed by Mazza, 2018).

Two groups of genes are required for anthocyanin biosynthesis: structural genes encoding enzymes directly involved in the metabolic reactions and transcription factors controlling the expression of structural genes. The regulators of transcription are termed the MBW ternary complex, because they are formed by MYB and bHLH transcription factors, together with WD40 repeat proteins. The MYBs involved in anthocyanin biosynthesis have been identified in a number of species, including model plants and fruit crops. MYB transcription factors can act as either inductors or repressors of the anthocyanin pathway. The *trans*-activation efficiency, the specificity for DNA binding and the interactions of MYBs are determined by key residues located in the N-terminal region (Hichri et al., 2011). Recently, it has been found that in *Citrus* species most of the natural variation in pigmentation associated with anthocyanins can be explained by differences in the activity of the *Ruby* gene, an MYB transcription factor (Butelli et al., 2017).

7.5 FRUIT SOFTENING

During ripening, the cell walls of the fruit suffer large modifications that make them softer and more attractive for seed dispersal. However, oversoftening produces important economic losses, since it reduces transportability, storage time, and postharvest shelf-life. Although the cell wall composition of the fruit tissues varies between species and developmentally, in general, cell walls can be defined as a mixture of rigid cellulose microfibrils embedded in a hydrated gel-like matrix phase containing noncellulosic polysaccharides: hemicelluloses (which include heteroxylans, heteroglucans, and heteromannans), pectins (including homogalacturonans (HGs), rhamnogalacturonans I and II (RG I and RGII)), glycoproteins, and in some differentiated cell types, lignin (Johnson et al., 2017). While ripening progresses, the cell wall and the middle lamella (the pectin-rich layer that binds the cell walls of two adjacent cells) suffer depolymerization of the matrix glycans, solubilization and depolymerization of pectins, and the loss of neutral sugars from the pectin lateral chains. These modifications result from the activity of enzymes such as polygalacturonase (PG), pectin methylesterase (PME), pectate lyase, and cellulase. In

addition, other nonhydrolytic cell wall proteins, such as expansins, also contribute to cell wall disassembly (Ruiz-May and Rose, 2013; Marowa et al., 2016). In other fruits, the loss of cell turgor has also been shown to be the major contributor to fruit softening. Studies on grape have suggested that changes in cell turgor can be due to the accumulation of apoplastic solutes and it has also been proposed that the loss of transpirational water through the cuticle contributes together with cell wall disassembly to the softening of tomato fruits (Gapper et al., 2013).

Tomato fruits have also been the model system to study the biochemical and molecular changes associated with softening during fruit ripening. In tomato and other type of fruits, the participation of hydrolytic enzymes, such as PG, cellulase, PME, β-galactosidase, and expansin, on cell wall metabolism and during ripening have received special attention. The exact contribution of each enzyme on the changes in fruit texture remains unclear probably due to the different compositions of cell walls in the different type of fruits, indicating the complex mechanism operating in the loss of fruit firmness (Ruiz-May and Rose, 2013). This situation is well illustrated by the fact that silencing or downregulation of some genes of cell wall-degrading enzymes produced very limited or negligible changes in fruit softening. More recently, transgenic tomato pectate lyase silencing lines with reduced gene expression and enzymatic activity displayed higher fruit firmness than control azygous wild-type lines (Uluisik et al., 2016). Importantly, the increase in fruit firmness in the RNAi lines was substantial, especially compared with other silencing lines of cell wall-degrading genes (Uluisik et al., 2016).

Evidences indicate that many cell wall modifications occurring during ripening could be general to a large diversity of fruits. However, different reactions and/or differences in the sequence of events taking place during ripening-associated softening could be fruit-specific (Ruiz-May and Rose, 2013). For example, apple fruits are quite peculiar in terms of softening because they become soft during ripening but still maintain a crispy texture, while ripe bananas are not crunchy but soft during ripening. QTL analysis has revealed candidate genes for the regulation of apple fruit texture such as *Md-ACS1*, and *Md-PG1* genes which are related to ethylene biosynthesis and cell wall hydrolysis and expansion. Similarly, several studies have associated changes in banana pulp firmness with the activities and/or gene expression levels of cell wall-degrading enzymes like PME, PG, pectate lyase, and cellulase or expansins. Moreover, the degradation of starch, which is the main component of the pulp of unripe bananas, seems to contribute to banana pulp softening (Ruiz-May and Rose, 2013).

During ripening of Charentais melon, the solubility of pectins changed significantly, although the total amount of pectin in the cell wall showed little reduction. The pattern of mRNA abundance of PG suggested that, at early ripening stages, the changes in pectin solubility are PG-independent. At later ripening stages the depolymerization of polyuronides occurred after the appearance of

PG mRNAs, suggesting that at late ripening stages pectin degradation is PG-dependent. Postharvest treatments have also shed some light on the regulation of melon softening, reinforcing the importance of the activities of PG and β-galactopyranosidase in cantaloupe melon and PME, PG, endo-1,4-β-glucanase, and β-galactosidase in "Hami" melon (Ruiz-May and Rose, 2013).

7.6 FLAVOR AND AROMA VOLATILES

Volatile emission and flavor perception are major attributes required for fruit consumer acceptance. There are four main classes of volatile compounds in fruits: terpenoids, fatty acid derivatives, amino acid derivatives, and esters. Terpenoid volatiles are synthesized through two parallel pathways: the cytosolic mevalonate (MEV) pathway and the plastidic MEP pathway. The mevalonate pathway starts from acetyl CoA and gives rise to sesquiterpenes (C15) and triterpenes (C30), while the MEP pathway starts with the condensation of pyruvate and D-glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose 5-phosphate that originate hemiterpenes (C5), monoterpenes (C10), and diterpenes (C20). Both pathways produce isopentenylpyrophosphate and dimethylallyl diphosphate, which are initial precursors, and after the sequential activity of prenyl transferases and terpene synthases (TPS) generate the hemi-, mono-, di-, and sesquiterpenes. Moreover, the compounds originated by TPSs may suffer hydroxylation, dehydrogenation, and acylation (reviewed in Dudareva et al., 2013). *Citrus* fruits produce terpenoid volatiles and are particularly interesting because they are synthesized in specific structures, the oil glands in the peel and the oil bodies of the pulp. The composition in terpenoids depends on the citrus species and variety, and on the developmental stage. In peel, limonene predominates, accounting for 90% of the volatiles, followed by a complex mixture of monoterpenes and sesquiterpenes. Interestingly, more than 55 functional TPSs have been identified in orange, with this being one of the largest families so far described (Alquézar et al., 2017). In strawberry, β-myrcene and β-pinene have a significant contribution to fruit aroma. In apple (E-E)-α-farnesene is one of the major volatiles of ripe fruits and it has been suggested as a causal agent of scalding, a physiological disorder occurring during storage. The biosynthesis of this volatile compound is associated with ethylene production and the treatment with 1-MCP has been proved to prevent its accumulation and delay the development of scald (El Hadi et al., 2013).

An important group of terpenoid volatiles, referred to as norisoprenoids, is derived from oxidation of carotenoids by a large group of enzymes termed carotenoid cleavage oxygenases (CCDs). In tomato, LeCCD1A and LeCCD1B have been demonstrated to cleave multiple cyclic carotenoids at the 9,10 (9′,10′) double bond, yielding C13 apocarotenoids, geranylacetone, pseudoionone, and β-ionone. In the flesh of peach fruit, high transcript levels of a *CCD4* gene have been correlated with the production of volatile apocarotenoids and degradation of carotenoids. In citrus, a CCD type 4 cleaves

β-carotene and β-cryptoxanthin and produces the volatile C10 β-cyclocitral (Ahrazem et al., 2016).

The aroma compounds derived from fatty acids are usually formed by enzymatic degradation processes, that are preceded by the action of acyl hydrolase, which liberates free fatty acids from aglycerols. The lypoxygenase reaction (LOX) and α- and β-oxidations are the most important reactions in the generation of volatiles from fatty acids. The LOX enzyme catalyzes the dioxygenation of unsaturated fatty acids (linoleic and α-linoleic) to produce hydroperoxides. Subsequently, hydroperoxide lyases (HPL) act and release aroma compounds, such as 3Z-hexenol, 2E-hexenal, and 2E-6Z-nonadienal. Then, the C6 and C9 aldehydes are metabolized to alcohol by alcohol dehydrogenases (ADHs). Some LOXs and ADHs respond to ethylene and are regulated during fruit ripening (El Hadi et al., 2013).

Volatile esters are aromatic compounds very abundant in fruits such as strawberry, peach, and some citrus species. Lipids are the precursors of aliphatic acids and are synthesized through β-oxidation. Subsequently, aliphatic acids are substrates for the formation of acylCoAs. One of the most studied key points in the biosynthesis of aliphatic esters is the step catalyzed by the alcohol acyl transferases (AATs). These enzymes combine several alcohols and result in a wide spectrum of aliphatic esters. AATs have been identified in a number of fruits including melon, strawberry, apple, and kiwifruit (Rodrigo et al., 2012; Rambla and Granell, 2013).

The volatile aromas derived from amino acids are phenylpropanoid and benzenoid compounds. Phenylpropanoid compounds like 2-phenylacetaldehyde and 2-phenylethylacohol importantly participate in tomato flavor. They both originate from phenylalanine, but their biosynthetic pathway is still not well understood. Compounds derived from leucine, 3-methylbutanal, 3-methylbutanol, and 2-methylbutanoic acid are present at important levels in strawberries, tomatoes, and several grape varieties. The esterification of amino acids and their alcohol derivatives yields compounds like 3-methylbutyl butanoate and 3-methylbutyl acetate that highly influence banana fruit aroma. Branched-chain volatiles are a group of compounds related to amino acids, displaying low molecular weight and high volatility. Key components of banana, apple, tomato, and strawberry aroma derive from the branched-chain amino acids valine, leucine, isoleucine, and methionine (Dudareva et al., 2013, Rambla and Granell, 2013).

7.7 ORGANIC ACIDS

The ratio between sugars or total soluble solids and the titratable acidity is often used as a maturity index because the combination of both parameters reflects, together with other factors such as firmness, the edibility of the fruit and helps to determine the harvest date. The acids that predominate in fruits differ between species. Thus, malic acid is the most abundant acid in many

fruits such as apple, and loquat, while malic and tartaric acid accumulate in grape fruits and citric acid in citrus fruits.

Malic and citric acids are intermediates of the tricarboxilic acid cycle (TCA), while tartaric acid derives from the catabolism of L-ascorbic acid (AsA, vitamin C) (Etienne et al., 2013). The initial biosynthesis of these organic acids consisting of the carboxylation of phosphoenolpyruvate (PEP) occurs in the cytosol, the degradation (decarboxylation of malate and oxaloacetate) is produced in the cytosol, and the conversion between tri- and dicarboxylates is located in the mitochondria (tricarboxylic acid cycle, TCA), the glyoxylate cycle in the glyoxysome, and citrate catabolism in the cytosol. Moreover, apart from the metabolic regulation, the accumulation of acids in the vacuole also seems to be key for the control of the acid concentration in fruits. In the case of malate, the thermodynamic conditions of its transport into the vacuole may limit its accumulation, while citrate accumulation could be due to metabolic reactions (Etienne et al., 2013).

The synthesis of dicarboxylates by the carboxylation of PEP produces malate and oxalacetate (OAA) and is catalyzed by PEP carboxylase (PEPC). This step has been pointed out to be key for the regulation of malate accumulation in grapes during ripening but, contrastingly, PEPC transcript abundance and enzymatic activity seemed not to correlate with malate contents in peach, apple, and loquat. During ripening there is usually loss of acidity due to the decarboxylation of tricarboxylates into dicarboxylates, and decarboxylation of dicarboxylates, like malate and OAA, that results in the degradation of acids. It is worth noting that the decarboxylation of malate and OAA leads to the production of sugars from PEP, which can originate from OAA through the activity of phosphoenol carboxykinase (PEPCK), which catalyzes the reversible reaction, although the most likely function is the synthesis of PEP. The origin of OAA could be the oxidation of malate by NAD-dependent cytosolic malate dehydrogenase (NAD-cytMDH) or the conversion of pyruvate by the activity of pyruvate orthophosphate dikinase (PPDK). And pyruvate, in turn, could be generated by the carboxylation of malate by cytosolic NADP-dependent malic enzyme (NADP-cytME), which has been related to the decrease in malic acid observed during ripening of tomato or loquat (Leegood and Walker, 2003).

In grape berries, the tartaric acid content is higher than the malic acid content, however the biosynthesis of this compound has been very poorly studied. The tartaric acid pathway is the result of L-ascorbic acid catabolism via the conversion of L-idonate to 5-keto-D-gluconate under the action of L-idonate dehydrogenase. Malic acid is metabolized during grape ripening while it accumulates during development, at véraison it is released from the vacuole and metabolically utilized in the TCA cycle, respiration, gluconeogenesis, and biosynthesis of secondary compounds (Cholet et al., 2016).

During citrus fruit ripening, there is a dramatic decrease in citric acid that is metabolized to isocitrate, 2-oxoglutarate, and glutamate. Aconitase (Aco), which transforms citrate to isocitrate, is the first step in citric acid catabolism.

Recent molecular and phylogenetic studies have shown that the pattern of expression of *Aco1* and *Aco2* was generally associated with the timing of acid concentration decrease observed in most citrus genotypes. Gene expression together with metabolite analyses carried out on Clementina mandarin led to the proposal of a mechanism consisting of the catabolism of glutamate by the GABA (α-aminobutyrate) shunt that consequently decreases the acidity of the cytoplasm (Cercós et al., 2006).

7.8 CARBOHYDRATE METABOLISM

The accumulation of sugars in fruits is generally due to the translocation of sucrose from the leaves and the bark, where it is stored as starch, rather than from the photosynthetic activity of the fruit itself. Sucrose is the major transport sugar in most plant species, however in some families like the *Rosaceae* (e.g., apple) a sugar alcohol (sorbitol) is also transported. Due to the size and polarity of these sugars they need proteins, that act as sugar transporters, to allow their diffusion across membranes. Sugar transporters for sucrose, hexoses, and sugar alcohols have been described to participate in efficient unloading into cell wall spaces, uptake of sugars leaked or transported via the apoplasm, loading from the cytosol into storage vacuoles, and the fine-tuning of sugar fluxes for homoeostasis and interactions with other proteins for sugar sensing and signaling. Hence, apart from the metabolic reactions that regulate sugar contents, the role of the transporters in sugar accumulation seems to be key for the regulation of the concentration of sugars in fruit tissues (Slewinski, 2011).

In most plant species, sugars are synthesized in the leaves and accumulated as starch, which is converted into sucrose, the transport sugar, and transported to the fruits where it is converted into fructose and glucose by sucrose invertase. In apple and other fruits from the *Rosaceae* family, both sorbitol and sucrose are synthesized in source leaves, and then translocated and utilized in sink organs, like fruits. In the sinks, sorbitol is converted to fructose by sorbitol hydrogenase and sucrose is hydrolyzed to fructose and sucrose by invertase (Koch, 2004).

Many fruits contain large contents of starch at early stages of development which are progressively degraded during ripening, originating soluble sugars (reducing and nonreducing sugars; Xiao et al., 2017). During ripening, starch degradation is catalyzed by a series of enzymes in the plastids, yielding glucose-1-phosphate, which is then transported to the cytoplasm and gives rise to an increase of glucose and fructose in fruit flesh. These sugars can enter the metabolic pool and be used as substrates for respiration during glycolysis, be utilized as reducing power or as carbon precursors for the biosynthesis of amino or nucleic acids, among others. During glycolysis, sugar breakdown generates energy that is required for the progression of ripening and during respiration the pyruvate generated in the glycolysis is converted to acetyl coenzyme A, which enters the TCA cycle to be completely oxidized to carbon dioxide. During gluconeogenesis, some organic acids from the TCA cycle are

converted into sugars (reviewed in Rodrigo et al., 2012). Gluconeogenesis from malate can occur by two alternate pathways: either via malate dehydrogenase (MDH) in conjunction with phosphoenolpyruvate carboxykinase (PEPCK) or alternately via the malic enzyme (ME) together with pyruvate orthophosphate dikinase (PPDK; Leegood and Walker, 2003). PEPCK catalyzes gluconeogenesis from malate/citrate in ripening tomato fruits. PPDK has been detected in the ripe flesh of tomato, and at lower amounts in peach and pepper flesh, and it could not be detected or was only present at very low amounts in apricot, aubergine, blackberry, blueberry, cherry, grape, plum, raspberry, and redcurrant fruits (Famiani et al., 2016). In contrast, PEPCK was present in the flesh of all the fruits investigated by Famiani et al. (2016). The presence of both enzymes in tomato flesh suggested that in those fruits both pathways, PEPCK and PPDK, could be potentially utilized, while in the other fruits the PEPCK route predominated.

7.9 VITAMINS

One of the most important benefits of fruits and vegetables in human nutrition and health is due to their contents of vitamins, minerals, and fiber, and also a wide range of bioactive phytochemicals (reviewed in Rodriguez-Casado, 2016). Table 7.1 summarizes a list of the 10 most highly produced fleshy fruits in the world (FAOSTAT-2014) and their vitamin contents. In these fruits, vitamin C (also known as ascorbic acid, AsA) is the most abundant vitamin and is especially high in oranges, followed by vitamin E (α-tocopherol), which is very abundant in mango, and vitamin A, which is highly present in cantaloupe melon.

Vitamin C, apart from being an essential component of the human diet, is also a powerful antioxidant that may also reduce the incidence of several diseases. Besides the relevance of AsA for humans, it is also essential for plants, however, AsA biosynthesis in plants remained elusive until 1998. The first significant milestone was the discovery of the L-galactose pathway for AsA biosynthesis (Wheeler et al., 1998). There is a general consensus of four possible AsA biosynthetic pathways in plants: L-galactose, L-gulose, *myo*-inositol, and D-galacturonic acid pathways (Lorence and Nessler, 2007). Apart from AsA biosynthesis, AsA degradation and recycling are also important routes that regulate AsA homeostasis. Hence, AsA can be transformed into monodehydroascorbate (MDHA) by the enzymes ascorbate oxidase and ascorbate peroxidase. Then, the MDHA radical can either be recycled into AsA by monodehydroascorbate reductase (MDHAR) or undergo disproportionation into dehydroascorbate (DHA) and AsA. In addition, DHA can be recycled into AsA by dehydroascorbate reductase (DHAR) before being irrevocably hydrolyzed (Mellidou and Kanellis, 2017).

Studies of AsA metabolism in fruit species have shown that in kiwifruit and immature peach fruits the L-galactose pathway predominates, whereas in strawberry fruit the D-galacturonic acid and the *myo*-inositol pathways seemed

to prevail. In tomato, most of the genes involved in the L-galactose pathway followed a temporal pattern of expression during fruit ripening opposite to that of AsA accumulation, but interestingly, *L-galactose-1-phosphate phosphatase* (*GPP/VTC2*) expression was closely correlated with AsA levels, suggesting a regulatory role of this gene. In citrus fruits, a study of the transcriptional regulation of AsA accumulation in the peel and pulp of fruits revealed that AsA accumulation correlated with the transcriptional profiling of the L-galactose pathway genes. Moreover, the *myo*-inositol pathway appeared to be also relevant in the peel of immature-green oranges. Furthermore, differences in AsA content between varieties with different AsA contents were associated with differential gene expression of *GDP-mannose pyrophosphorylase* (*GMP*), *GDP-L-galactose phosphorylase* (*GGP*), and *GPP*, *myo*-inositol oxygenase in peel, and *GGP* and *GPP* in the fruit pulp. Collectively, the results indicated a differential regulation of AsA concentration in the peel and pulp of citrus fruits that changes during fruit development. The L-galactose pathway appeared to be predominant in both tissues, but the AsA concentration seemed to be regulated by complex mechanisms in which degradation and recycling also play important roles (Alós et al., 2014). In summary, the predominating AsA biosynthetic pathways seem to be species- and tissue-specific and also change during fruit development and ripening. In addition, apart from the de novo biosynthesis of AsA, other mechanisms, such as rates of degradation and recycling, can contribute to the regulation of AsA concentrations in plants (Mellidou and Kanellis, 2017).

Vitamin A is a group of compounds that includes retinol, retinal, and retinoic acid, which are derived from the oxidative cleavage of carotenoids with at least one unsubstituted β-ione ring in their structure (provitamin A carotenoids) such as β- and α-carotene and β-cryptoxanthin, mostly present in fruits and vegetables. Vitamin A is necessary for the normal functioning of the visual system, maintenance of epithelial integrity, and immunity, among other important roles (Britton and Khachik, 2009). Tocopherols (vitamin E) are isoprenoid-derived compounds that are synthesized from the condensation of homogentisate and phytyl-diphosphate from the shikimate and MEP pathways, respectively (DellaPenna and Pogson, 2006). In tomato fruits tocopherol biosynthesis is controlled both temporally and spatially, however, total tocopherol content remains constant during ripening. The transcriptional profiles of genes from the precursor pathways would suggest an increase in vitamin E contents during fruit development. Nevertheless, the phytyl diphosphate supply limited tocopherol biosynthesis in late fruit stages, which was partly due to the decreasing transcript levels of geranylgeranyl reductase (*GGDR*) which restricted the isoprenoid precursor availability (Quadrana et al., 2013).

Vitamin K1 (phylloquinone) is an essential cofactor for the conversion of glutamic acid to γ-carboxyglutamic acid residues in vitamin-K-dependent proteins, including hemostasis factors II, VII, IX, and X, and proteins C and S involved in blood coagulation (Furie et al., 1999). Phylloquinone is

Table 7.3 Vitamin Concentrations in the 10 Most Highly Produced Fleshy Fruits Around the World

Commodity	Production (tons) [a]	Vitamin A (μg/100 g)[b]	Vitamin C (mg/100 g)[b]	Vitamin E (mg/100 g)[b]	Vitamin K1 (μg/100 g)[b]	Vitamin B9 (μg/100 g)[b]
Tomato	171	42–80	9–22	0.52–0.89	0–7.90	15–30
Banana	114	3–4	8–9	0.10–0.16	0.20–0.50	14–20
Watermelon	111	18–28	8–11	0.05	0–0.10	1.70–4.50
Apple	84	1–3	0–8	0.09–0.39	1.00–3.40	1.50–5.20
Grape	74	3–5	2–6	0.18–0.20	14.60	2–6
Orange	72	9–12	45–59	0.15–0.18	0.10–0.20	17–34
Coconut	60	0	2–3	0.00–0.24	0.00–0.20	3–26
Mango	45	31–54	36–228	0.73–0.90	2.60–4.20	43–49
Mandarins	30	8–34	27–49	0.20–0.58	0.00	16–24
Melon	29	1–169	8–37	0.05–0.07	1.5–2.50	13–21

[a] *FAOSTAT2014 (http://www.fao.org/faostat/).*
[b] *National Nutrient Database for Standard Reference (USDA, 2017).*

synthesized from a naphthoquinone ring derived from chorismate in the shikimate pathway and a prenyl side chain derived from phytyl diphosphate from the MEP pathway, similarly to tocopherol (Spicher and Kessler., 2015). Phylloquinone is very abundant in some berries, such as blackberries and blueberries, at around 20 μg/100 g of fruit, while tomato contains 7.90 μg/100 g (Dismore et al., 2003, Table 7.3), whether it changes or not during fruit ripening has not yet been described.

Folate is the generic term for tetrahydrofolate (THF) and related compounds exhibiting the biological activity of folic acid, also known as vitamin B9. Folates are a key nutrient for human health, with protective effects against cancer, cardiovascular diseases, and impaired fetal development. THF is a tripartite molecule composed of pterin, *p*-aminobenzoate (*p*ABA), and glutamate moieties that are assembled in the mitochondrion. Transcriptomic analyses in tomato have provided evidence for both feedback and feedforward regulation of the expression of folate pathway genes and, in fact, still little is known about folate biosynthesis. In tomato fruits, the folate content fell markedly as tomato turned from green to red and in strawberries the concentrations during ripening increased or decreased depending on the harvest year. Hence, it seems that the environmental conditions could affect the folate contents in strawberry fruits (reviewed in Hanson and Gregory, 2011; Table 7.3).

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CHAPTER 8
Transpiration

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8.1 INTRODUCTION

Water is a compound directly associated with life processes. It is the essential medium where biochemical reactions occur and a major constituent in plant cells. Water is the most abundant component in fruit and vegetables. Water content in fruits and vegetables can range from about 10% in dried seeds to about 95% in fresh vegetables and fruit, such as watermelon.

Together with temperature, transpiration is one of the most important factors determining deterioration and shelf life in fruits and vegetables. A strong understanding of the environmental and biological factors that determine produce transpiration and the ways transpiration affects the quality of fruits and vegetables is essential to developing effective approaches to manage produce transpiration and prolong the shelf life of fruits and vegetables.

8.2 PHYSICAL PROPERTIES OF WATER

Water has distinctive physical properties that make living processes possible. Those properties derive from the structure of water and from the ability of hydrogen and hydroxyl ions to dissociate in solution.

Some important water properties include the following:

- Water is the substance with the highest specific heat, except liquid ammonia; this property tends to stabilize temperatures.
- Water is in liquid state at normal temperatures.
- Water is a strong solvent, thus it serves as a means for transport of nutrients and provides a substrate for cell biochemical reactions.
- Water has high heat of vaporization (540 cal/g at 100°C) and high heat of fusion (80 cal/g). These properties allow water evaporation to have a cooling effect and water condensation to have a warming effect.

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- Water has high heat conductivity.
- Water is transparent to visible radiation.
- Water is an effective solvent for nonelectrolytes because the positive (H^+) and negative (OH^-) charges of water molecules attract ions. Water is a dipole because of the asymmetry of the angles of the two covalent O−H bonds. This polarity allows for the formation of H bonds between water molecules.
- Water has a higher surface tension than the majority of liquids.
- Water has a high density and its maximal density is at 4°C.
- Water expands at freezing. A given volume of water at the frozen state is 9% greater than the same amount of water at the liquid state.
- Water has low ionization. Only one water molecule is ionized (dissociated) out of 55.5×10^7.
- Water may be bound or adsorbed to surfaces of cellulose, starch, proteins, etc.

8.3 PHYSICS OF WATER LOSS

Water *evaporation* is a phase transition from the liquid phase to vapor that occurs at temperatures below the boiling temperature. It occurs on the surface of the liquid (Britannica, 2018). Water evaporation occurs when the partial pressure of water vapor is less than the equilibrium water vapor pressure.

Transpiration (from Latin *trāns* through + *spīrāre* breath) is a type of water evaporation or water loss from plant parts (Kramer and Boyer, 1995). Transpiration involves the evaporation of water from cell surfaces into intercellular spaces and the diffusion of water molecules out of the plant tissue or organ into the surrounding air.

Transpiration in plants and harvested fruits and vegetables works following the physical laws that determine water evaporation from moist surfaces. Transpiration is proportional to the water vapor pressure gradient between the surface of the commodity and the surrounding air, and inversely proportional to the resistance to transpiration (e.g., cuticular resistance).

The *vapor pressure of water* is the equilibrium pressure of water vapor above liquid (or solid) water. It is the pressure of the water vapor molecules resulting from evaporation. The vapor pressure of water increases with temperature, that is, more water molecules are evaporated at high compared to low temperatures (Table 8.1). The difference between the vapor pressure of the air and the vapor pressure of the evaporating surface is what determines the rate of transpiration from produce. This difference in vapor pressure is called the *vapor pressure difference* or *vapor pressure deficit* (VPD). *Relative humidity* (RH) is the water content of the air expressed as a percentage relative to the vapor pressure of the same air, saturated, and at the same temperature. Table 8.2 provides an example on how to calculate VPD.

Table 8.1 Saturation Vapor Pressure Over Water[a]

Temperature (°C)	Vapor Pressure (kPa)	Temperature (°C)	Vapor Pressure (kPa)
1	0.657	26	3.361
2	0.705	27	3.565
3	0.758	28	3.78
4	0.813	29	4.005
5	0.872	30	4.243
6	0.935	31	4.493
7	1.001	32	4.755
8	1.072	33	5.031
9	1.147	34	5.32
10	1.227	35	5.624
11	1.312	36	5.942
12	1.402	37	6.276
13	1.497	38	6.626
14	1.598	39	6.993
15	1.704	40	7.378
16	1.817	41	7.78
17	1.937	42	8.202
18	2.063	43	8.642
19	2.196	44	9.103
20	2.337	45	9.585
21	2.486	46	10.089
22	2.643	47	10.616
23	2.809	48	11.166
24	2.983	49	11.74
25	3.167	50	12.34

[a]*Fruits and vegetables have a high water content (90% or more). Thus, for calculating vapor pressure differences between the produce and its surrounding air, the produce is considered to be "at water saturation."*

Source: Data from Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W., 1991. Plant Physiological Ecology. Field Methods and Instrumentation. Chapman and Hall, London.

8.4 TRANSPIRATION OF FRUITS AND VEGETABLES ATTACHED TO THE PLANT

In plants, transpiration is beneficial because it cools the leaves and produces the water potential gradient that allows for water and nutrient uptake from the soil (Kramer and Boyer, 1995). It is an "unavoidable evil" because, in order for leaves to receive CO_2 from the air, leaves have to "pay the price" of losing water.

When fruits and vegetables are still attached to the plant, they maintain a relative balance between the amount of water they lose through transpiration and the water they receive from the soil. Fruits' and vegetables' water status is not constant, but rather changes during the day. During the night and early

Table 8.2 Example on How to Determine Vapor Pressure Difference Between a Commodity and the Surrounding Air

Storage or ambient temperature: mean = 20°C
Storage relative humidity (RH) = 70%
Vapor pressure difference (VPD)
To calculate VPD, we need to know the ambient (storage conditions) temperature and RH, and the temperature of the commodity. After a few hours under storage, we can assume that air temperature and fruit temperature are the same. We assume that the RH of the commodity is 100%
With the temperature information, we find the vapor pressure of the air at saturation (Table 8.1)
Vapor pressure of air at 20°C at saturation (100% RH) = 2.337 kPa
Partial vapor pressure of air at 20°C at 70% RH = $2.337 \times 0.70 = 1.636$ kPa
Partial vapor pressure of commodity at 20°C and 100% RH = 2.337 kPa
Vapor pressure difference between commodity and air = 2.337 kPa − 1.636 kPa = 0.701 kPa

morning, when the plant has low water loss through transpiration, the produce is well hydrated (i.e., it has a high water status). As the plants show an increasing rate of water loss, as air temperature and evapotranspiration increase, fruits and vegetables show decreased water status because the produce loses more water than the amount of water it receives from the roots. Fruits may show diurnal fluctuations in size in addition to long-term irreversible enlargement. Diurnal fluctuations are related to plant water status (Johnson et al., 1992; Thompson et al., 1999). In tomato fruit, the maximum relative growth rate occurs in the morning and the minimum relative growth rate at midday (Díaz-Pérez and Shackel, 1991). Vegetables (e.g., leafy vegetables) also show changes in plant water status during the day because of imbalances in the amount of water taken up from the soil and the amount of water lost by transpiration. These physiological responses of crops to the environment explain the common practice of harvesting the produce in the morning, when fruits and vegetables are highly hydrated. Another example is melon fruits which, when harvested in the morning, are heavier than those harvested at midday; melon fruit harvested in the morning, however, tend to have reduced soluble solid content compared to melons harvested at midday.

8.5 TRANSPIRATION OF FRUITS AND VEGETABLES AFTER HARVEST

The commercial postharvest life of fruits and vegetables may be determined by transpiration, decay, physiological disorders or processes (chilling injury, overmaturity, decay, spouting, rooting, undesirable color changes, off-flavors, and elongation), mechanical injury, etc. (Ben-Yehoshua and Weichmann, 1987). Transpiration is one of the most important factors determining deterioration

in fruits and vegetables (Kader, 1992), as in leafy vegetables (spinach, lettuce, and chard), cabbage, mushrooms, and green onion.

In harvested fruits and vegetables, transpiration is undesirable because water loss cannot be compensated by more water uptake from the soil since the produce are not attached to the plant any more. The amount of water in a commodity at harvest time is the maximum of water the commodity will have. Thus, any water loss from the commodity after harvest will result in produce dehydration.

The water loss from fruits and vegetables induces water stress in their tissues. This water stress may enhance or accelerate senescence in commodities, probably because of an increased rate of cellular membrane disintegration and leakage of solutes (Ben-Yehoshua et al., 1983). Excessive water loss results in produce softening and shriveling, loss of peel gloss and calyx browning due to dehydration in eggplant (Risse and Miller, 1983).

Symptoms of produce water loss. Postharvest transpiration causes a loss in cell turgor that is manifested by produce softening (e.g., bell pepper, eggplant, etc.), shriveling (e.g., citrus, cucumber, lettuce, spinach, mushroom, potato, etc.), and loss of shine (e.g., eggplant, mango, cucumber, etc.), epidermis color change (e.g., whitening of carrots), among other factors detrimental to produce quality (Fig. 8.1).

Allowable weight loss in commodities. Commodities vary in their tolerance to water loss. The *allowable weight loss* before the commodity becomes unmarketable ranges from about 3% to 10% of the weight of the commodity immediately after harvest (Table 8.3). An allowable weight loss of 5% is typical for many commodities. As a rule, shriveling is visible at about half the total figure of the allowable weight loss. This means that half of the commercially allowable weight loss is not visible. Above the maximum allowable weight

FIGURE 8.1

Bell pepper fruit immediately after harvest (left), 3 days after harvest (center), and 7 days after harvest (right). Fruit kept at 20°C and 70% RH.

Table 8.3 Transpiration (Water Loss) Rates From Various Commodities After Harvest

Commodity	Water Loss Rate[a] (% of Initial wt./day/kPa)	Temperature of Measurement (°C)	Maximum Allowable Water Loss (% Original Commodity Weight)
Asparagus	36[a]	10	8[a]
Beans, broad	21[a]	15	6[a]
Beans, runner	18[a]	15	5[a]
Beetroot storing	16[a]	10	7[a]
Beetroot, bunching w/ leaves	16[a]	15	5[a]
Bell pepper	0.6[d]	20	7[a]
Blackberries, Bedford	5[a]	10	6[a]
Broccoli, sprouting			4[a]
Brussels sprouts	28[a]	15	8[a]
Cabbage Primo	10[a]	10	7[a]
Cabbage Decema	1[a]	10	10[a]
Carrots, storing	19[a]	10	8[a]
Carrots, bunches with leaves	28[a]	15	4[a]
Cauliflower, April Glory	19[a]	15	7[a]
Celery, white	18[a]	15	10[a]
Cucumber	4[a]	15	5[a]
Eggplant, Classic	0.6[b]	20	
Eggplant, Japanese	5.7[b]	20	
Leeks (“Musselburgh”)	9[a]	15	7[a]
Lettuce, Unrivalled	75[a]	15	5[a]
Onion, Bedfordshire Champ	0.2[a]	15	10[a]
Parsnip, Hollow Crown	24[a]	15	7[a]
Peas in pod, early	13[a]	15	
Peppers, green	6[a]	10	

(*Continued*)

Table 8.3 (Continued)

Commodity	Water Loss Rate[a] (% of Initial wt./day/kPa)	Temperature of Measurement (°C)	Maximum Allowable Water Loss (% Original Commodity Weight)
Potato, main crop, King	0.5[a]	15	7[a]
Potato, new (immature)	5[a]	15	7[a]
Raspberries, Malling Jewel	25[a]	10	6[a]
Rhubarb, forced	23[a]	10	3[a]
Sapote mamey	1.4[c]	20	
Spinach, Prickly True	110	15	3[a]
Sprouting broccoli	75[a]	15	4[a]
Strawberries, Cambridge	7[a]	15	6[a]
Sweetcorn	14[a]	15	7[a]
Tomato, Eurocross	1[a]	10	7[a]
Turnip, bunching with leaves	11[a]	10	5[a]
Watercress	350[a]	15	7[a]

Transpiration is expressed as commodity weight loss relative to the initial weight of the commodity.
[a]*After Robinson, J.E., K.M. Browne, Burton, W.G., 1975. Storage characteristics of some vegetables and soft fruits. Ann. Appl. Biol. 81, 399–408. Data were transformed from "% of initial wt./day/mbar" into "% of initial wt./day/kPa".*
[b]*Díaz-Pérez, J.C. 1998a. Packaging of 'Classic' and 'Japanese' aubergines (*Solanum melongena*) with polyethylene films. Agrociencia 32, 71–74.*
[c]*Diaz-Perez, J.C., Bautista, S., Villanueva, R., 2000. Quality changes in sapote mamey fruit during ripening and storage. Postharvest Biol. Technol. 18, 67–73.*
[d]*Díaz-Pérez, J.C., Muy-Rangel, M.D., Mascorro, A.G., 2007. Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (*Capsicum annuum *L.). J. Sci. Food Agric. 87, 68–73.*

loss, commodities may be edible but their quality low (soft, wilted, shriveled, etc.) and have little commercial value.

The *epidermis* of fruits and vegetables plays a major role in the gas exchange between the produce and its surrounding air (Díaz-Pérez et al., 2007). The epidermis prevents rapid dehydration of produce by forming a layer of reduced permeability to water loss. This protection against water loss is of even greater importance after harvest, when fruits and vegetables are detached from the plant, the source of water.

Water can be transpired from produce through the *cuticle* (cuticular transpiration), stomata (stomatal transpiration), and suberized surfaces (peridermal transpiration) (Larcher, 1995). In many fruits, most of the transpiration is

cuticular because fruit skin has few stomata. In some fruits, however, transpiration may occur primarily through other fruit parts, such as the calyx, as in eggplant fruit (Díaz-Pérez, 1998b).

In roots, tubers, and bulb vegetables, produce (e.g., onions, garlic, sweet potato, white potato, cassava, etc.) is submitted to *curing* before long-term storage, to induce the formation of periderm in the epidermis (Wills et al., 1998). The periderm is composed of layers of suberized cells. Immediately after harvest, onions are placed on the surface of soil for curing (drying) under field conditions. Onions can also be cured using a forced-air oven (Petropoulos et al., 2017). Cured produce has increased shelf life and reduced both transpiration and incidence of decay compared to uncured produce. A fast curing (9 days at 30°C) was found to reduce the rate of onion bulb deterioration (Eshel et al., 2014). In sweet potato, curing is done for 7 days at 29°C and 90%–95% RH (Wills et al., 1998).

8.6 MEASUREMENT OF TRANSPIRATION IN FRUITS AND VEGETABLES

The *rate of transpiration* of fruits and vegetables depends on the water vapor concentration or water vapor pressure at the evaporating surface of the produce and the diffusive resistance of all paths from inside the produce to the air.

Periodic weighing in conditions of known air VPD and air temperature is the most common method to measure transpiration of fruits and vegetables. This method assumes that all weight loss in produce derives from water loss, although some weight loss in produce may be attributed to CO_2 respirational losses. In tomato, 5% of the fruit weight loss is due to respiration (Shirazi and Cameron, 1993). The rates of water loss for various commodities have been determined (Table 8.3). However, these rates of water loss vary significantly in the literature. Values also are often expressed using different units. Additionally, some studies report water loss rates without information on the vapor pressure difference in which commodities were stored, sometimes making it difficult to compare data from various studies. Values of water loss rate in Table 8.3 are shown as a percentage of weight loss of produce per unit of vapor pressure difference (kPa) between the produce and the surrounding air.

8.7 FACTORS THAT AFFECT POSTHARVEST TRANSPIRATION

Transpiration accounts for most of the weight loss in the majority of fruits and vegetables (Burton, 1982). In tomatoes, transpiration accounts for 92%–97% of fruit weight loss (Shirazi and Cameron, 1993). Postharvest transpiration is determined by various biological and environmental factors (Fig. 8.2).

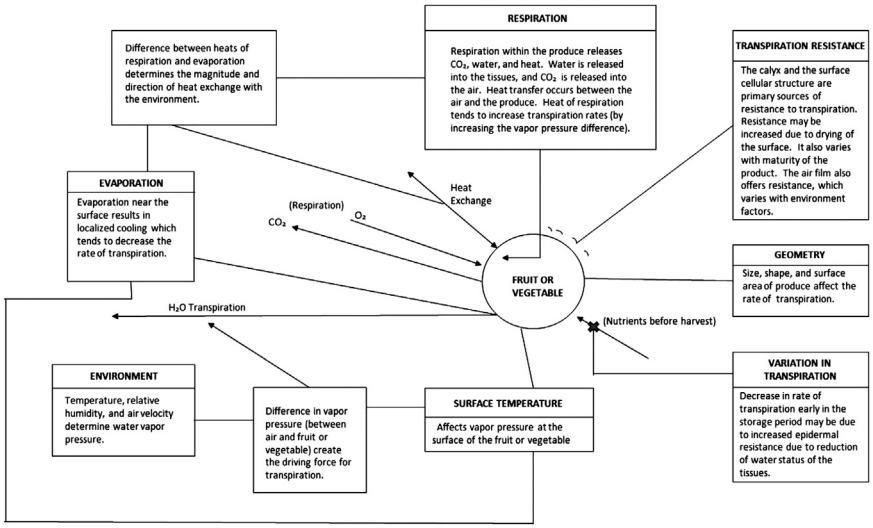

FIGURE 8.2

Factors that determine postharvest transpiration in fruits and vegetables. *Adapted from Sastry, S.K., Baird, C.D., Buffington, D.E., 1978. Transpiration rates of certain fruits and vegetables. ASHRAE Trans. 84, 237–255.*

8.8 ENVIRONMENTAL FACTORS

Temperature and *RH* are the most important environmental factors that determine the postharvest life of commodities (fruits and vegetables). In postharvest handling, emphasis has been on temperature management (cooling) as a means to reduce produce transpiration and thus extend produce shelf life. *Wind speed* as in controlled-temperature rooms may also influence transpiration of commodities. The type of packaging used may influence the wind speed to which produce is exposed.

8.9 BIOLOGICAL FACTORS

8.9.1 Fruit Size

Fruit transpiration decreases with increasing fruit size. This response has been found in tomato, bell pepper, and eggplant (Díaz-Pérez, 1998b; Díaz-Pérez et al., 2007; Lownds et al., 1993). The effect of fruit size on fruit transpiration rate may be of commercial importance due to the direct impact of transpiration on reducing sealable fruit weight and the impact on decreasing fruit shelf life. In eggplants, fruit of commercial size-32 (180 g/fruit) was found to have a transpiration rate of 1.12%/day/kPa compared to fruit size-16 (550 g/fruit) with a rate of 0.62%/day/kPa (Díaz-Pérez, 1998b). Because of these differences in transpiration rate, smaller eggplant fruit become shriveled and have a shorter shelf life compared to larger eggplant fruit.

8.9.2 Surface Area/Weight Ratio

The commodity transpiration rate is directly proportional to its surface area (Burton, 1982). For commodities of similar shape, the surface area/weight ratio decreases as the commodity increases in size (Fig. 8.3). A similar response is obtained when relating transpiration with surface area/volume ratio. In eggplants, small fruits had increased surface area/weight ratio and increased fruit transpiration rates relative to large fruits (Díaz-Pérez, 1998b). In different tomato lines, fruit water loss was found to be related to fruit surface area/volume ratio (Bouzo and Gariglio, 2016). Thus, when comparing fruits of different sizes and cultivars it is necessary to express fruit transpiration on a per surface area basis to normalize for differences in surface area/weight among fruit.

8.9.3 Stage of Development/Maturity Stage

The maturity stage may influence the transpiration rate of the commodity. As commodities develop, the transpiration rate may change in response to their increased size and decreased surface area/weight ratio, as well as by the morphological transformations, such as changes in cuticle thickness and composition. Eggplant is one example of how several factors may participate to reduce fruit water loss as fruit develop (Díaz-Pérez, 1998b). Eggplant fruit have decreased surface/weight ratio with increasing fruit size. Eggplant fruit are partially covered by an enlarged calyx. This calyx is a major route for transpirational water loss. The rate of water loss through the calyx is higher than through the fruit, and the proportion of eggplant fruit covered by the calyx decreases with increasing fruit size. In tomato, fruit at the red stage was found

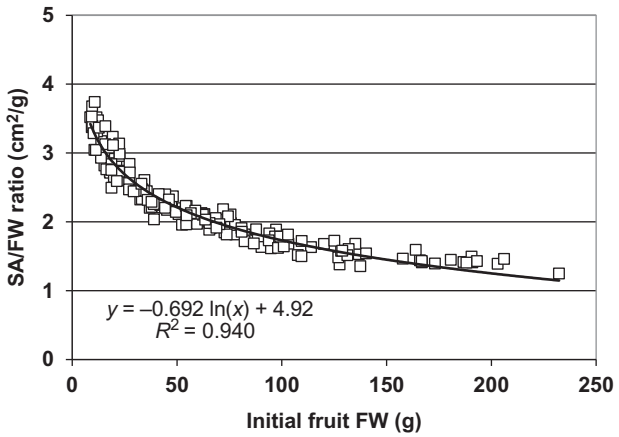

FIGURE 8.3

Relationship of bell pepper fruit surface area/fresh weight (SA/FW) ratio to initial fresh weight (FW). Each point represents an individual fruit. *After Díaz-Pérez, J.C., Muy-Rangel, M.D., Mascorro A.G., 2007. Fruit size and stage of ripeness affect postharvest water loss in bell pepper fruit (Capsicum annuum L.). J. Sci. Food Agric. 87, 68–73.*

to have a higher transpiration rate than fruit at the mature-green stage (Díaz-Pérez and Araiza, 1997). Whether a produce is climacteric or nonclimacteric may also affect its susceptibility to water loss. In nonclimacteric produce, RH (high) may have a stronger effect on delaying produce deterioration than temperature (low) (Lurie et al., 1986). Thus, reducing water loss particularly in nonclimacteric produce is an important factor affecting postharvest life.

8.9.4 Epidermis (Skin)

The exterior surfaces (skin) of leaves, stems, flowers, and fruits are covered by a waxy, relatively impermeable, layer called the *cuticle* (Kramer and Boyer, 1995). This skin plays an important role in gas exchange between the commodity and the environment. The skin allows the commodity to maintain a high water content despite low air humidity values around the commodity. For example, tomato fruit have a moderately thick waxy cuticle with no pores (Wilson and Sterling, 1976). Cuticles are more impermeable to water when dry than when they are wet. The cuticle is typically thinner on leaves grown under shaded and well-irrigated conditions than under sunny and soil water-limiting conditions. In bell pepper grown under different levels of shade, however, fruit transpiration was unaffected by shade level (Díaz-Pérez, 2014). This apparent inconsistency may be because cuticle permeability to water vapor diffusion may not necessarily depend on cuticle thickness or degree of wax coverage, but on the cuticle chemical composition (Kerstiens, 2006). In tomato, cuticle thickness was found not to explain the differences in fruit transpiration among several tomato lines (Bouzo and Gariglio, 2016).

8.9.5 Injuries, Wounds, and Cracks

Tissue wounds caused by disease or mechanical injury may increase the transpiration rate of commodities. In laboratory drop tests, impacted oranges were found to have a fruit transpiration rate 0.5% higher than the control (Miranda et al., 2015). In addition to causing increased transpiration, tissue wounds may be a route for penetration of pathogenic microorganisms into fruits and vegetables.

The presence of cracks in commodities is usually associated with increased transpiration. Cracking is common in many produce, such as sweet cherries and tomatoes. Tomato fruit cracking is frequent in fruit that have a thin skin, such as heirloom cultivars (Fig. 8.4).

8.9.6 Presence of Leaves, Stems, Flowers, or Calyx Attached to Commodities

The transpiration rate in commodities may increase when the commodity is attached to plant parts (leaves, stems, peduncles, flowers, etc.) that have a high transpiration rate (Fig. 8.5). The contribution of leaves and stems attached to produce transpiration is often not fully understood. There seems

FIGURE 8.4
Fruit cracking in heirloom tomatoes.

FIGURE 8.5
Bunched onions and carrots have a shorter shelf life and a higher transpiration rate compared to onions and carrots whose leaves have been removed.

to be a functional two-way hydraulic connection (predominantly through xylem) between the produce and its attached organs, as found in tomato and sweet cherries (Athoo et al., 2015; Windt et al., 2009). This xylem connection allows water movement from the produce (e.g., carrot root) to the highly transpiring organs to which the produce may be attached (e.g., leaves), resulting in increased produce water loss. Personal observations indicate that bunch carrots, bunch onions, and bunch tomatoes wilt faster compared to the same produce detached from leaves and stems (Fig. 8.5).

In sweet cherries (*Prunus avium*), the peduncle is an indicator of fruit freshness for marketing purposes. Shriveled and brown pedicels occur because of water loss from the peduncle (Drake and Elfving, 2002). In cherries, it is possible that water may move from the peduncle to the fruit.

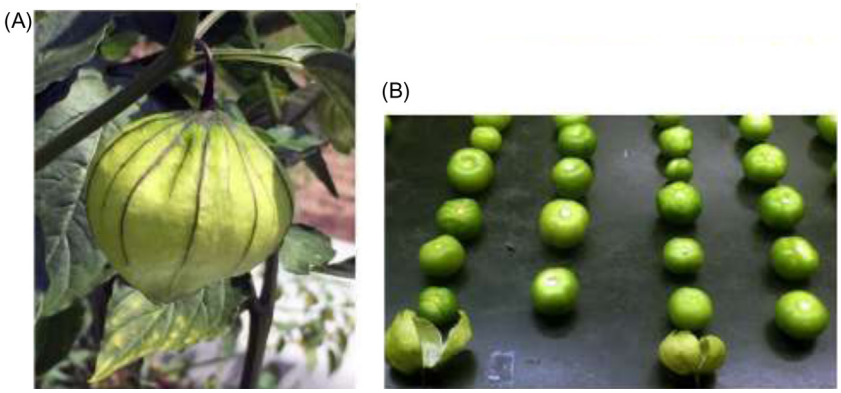

FIGURE 8.6

(A) Fully developed tomatillo (*Physalis ixocarpa*) fruit before harvest. (B) Tomatillo fruit kept 7 days at 20°C and 70% RH; fruits on rows 1 and 3 (left to right) were kept with the husk intact, covering the fruit, while fruits on rows 2 and 4 were kept without their husk. The presence of husk on fruit resulted in enhanced fruit shriveling.

Tomatillo (*Physalis ixocarpa*), also called "husk tomato," is a popular fruit in Mexico and Guatemala. Tomatillo fruit is covered by an accrescent calyx (husk) (Fig. 8.6). This husk remains attached to the fruit during fruit development. Commercially, tomatillos are typically sold with their calyx attached. I have found (unpublished data) that tomatillo fruit without the husk has a significantly reduced fruit transpiration relative to fruits attached to their husk. Thus, removing tomatillo husk will probably reduce fruit water loss in tomatillo and may help prolong its postharvest life.

The stem scar is a major pathway for transpiration in tomato and other fruit (Yang and Shewfelt, 1999). It has been reported that there is a positive correlation between the surface area of the stem scar to the fruit surface area ratio and water loss of the tomato fruit (Bouzo and Gariglio, 2016). Tomato may be jointed-stem or jointless-stem (Zahara and Scheuerman, 1988). The calyx and stem of jointless cultivars usually remain attached to the plant when the fruit is harvested. The stem of jointed cultivars typically separates from the plant at the stem joint, so that the stem and calyx remain attached to the harvested fruit. The harvester then has to remove the stem and calyx from the fruit to prevent injury to other fruit in the containers. Such punctures of the fruit may lead to fruit decay. Although jointless cultivars may have increased fruit transpiration rates, these cultivars provide considerable economy in picking time. With the increased efficiency of picking jointless over jointed cultivars, a crew can harvest more fruit in a day and reduce injury to tomato fruit and plants. Although sealing of the stem scar with lanolin, petrolatum, or other lipids may reduce fruit water loss, doing so may diminish diffusion of oxygen and carbon dioxide, resulting in fermentation of the fruit. In blueberries, the stem scar accounts for about 40% of fruit transpiration and the water loss rate through the stem scar is 170 higher than through the cuticle. The

influence of the stem scar on water loss was found to increase as temperature declines (Moggia et al., 2017).

In eggplant (*Solanum melongena*) the fruit calyx is the main route for fruit water loss, accounting for at least 60% of fruit transpiration (Díaz-Pérez, 1998b). In some countries, such as Japan and Israel, the greenness and freshness of the calyx is an important fruit quality attribute. Thus, postharvest treatments are necessary to maintain the health and freshness of the calyx to extend shelf life (Temkingorodeiski et al., 1993).

8.9.7 Cultivars

The transpiration rate may vary among cultivars of the same crop. This difference may be due to differences in permeance of the epidermis or to differences in surface area/weight ratio as a result of differences in size or shape. In blueberries, the water permeance of the fruit cuticle was found to vary twofold and the apparent permeance of the scar to vary threefold among nine lines. One line exhibited a 75% lower rate of water loss from its stem scar than the other lines than would be predicted based on its scar diameter (Moggia et al., 2017). In tomato, fruit transpiration was found to vary among 10 different tomato lines and the differences were attributed to differences in surface area/volume of fruit (Bouzo and Gariglio, 2016).

8.9.8 Storage

The transpiration rate of commodities often declines over time after harvest. In tomato, after a 14-day storage period, transpiration was reduced by 50% of its initial value (Díaz-Pérez and Araiza, 1997). The causes of this reduced transpiration rate are probably due to increased resistance to water movement inside the commodity from the commodity to the outside air as the commodity dehydrates. In blueberry, cuticle permeance was found to be unaffected by temperature (Moggia et al., 2017). The cuticle characteristics may change over storage thus affecting fruit transpiration of fruit, as in apples (Fig. 8.7).

8.10 CONTROL OF TRANSPIRATION

8.10.1 Storage

Low temperature reduces the vapor pressure difference between the commodity and the surrounding air. A combination of high-RH and low-temperature storage is more effective in extending shelf life compared to only using low-temperature storage.

8.10.2 Surface Coatings

Surface coatings (waxes, edible films, etc.) are used to modify the internal atmosphere and to reduce water losses of fruits and vegetables (Ergun et al., 2005; Hagenmaier and Baker, 1994). The permeance values of produce treated with coatings may differ compared to the permeance of the coatings

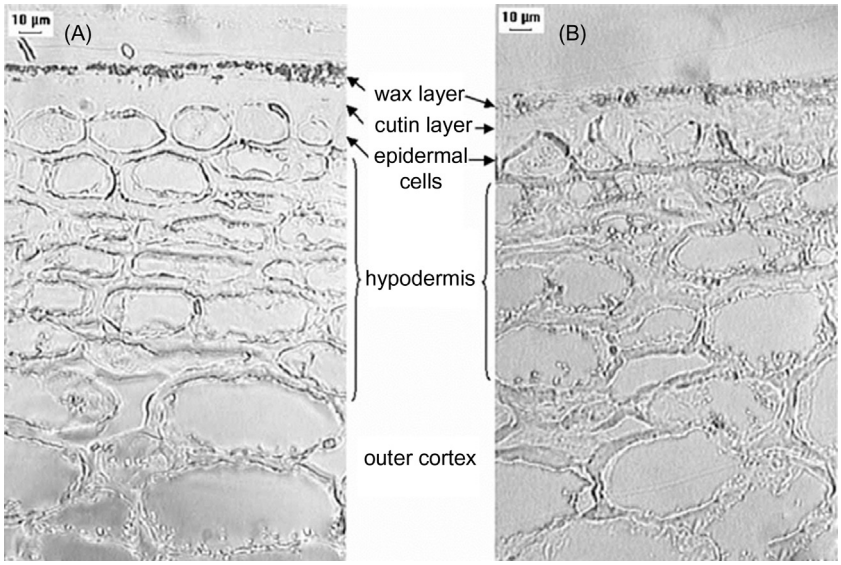

FIGURE 8.7

Cross sections of the skin and outer cortex of "Jonica" apples sampled in (A) March and (B) June, after a 3-month storage. *Photo from Schotsmans, W., Verlinden, B.E., Lammertyn, J., Nicolai B.M., 2004. The relationship between gas transport properties and the histology of apple. J. Sci. Food Agric. 84, 1131–1140.*

FIGURE 8.8

Greenhouse-grown bell pepper packed with polymeric film. The package extends fruit postharvest life by reducing fruit transpiration. The package also facilitates fruit handling and provides protection to reduce fruit mechanical damage. The package may help in marketing by allowing labeling and providing an appealing appearance of the fruit.

(Amarante et al., 2001). Coatings block the pores on the produce skin, reducing fruit water loss. Wax is commonly used in many fruits such citrus, tomato, bell pepper, and cucumber to improve fruit appearance and reduce transpiration; however, the effect of the wax on reducing fruit transpiration is limited (about 30% reduction).

8.10.3 Polymeric Films

Modified atmosphere packaging with polymeric films is effective in reducing transpiration and extending shelf life in commodities (Fig. 8.8).

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CHAPTER 9
Carbohydrates

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9.1 INTRODUCTION

Carbohydrates are compounds that contain mainly molecules of carbon, hydrogen, and oxygen, although they can also contain nitrogen and phosphorus. They are the most abundant compounds and are widely distributed in horticultural commodities. Their contents in fruits and vegetables vary between less than 1.0% to up to more than 60% (Tables 9.1 and 9.2). They are normally estimated at around 50%–80% of the dry weight of vegetables and fruits. In general, carbohydrates in leafy and stem vegetables are around 2%–9%, in root vegetables and tubers are from 15% to 25%, and in citrus fruits are from 10% to 12% of fresh weight. Carbohydrates are very important in horticultural commodities because of their contribution to the texture, flavor, color, and nutritional value of these products. Celluloses, hemicelluloses, and pectins are essential to maintaining the structure of plant cells. Starch is the storage polysaccharide in unripe fruits and vegetables, and is transformed into simple carbohydrates during ripening. Sugars contribute to the sweetness of fruits and vegetables. The palate of horticultural commodities depends on the balance between sugars, organic acids, and other compounds such as phenolic compounds. Most of the flavonoids that contribute to the red and blue colors of horticultural products are glycosides. The hexose sugars are the source of energy in the cell through their degradation during the process of respiration. Ascorbic acid (vitamin C) is considered a derivative of sugars. Based on molecular structure, carbohydrates are classified as monosaccharides (the simplest units of carbohydrates), oligosaccharides (formed by 2–10 units of monosaccharides linked by glycosidic bonds), and polysaccharides (theoretically formed by more than 10 units).

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Table 9.1 Total Contents of Sugars in Some Horticultural Commodities (g/100 g Fresh Weight)

Commodity	Average	Maximum Content	Minimal Content	Commodity	Average	Maximum Content	Minimal Content
Apples	12.00	16.60	6.00	Nectarines	7.90		
Apricot	4.00	11.80	1.60	Olives	2.20		
Avocado	0.40			Oranges	7.90	12.00	3.96
Bananas	18.00	21.7	11.4	Papaya	9.00		
Blueberries	4.50	10.40	2.40	Passion fruit	10.0	13.3	7.4
Cantaloupe	6.90			Peaches	8.50	11.70	6.30
Cherries	9.40	15.30	6.40	Pears	7.00	13.20	6.50
Dates				Persian limes	2.20	3.60	0.90
Feijoa	6.6			Persimmon	16.00		
Figs	16.00	18.20	13.10	Pineapples	12.30	18.40	7.50
Grapefruit	6.80	9.96	3.30	Plums	7.80	13.20	2.90
Grapes	15.00	18.20	13.10	Quince	8.0	10.0	6.5
Guava	5.70	10.00	3.30	Strawberries	5.70	9.80	2.80
Honeydew melons	7.50			Tomatoes	2.80	4.30	1.80
Mango	14.00						

Table 9.2 Contents (% of Fresh Weight) of Total Sugars, Glucose, Fructose, and Sucrose in Some Fruits (g/100 g Fresh Weight)

Fruit	Total Sugars	Glucose	Fructose	Sucrose
Apples	11.6	1.7	6.1	3.6
Apricots		1.9	0.4	4.4
Avocado	0.4			
Bananas		5.80	3.8	6.6
Dates	61.0	31.0	23.7	8.2
Grapefruit		2.0	1.2	2.1
Grapes	14.8	8.2	7.3	
Green figs		5.5	4.0	0.0
Peaches		1.5	0.90	6.7
Pears	10.0	2.4	7.0	1.0
Persian limes	0.7			
Pineapples	12.3	2.3	1.4	7.9
Plums		4.0	1.3	4.3
Strawberries		2.6	2.3	1.3
Tomato	2.8	1.6	1.2	

9.2 TYPES

9.2.1 Reducing and Nonreducing Sugars

Sugars containing a free or potentially aldehyde-free group are referred to as reducing sugars. The aldehyde group has the ability to act as an electron reductant or acceptor in an alkaline solution. Examples of reducing sugars include glucose, fructose, galactose, ribose, xylose, and mannose. Examples of nonreducing sugars include sucrose and raffinose. The content of reducing to nonreducing sugars is very important in several products. Preharvest and postharvest handling of horticultural commodities can influence the contents of these sugars, and thus the quality of these commodities. Early potato crops can accumulate excessive reducing sugars in a few days when stored at 10−13°C. This is detrimental for the quality of processed potatoes (to elaborate chips). A high content of reducing sugars in early potatoes results in the production of undesirable dark-colored chips due to the Maillard reaction (browning) during frying.

9.2.2 Monosaccharides

Monosaccharides are the simplest carbohydrates since they cannot be hydrolyzed to smaller carbohydrates. Chemically they are aldehydes or ketones possessing two or more hydroxyl groups, and are important as building blocks for the synthesis of nucleic acids, as well as fuel molecules, that is, in glycolysis. Monosaccharides are classified according to three different characteristics: the location of their carbonyl group, the number of carbon atoms they contain, and their chiral property. If the carbonyl group is an aldehyde, the monosaccharide is an aldose. If the carbonyl group is a ketone, the monosaccharide is a ketose. Monosaccharides with three carbon atoms are called trioses and these are the smallest monosaccharides, such as dihydroxyacetone and D- and L-glyceraldehyde. Those composed of four carbon atoms are called tetroses, those with five carbons are called pentoses, those of six carbons are hexoses, and so on. The most important monosaccharides in fruits and vegetables are the hexoses, glucose and fructose (Fig. 9.1). Other minor monosaccharides include mannose, galactose, xylose, and arabinose. The most commonly detected pentoses are arabinoses and xyloses. Monosaccharides are usually found in the cytosol (cell sap). Their content is very high in some fruits and vegetables such as corn, peas, and sweet potatoes.

9.2.3 Oligosaccharides

Oligosaccharides are carbohydrates formed by 2−10 units of monosaccharides linked by glycosidic bonds, so they can be disaccharides, trisaccharides, tetrasaccharides and pentasaccharides. Many of them are components of fiber from plant tissue. Fructo-oligosaccharides (FOS) are oligomers of fructose molecules with a degree of polymerization (DP) equal to or less than 10, and are considered soluble dietary fiber. FOS are extracted from the blue agave plant as well as

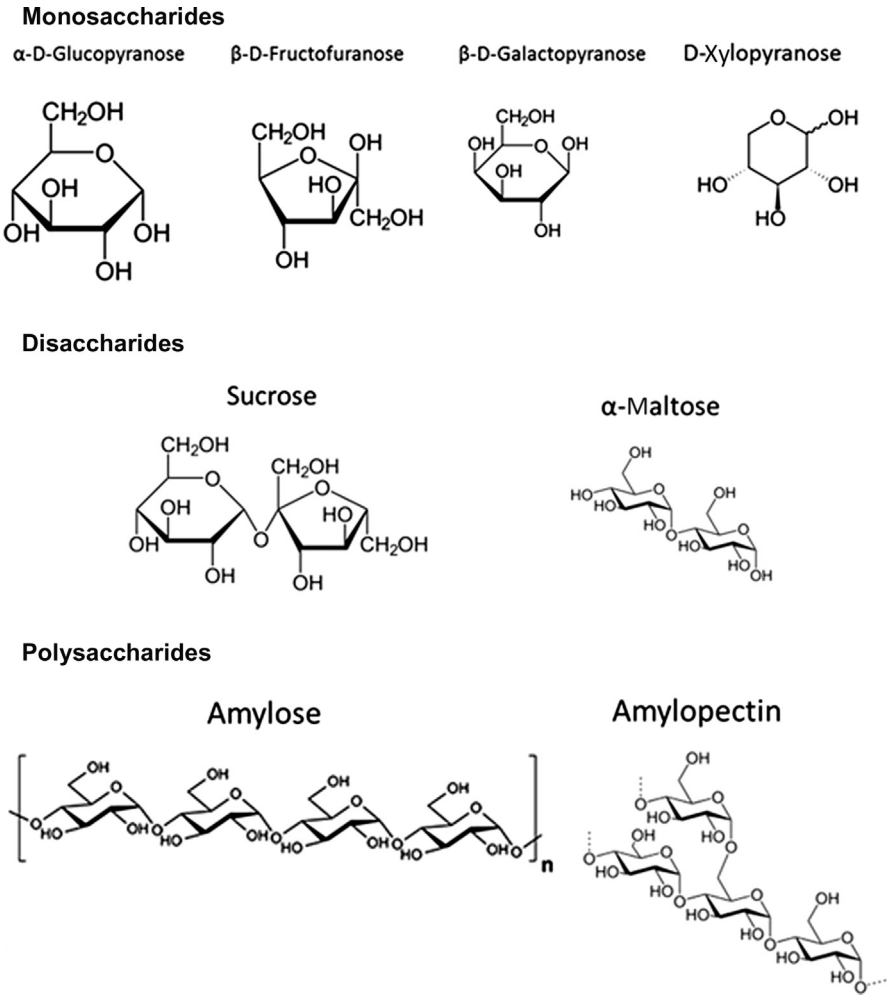

FIGURE 9.1
Basic structures of some monosaccharides, disaccharides, and polysaccharides.

some fruits and vegetables such as bananas, onions, chicory root, garlic, asparagus, Jerusalem artichoke, jicama, and leeks. Some grains and cereals, such as wheat and barley, also contain FOS. Both Jerusalem artichoke and blue agave plant have been found to have the highest concentrations of FOS of cultured plants. Galacto-oligosaccharides (GOS), which also occur naturally, consist of short chains of galactose molecules also sometimes called oligo-fructose or oligo-fructan, are oligosaccharide fructans, used as an alternative sweetener.

9.2.3.1 DISACCHARIDES

These are composed of two units of monosaccharides linked by a covalent bond known as a glycosidic bond formed by a dehydration reaction, which

results in the loss of one hydrogen atom from one monosaccharide and one hydroxyl group from the other. Sucrose (*O*-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside) is the most important of the disaccharides composed of two hexoses, glucose and fructose, that unlike most disaccharides their monomers are linked head-to-head, that is reducing end to reducing end (anomeric carbon atom from glucose to anomeric carbon atom from fructose). This is the reason why sucrose (Fig. 9.1) has no reducing end and is classified as a nonreducing sugar. This sugar is the main form of transporting carbohydrates in fruits and vegetables, with sugar cane, sugar beet, pineapple, apricot, and peach being the main sources. Maltose is a disaccharide formed from two units of glucose joined by an *O*-glycosidic bond between the first carbon (C1) of the first glucose linked to the fourth carbon (C4) of the second glucose. The bond is indicated as α(1→4) bond.

9.2.3.2 TRISACCHARIDES

Raffinose consists of three sugar molecules: galactose, glucose, and fructose, sequentially linked as αGalp(1→6)αGlc(1↔2)βFruf, and occurs naturally in cabbage, sugar beet, beans, and broccoli, among other commodities.

9.2.3.3 TETRASACCHARIDES

Stachyose is an example of these sugars, formed by two units of α-D-galactoase, one α-D-glucose unit, and one β-D-fructose unit, which are sequentially linked as αGal*p*(1→6)αGal*p*(1→6)αGlc*p*(1↔2)βFru*f*. Stachyose occurs naturally in green beans, soybeans, peas, and peanuts, among some other commodities.

9.2.3.4 PENTASACCHARIDES

Verbascose is composed of five monosaccharide molecules: three galactoses, one glucose, and one fructose (α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Glup(1↔2)-β-D-Fruf), and occurs naturally in soybean. In the large intestine, raffinose, stachyose, and verbascose act as a soluble dietary fiber.

9.2.4 Polysaccharides

Starch is the main reserve polysaccharide in fruits and vegetables. The structural polysaccharides (cellulose, hemicellulose, and pectins) are normally found in the cell wall. Their content is very abundant and they are very important for the texture and hardness of plant cells.

9.2.4.1 STARCH

Starch is the main form to store carbohydrates inside the cell (in the chloroplasts in leaves and amyloplasts in nonphotosynthetic tissues of plant cells). It is organized into small insoluble and semicrystalline granules. It is composed of two polymers of glucose named amylose and amylopectin. Amylose is a linear molecule of about 200−1000 units of glucose units connected through

glycosidic 1,4-alpha linkages, while amylopectin is a branched molecule of about 2000−2,000,000 units of glucose units linked with glycosidic 1,4-alpha linkages in the linear sections and 1,6-alpha linkages in the branching points. In the molecule of amylopectin, for every 20−25 units of glucose connected in a linear form with the glycosidic 1,4-alpha linkage there is a branching with the glycosidic 1,6-alpha linkage (Fig. 9.1). Starch is present in many horticultural commodities, especially in the unripe state, and when they are consumed uncooked, it can contribute to dietary fiber fraction. Starch from fruits (such as kiwi, unripe banana, apple) present some physicochemical, functional and nutritional characteristics that can show some advantages over cereal and tuber starches, which are used in diverse food and nonfood applications.

9.2.4.2 PECTIC SUBSTANCES

Pectic substances are heteropolysaccharides that contain galacturonic acid residues that are methylesterified to a variable extent (Fig. 9.2A). They include

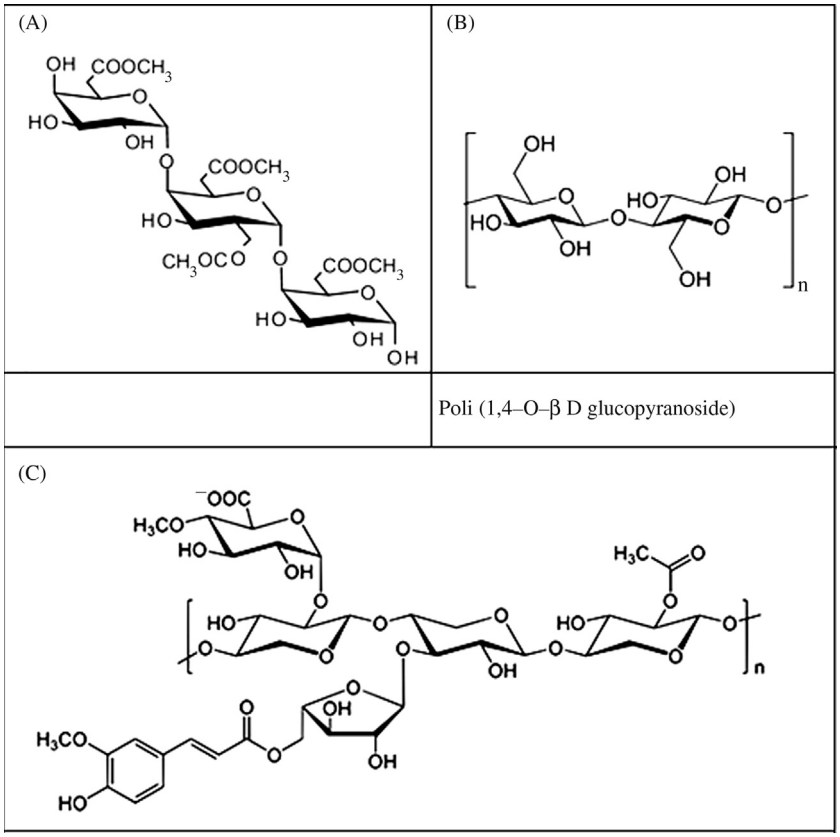

FIGURE 9.2

Basic structure of some polysaccharides in fruits and vegetables. (A) Basic structure of pectin; (B) basic structure of cellulose; (C) basic structure of hemicelluloses (xylan).

protopectin, which is insoluble in water and a precursor of the water-soluble pectin. It also includes pectinic acid which is a colloidal polygalacturonic acid with groups of methylated esters capable of forming gels. It can exist without esterification in which case it is referred to as pectic acid, or in association with divalent ions as pectate. In fruits and vegetables, it is believed that pectins are the components with the greatest contribution to the texture. The main structure of pectins consists of unbranched chains of galacturonic acids (GalA) linked together by α(1→4) bonds, which are known as homogalacturonan (HG) or also as a smooth region. Pectins may be esterified with a methyl group on carbon 6, or with an acetyl group on carbon 2 or 3. In immature fruits, HGs are found with a high methyl esterification and during the maturation of the fruit the enzyme pectin methyl esterase (PME) is expressed, which breaks the methyl-ester bond of carbon 6 and favors the formation of ionic bonds with Ca^{+2} residues. Two antiparallel chains of HGs can join and form a structure known as an egg box, which provides a structure resistant to digestion by the enzyme polygalacturonase (PG) and can only be hydrolyzed by the enzyme pectate lyase. The rhamnogalacturonans type I (RG-I), in addition to the HGs, are the main components of pectin and correspond to the branched region. RG-Is are responsible for the chemical and structural diversity of pectins and are located both in the primary cell wall and in the middle lamina of dicotyledonous plants. They are composed of the repeating units of a disaccharide composed by galacturonic acid and rhamnose [-α-D-GalpA- (1→2) -α-L-Rhaf-]. Such disaccharides are joined by α-(1→4) linkages and form a linear backbone with side chains of residues of (1→4) β-D-galactose and / or (1→5) α-L-arabinose, mainly bound to the backbone rhamnose in the O-4 position. Arabinogalactan type I contains side chains of (1→4) β-D-galactan with arabinose terminal residues in the O-3 position. The pectic polysaccharides of vegetables vary mainly in the composition of the side chains. Xylogalacturonan and rhamnogalacturonan type II (RG II) are compounds considered to be structurally modified HGs. RG II has a great diversity of sugars, among them apiose, aceric acid (3-C-carboxy-5-deoxy-L-xylose), 2-*O*-methyl fucose, 2-*O*-methyl xylose, Kdo (3-deoxy-D-man-2-octulosonic), and DHA (3-deoxy-D-lixo-2-heptulosaric acid). RG II is in a low proportion compared to RG I in the cell wall, but its structure is highly conserved, which suggests an important cellular function; its skeleton is HG with short side chains of the aforementioned sugars that allow the conformation of dimers by disteric bond of borate. Xylogalacturonan is a separate class of substituted HG, where about half of the GalA units are branched with α-D-Xyl side chains at position O-3.

9.2.4.3 CELLULOSE

Cellulose is a polymer of a linear and very long chain of glucose molecules (1000−10,000 molecules) with a molecular weight of around 200,000−2,000,000. The glucose molecules are connected through 1,4-beta linkages in a basic unit called cellobiose (Fig. 9.2B). Cellulose is very low in organs that contain carbohydrates from reserves such as starch, however,

it is very abundant in plant cell walls as a structural component where it constitutes 15%–30% and is found in the form of cellulose microfibrils, which are semicrystalline linear chains of β-(1–4) D-glucose linked by hydrogen bonds. Each cellulose microfibril contains approximately 36 linear glucose chains, and its organization determines the mechanical properties of the cell and contributes to the resistance of the cell wall. The glucose chains may reach a length of hundreds of micrometers, and the microfibrils are 5–15 nm wide and are separated from each other by 20–40 nm. Current models of microfibrillar organization suggest that cellulose has a structure composed of highly crystalline domains linked by amorphous regions.

9.2.4.4 HEMICELLULOSES

Xyloglucans (XG), commonly called hemicelluloses, are the main crosslinking polysaccharides in cell walls and are bound by hydrogen bonds to the cellulose microfibrils. The xyloglucans are mainly composed of linear chains of (1→4) β-D-glucan, linked to this chain are several units of α-D-Xyl, in the O-6 position of the glucose units (Fig. 9.2C). Basically, XGs are formed by repeating units of hepta- and octasaccharides. The repeated basic unit consists of four linked residues of glucose from which three consecutive residues are substituted with 1–6-linked xylose sidechains. Approximately half of these heptasaccharide units contain β-D-galactose-(1→2) branches on the xylose that is closest to the reducing end of the glucan, or on the middle xylose or between both residues. One α-L-fucose-(1→2) residue binds to the galactose residue in the first position. The structure and molecular distribution of the lateral chains of XG vary according to the species and plant tissues. In raspberry, it was found that the main neutral sugars of the hemicellulosic fraction were xylose and glucose, suggesting the presence of xylans and xyloglucans, in addition relatively high amounts of arabinose and galactose are found, suggesting the presence of arabinoxylans and/or branched pectins, which could be associated with hemicellulose. These results support the theory that conjugated xyloglucan-RG-I could exist in plant cell walls. In boysenberry it was found that xylose was the most abundant neutral sugar of the hemicellulose fraction, suggesting that xylan is the most important component in the cell wall of this fruit. Xyloglucan represents approximately 20% of the components of the cell wall in noni (*Morinda citrifolia*) ripe fruit.

9.2.4.5 INULIN

Inulin has a much higher degree of polymerization than FOS and is also a polysaccharide considered as soluble dietary fiber. Inulin is present in Jerusalem artichoke, chicory, leeks, garlic, onions, and asparagus, and is becoming a significant part of the daily diet in many countries.

9.2.5 Sugar Derivatives

9.2.5.1 ACID SUGARS

These are monosaccharides which possess a carboxyl group (Fig. 9.3). D-galacturonic acid, a molecule in which the terminal hydroxyl group of D-galactose has been oxidized to carboxylic acid, is the most common acid sugar found polymerized as polygalacturonic acid, the main component of pectin in horticultural commodities.

9.2.5.2 SUGAR ALCOHOLS OR POLYOLS

Sorbitol, myo-sorbitol, and xylitol are found in most fruits. The alcohol group is found in the carbon 5 of xilose (Fig. 9.3). Glycerol is found in olives. Sugar alcohol levels in the range of 0.003−6.8 g/100 g fw, mainly sorbitol and xylitol, are found in plum, apricot, sweet cherry, sour cherry, pear, and nectarine.

9.2.5.3 PHOSPHATE SUGARS

Phosphate sugars are intermediary esters in the metabolism of sugars and are usually found in low concentrations in mature fruits. The most important

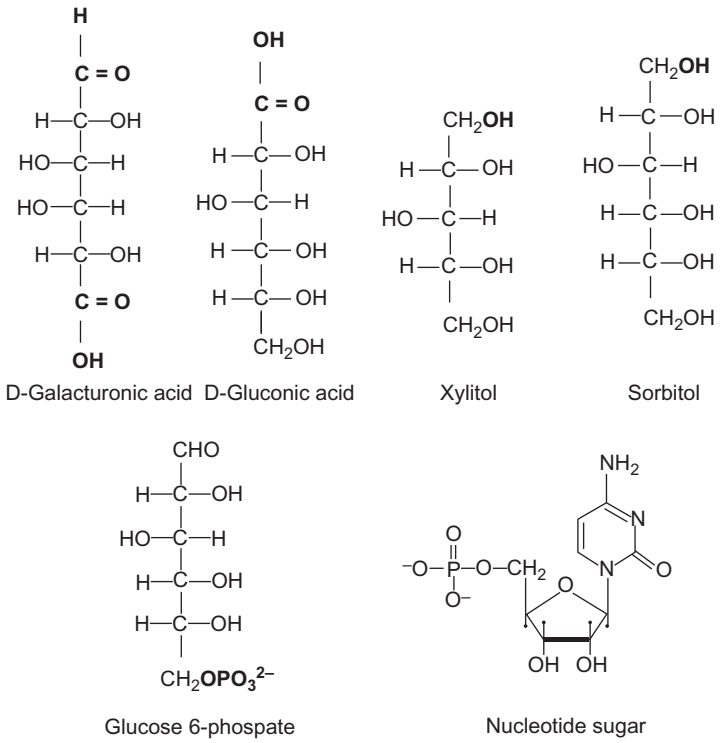

FIGURE 9.3

Examples of sugar derivatives: acid sugars (D-galacturonic acid and D-gluconic acid); polyols (xylitol and sorbitol); phosphate sugars (glucose 6-phosphate); and a nucleotide sugar (sugar linked to a phosphate group and to a nitrogenous base).

phosphate sugars include glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-diphosphate (Fig. 9.3). All of them take part in the glycolysis pathway in the cytosol. Both glucose 6-phosphate and fructose 6-phosphate also take part in the pentose phosphate pathway.

9.2.5.4 GLYCOSIDES

These are sugar derivatives where the sugar group is linked through its anomeric carbon to a functional group of another molecule via a glycosidic bond, as with an alcohol or a phenol group. Flavonoid glycosides are naturally occurring in some fruits and vegetables. For example, hesperidin, commonly found in citrus fruits, is a glycoside whose aglycone is the flavonoid hesperetin and its sugar residue is the disaccharide rutinose (6-*O*-α-L-rhamnosyl-D-glucose). Another example is rutin whose aglycone is quercetin and whose sugar residue is also rutinose. This latter glycoside is found in most fruits and vegetables.

9.2.5.5 SUGAR-COATED NUCLEOTIDES (NUCLEOTIDE SUGARS)

These serve as glycosyl donors in glycosylation reactions. Glucose and galactose are the sugars that are most commonly found as nucleotides with uridine, guanosine, cytidine, adenosine, thiamine, and 2-deoxyuridine (Fig. 9.3).

9.3 SYNTHESIS OF CARBOHYDRATES

The biosynthesis of sugars during photosynthesis is activated by sunlight. Firstly, by means of photosynthesis (the Calvin cycle), the leaves of plants produce triose phosphates (the phosphate three-carbon monosaccharides) that are then used to generate energy or synthesize precursors. The excess of triose phosphate is converted to sucrose in order to be translocated to other parts of the plant where it can be used as fuel, or stored. Starch is the main form of storage of sugars in most plants, but in some commodities, such as sugar beet and sugarcane, sucrose is the main form of storage. The balance between the two processes is tightly regulated, and both must be coordinated with the rate of carbon fixation. The synthesis of sucrose occurs in the cytosol, while starch synthesis occurs in the plastids, with these processes being coordinated by a variety of regulatory mechanisms that respond to changes in light and photosynthetic rate. Starch is synthesized and temporarily stored in the chloroplasts as a stable end product of photosynthesis, and for long periods in amyloplasts of the nonphotosynthetic parts of plants, such as seeds, roots, and tubers.

9.3.1 Hexoses

Hexoses, as hexose phosphate pools, are present in both plastids and cytosol, and there is direct transport between both as in the case of developing fruits. On the other hand, amyloplasts that store starch for the long term can directly transport glucose 6-phosphate, facilitating direct communication between cytosolic and plastid pools of hexose phosphate. In the amyloplasts of pea roots and cotyledons, glucose 6-phosphate is also transported, whereas in

amyloplasts of potato tubers the transported species is glucose 1-phosphate. This difference in the transport of different hexose phosphates across chloroplasts and amyloplasts appears to indicate that chloroplast is just a place of temporary storage of recently fixed carbon. Because of the absence of hexose phosphate carriers in chloroplasts, the exchange between cytosolic and chloroplast hexose phosphate pools generally takes place in the form of three-carbon intermediates, namely dihydroxyacetone phosphate and 3-phosphoglycerate. The transport is through a triose-phosphate translocator (TPT), which is an antiporter of the inner membrane of chloroplast. TPT exchanges three-carbon intermediates for inorganic phosphate. This exchange process takes place to counter the absence of a required substrate during the process of synthesis.

9.3.2 Sucrose

Sucrose is the most abundant disaccharide in nature. It is only produced by plants and cyanobacteria (photosynthetic organisms) and serves as a transportable carbohydrate and sometimes as a storage compound. Sucrose is a convenient form of carbon transport because of its unusual linkage between the anomeric C-1 of glucose and the anomeric C-2 of fructose. This unavailability of the anomeric carbons prevents sucrose from being hydrolyzed by common carbohydrate-cleaving enzymes such as amylases, and from reacting nonenzymatically with amino acids and proteins. Sucrose is synthesized in the cytosol of leaf cells. The very beginning of sucrose synthesis is when dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, after being exported from the chloroplast to cytosol, react with each other, forming fructose 1,6-bisphosphate catalyzed by aldolase. After condensation of the two trioses, hydrolysis by fructose 1,6-bisphosphatase yields fructose 6-phosphate. On the other hand, UDP-glucose is derived from glucose 1-phosphate + UTP releasing PPi, catalyzed by UDP-glucose pyrophosphorylase. The biosynthesis of sucrose proceeds via the precursors UDP-glucose and fructose 6-phosphate, catalyzed by the enzyme sucrose-6-phosphate synthase to form sucrose 6-phosphate (fructose-6-phosphate + UDP-glucose → sucrose 6-phosphate + UDP). Finally, sucrose phosphatase removes the phosphate group (sucrose phosphate + H_2O → sucrose + Pi), making sucrose available for export to other tissues. The reaction catalyzed by sucrose 6-phosphate synthase is a low-energy process, but the hydrolysis of sucrose 6-phosphate to sucrose is sufficiently exergonic to make the overall synthesis of sucrose essentially irreversible. Sucrose synthesis is regulated and closely coordinated with starch synthesis.

9.3.3 Starch

Starch is synthesized in chloroplasts and amyloplasts by three key enzymes: ADP-glucose-pyrophosphorylase, starch synthase, and branching enzyme. The steps in the starch biosynthesis are shown in Fig. 9.2.

The ADP-glucose-phosphorylase activates the glucose molecule through the formation of ADP-glucose. Starch synthase catalyzes the addition of single glucose residues, donated by ADP-glucose, to the reducing end of a starch molecule by a two-step insertion mechanism (Fig. 9.4A).

There are two types of starch synthases: soluble and granule-bound; the former synthesizes amylopectin and the granule-bound synthesizes amylose. Three isoforms of the starch synthase soluble (SSS) have been reported. SSSI produced short linear chains with a degree of polymerization (DP) of 6–12, SSSII synthesized chains with a DP higher than 24, and SSSIII intermediate chains with a DP of 13–24. In the case of granule-bound starch synthase (GBSS), which synthesizes amylose, no isoforms have been reported. GBSS transfers glucosyl units from ADP-glucose to glucan chain, producing long chains. This reaction is achieved within the semicrystalline matrix formed by amylopectin (Fig. 9.4B).

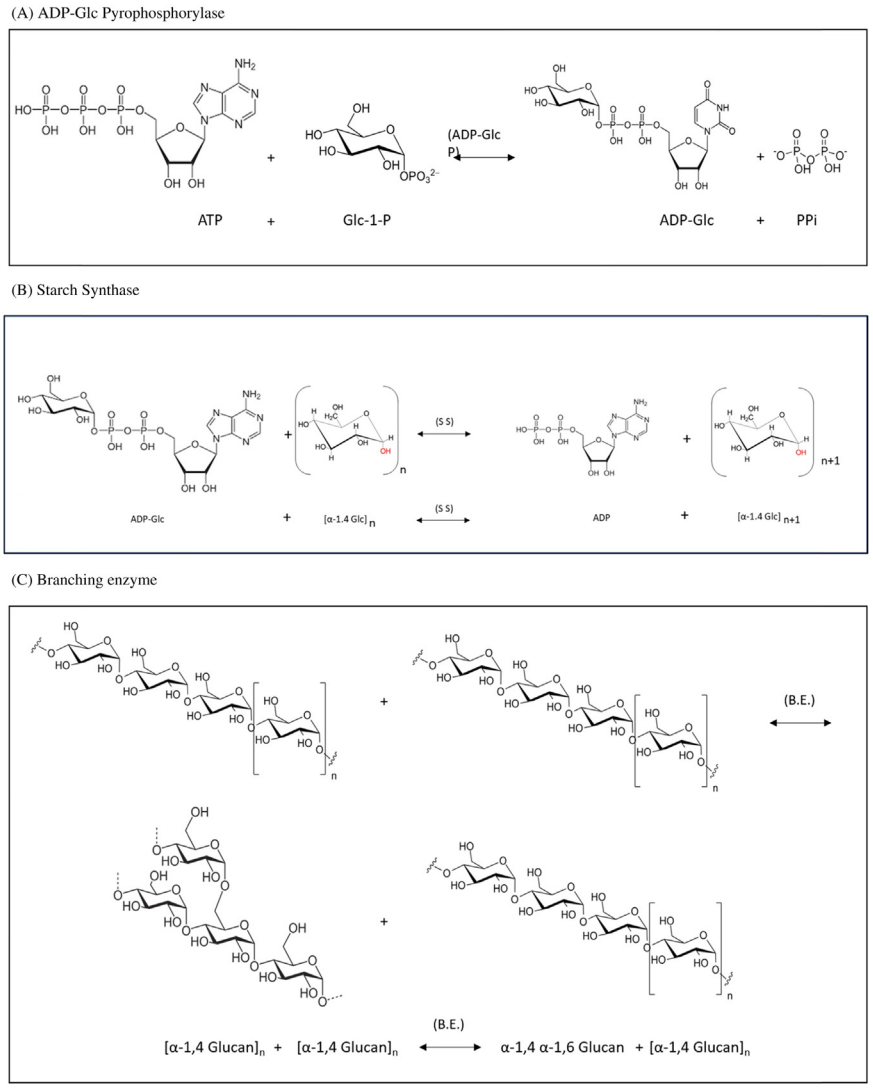

FIGURE 9.4

Main steps in starch biosynthesis. (A) (ADP-Glc P) ADP-Glc pyrophosphorylase; (B) (SS) starch synthase; (C) (BE) branching enzyme.

Branches in amylopectin are introduced by another enzyme (branching enzyme, BE), where isoforms SBEI, SBEIIa, and SBEIIb introduce long, intermediate, and short chains, respectively. In growing plants, starch is accumulated from rapid growth during the day. The synthesis of starch is not completely understood, but it is thought that this process begins with the conversion of sucrose to ADP-glucose, directly by the enzyme sucrose synthase or indirectly by the enzyme invertase. Also, it is not well known if amylopectin is produced in the first step and after debranching to produce amylose or if both molecules are produced simultaneously (Fig. 9.4C).

Those fruits that store starch during development switch to starch hydrolysis during ripening. Kiwifruit provide an example of biochemical changes in a starch-storing fruit. They are normally harvested with starch contents ranging from 4% to 10% in fresh weight, and soluble solids content between 6.2% and 12%. At harvest, the main sugars are sucrose, glucose, and fructose. As the fruit ripen after harvest, the sucrose content increases only slightly, while fructose and glucose increase in parallel, to become the predominant sugars in ripe fruit. There are increases in the activities of a number of sucrose-metabolizing enzymes, particularly sucrose phosphate synthase and invertase. The starch-degrading enzyme, α-amylase, increases twofold.

9.3.4 Cellulose

Cellulose is a major component of the plant cell wall, but its synthesis is not well known, even less known than that of starch. Cellulose is synthesized from intercellular precursors deposited and assembled outside the plasma membrane. The enzymatic machinery required for the initiation, elongation, and export of cellulose chains is of higher complexity than that needed to synthesize and accumulate starch inside the cell. Cellulose is synthesized in the plasma membrane whose enzymatic machinery that assembles cellulose chains crosses the plasma membrane, with one part positioned in the side of the cytosol to bind the substrate UDP-glucose, and another part extending to the outside of the plasma membrane which is responsible for elongating and crystallizing cellulose molecules in the extracytoplasmic space. These external parts, called rosettes, are composed of six large particles arranged hexagonally, where the catalytic subunit of cellulose synthase is part. This latter enzyme has not been isolated in its active form. However, from the nucleotide sequence of its gene, its amino acid sequence has been determined.

The formation of a lipid-linked intermediate seems to be involved in the initiation of new cellulose chains, a trait not seen in starch synthsis. The synthesis begins on the inner face of the plasma membrane where glucose seems to be transformed from UDP-glucose to sitosterol (a membrane lipid). Here, additional glucose residues are linked with a $\beta(1\rightarrow4)$ linkage to the first residue by the intracellular cellulose synthase. Thus, a short chain, referred to as sitosterol dextrin, is formed attached to sitosterol from the outer face of the plasma membrane from which most of the polymeric chain is removed by an

endo-1,4-β-glucanase. A second form of cellulose synthase is able to extend the chain to 500−15,000 glucose units, being extended onto the outer surface of the cell. Whether the addition of glucose residues occurs at the reducing end or at the nonreducing end of the growing chain has not been established. Once finished, cellulose chains are arranged in crystalline microfibrils made up by 36 parallel-oriented separate chains. It is believed that each large particle in the rosette is able to synthesize six separate cellulose chains simultaneously, so that 36 polymers are aligned together on the outer surface of the cell and crystallized as a microfibril of the cell wall. A critical length reached by the 36 polymers triggers an unknown mechanism indicating polymer synthesis termination.

9.3.5 Hemicelluloses

Hemicelluloses are synthesized by glycosyltransferases located in the Golgi membranes. Some glycosyltransferases needed for biosynthesis of xyloglucans and mannans are known.

9.3.6 Pectic Substances

The Golgi apparatus is the place where pectin is synthesized, and from there it is transported to the wall enveloped in membrane vesicles. The synthesis occurs simultaneously in numerous Golgi stacks in a process that seems to be carried out by specific and compartmentalized biosynthetic enzyme construction of increasingly complex pectin polysaccharides through the *cis*, medial, and *trans* Golgi cisternae. Such polymers are transported in Golgi vesicles targeted to reach the cell wall, presumably being directed by actin filaments that have myosin motors. The enzymes involved in pectin synthesis are Golgi-localized glycosyltransferases that are associated or membrane bound, able to transfer glycosyl residues from nucleotide sugars to oligosaccharides or polysaccharides. However, it is not clear how the synthesis of any of the pectic polysaccharides is initiated. Some enzyme-catalyzed modifications on glycosyl residues of pectin chains can be done, for example, esterification, acetylation, or feruloylation. When inserted into the walls, homogalacturonans seem to be highly methylesterified, however, these polymers are later de-esterified by wall-localized pectin methylesterases producing negatively charged polymers, a process that has been associated with growth cessation. Thus, the binding of negatively charged polymers with positively charged ions and proteins, is possible. Also, the association of homogalacturonan molecules to each other via Ca^{2+} binding is possible, contributing to cell−cell adhesion.

9.4 CHANGES DURING FRUIT DEVELOPMENT AND RIPENING

Carbohydrates are translocated from the leaves to the fruit, normally in the form of sucrose. In the early stages of fruit development, the carbohydrates that are synthesized in the fruit are polysaccharides such as cellulose,

hemicellulose, pectins, and starch. During ripening and senescence of the fruit, the polysaccharides begin to degrade to simple sugars and thus the sweetness of the fruit increases. Carbohydrate changes occur in a similar form in the climacteric and nonclimacteric commodities before harvest. However, after harvest, the change in carbohydrates is normally much more accelerated in the climacteric commodities after they reach their physiological maturity, and very slow or nonexistent in the nonclimacteric commodities. For this reason, nonclimacteric commodities should not be harvested before reaching their optimum ripening and quality. In tomato fruit, there are different genotypes that accumulate either hexoses or sucrose. Most cultivars are hexose accumulators, in which acid invertase is active during growth and ripening. In transgenic tomatoes in which acid invertase activity was suppressed by expression of an antisense invertase transgene, sucrose accumulation occurs in a normally hexose-accumulating cultivar. In melons, where sucrose is the main sugar that increases, there is a corresponding decrease in acid invertase and an increase in sucrose phosphate synthase activity, which synthesizes sucrose from hexose phosphate and the adenylated precursors.

9.5 INTERCONVERSION OF CARBOHYDRATES

Three metabolic intermediates, namely glucose 6-phosphate, glucose 1-phosphate, and fructose 6-phosphate constitute the hexose phosphate pool. These are kept in equilibrium through the conversion of glucose 1-phosphate to glucose 6-phosphate and vice versa by phosphoglucomutase activity, and the conversion of glucose 6-phosphate to fructose 6-phosphate by glucose 6-phosphate isomerase. However, the entry of carbon in the hexose phosphate pool takes place in the form of the production of starch and sucrose degradation, through gluconeogenesis and glycolytic pathways, whereas the exit of carbon from this pool takes place through starch and sucrose synthesis, the process of cell wall formation, and through the oxidation reactions of the pentose phosphate pathway.

9.5.1 Degradation and Losses During Respiration

As starch-storing fruit ripen, starch is degraded. Starch accumulation during fruit growth, and starch degradation during fruit ripening, are important phenomena since the sugar content is increased, providing the sweetness associated with the taste of ripe fruit. Sweetness is one of the most important quality attributes in most fruits, affecting the final fruit flavor and quality. The net loss of starch starts at the beginning of the fruit-ripening process on and off the tree. After harvest, a rapid increase in ethylene production and respiration rate in several climacteric fruits, such as apples, is simultaneously observed with the loss of starch content and sugars, since in fruits and vegetables the respiration process transforms glucose into carbon dioxide, which is released from the tissue into the surrounding atmosphere. The respiration rate depends on several factors, such as temperature and atmospheric

composition of the storage system, however, the nature of the produce is decisive in how fast it will lose sugars. While citrus, onion, and potato respire slowly, sweet corn and spinach respire extremely quickly.

9.5.2 Degradation of Starch to Sugars

In horticultural commodities, the degradation of starch is a very important process during ripening. In almost all horticultural products when the fruit is still immature, most of its nonstructural carbohydrates are stored in the form of starch. At the beginning of the maturation process, the degradation of starch to simple sugars begins. For example, the starch content in fruits of immature bananas and mangoes is around 14% and 18%, respectively, and only around 1.0%−2.0% in both fruits when they reach their ripening stage. The degradation of starch to simple sugars contributes to the formation of energy, flavors, and aromas. The degradation of starch to its base unit (glucose) is catalyzed by three enzymes: alpha-amylase, beta-amylase, and starch phosphorylase as indicated in the following.

$$\text{Amylase}: \ \text{Starch} + n\,H_2O \rightarrow n - \text{maltose}$$

Alpha-amylase hydrolyzes alpha 1,4-linkages of amylose at random, thus producing fragments of 10 glucose subunits called "maltodextrin." These are slowly hydrolyzed to maltose. The enzyme can also hydrolyze the alpha 1,4 part of the amylopectin but not the alpha 1,6 part. Beta-amylase catalyzes the removal of maltose units starting from the nonreducing side of the starch chain until it reaches the point with the linkage of alpha, 1,6, producing maltose and dextrins. The enzyme starch phosphorylase hydrolyzes the alpha 1,4 chain of the amylose, producing glucose-1-phosphate. None of the three enzymes is active with the alpha 1,6 chain of amylopectin and thus starch cannot be hydrolyzed in a complete form with these three enzymes.

$$\text{Maltase}: \ \text{Maltose} + H_2O \rightarrow 2\ \text{glucoses}$$

The resulting maltose and glucose are exported to the cytosol by the glucose transporter and maltose transporter, and glucose-1-phosphate is thought to be exported by a similar but as yet unknown mechanism. Once in the cytosol the maltose and glucose are converted to substrates for either sucrose synthesis, glycolysis, or the oxidative pentose phosphate pathway. Bananas accumulate soluble sugars after harvest from a stored starch reserve. Starch, which constitutes 20%−25% of the fresh weight of unripe bananas, is almost entirely converted to soluble sugars during ripening with approximately 2%−5% lost as CO_2 during respiration. Initially the predominant sugar is sucrose, hexose sugars appear after sucrose, and ultimately exceed sucrose concentration.

9.5.3 Conversion of Sugars to Starch

Potatoes stored for 2−3 months at low temperatures (1−3°C) may exhibit a loss of starch of as much as 30% of their initial content. However, if these potatoes are transferred to high temperatures the starch content of potatoes may increase, as a result of synthesis of starch from sugars, a conversion

carried out presumably by starch-synthesizing enzymes. Nevertheless, this resynthesis of starch may present a significant alteration to the structure of the starch granules. In sweet corn, a rapid and significant conversion of sugars to starch is also seen in the endosperm of its kernels. That is why sweet corn is harvested at an immature stage of development and eaten as a vegetable. Also, the sugars of peas are readily converted into starch, and therefore, green peas must be promptly cooled near 0°C immediately after picking in order to avoid loss of sugar content and flavor.

9.5.4 Conversion of Sucrose to Reducing Sugars

In the degradation reactions of sucrose to hexose monophosphates in plant tissues, the first step is the cleavage of the glycosidic bond by either invertase (catalyzing the following reaction: sucrose + H_2O → D-glucose + D-fructose) or sucrose synthase (catalyzing the following reaction: sucrose + UDP → UDP-glucose + D-fructose). In plant tissues, two types of invertases are able to hydrolyze sucrose to glucose and fructose in an essentially irreversible reaction. Acid invertase is present in the vacuoles, in the free space outside cells, and may be associated with the cell wall. This has an optimum activity near pH 5. The other is the alkaline or neutral invertase, located in the cytosol and maximally active at about pH 7−7.5. Sucrose synthase is a cytosolic enzyme that catalyzes a readily reversible reaction, but probably acts only in the breakdown of sucrose in vivo. Sucrose appears to be partitioned between alkaline invertase (Km values: 10−15 mM) and sucrose synthase (Km values: 20−30 mM) in the cytosol on the basis of differences in affinity of the two enzymes for the substrate. Km, also named Michaelis constant, is the substrate concentration at which the reaction rate is half of the maximum rate for a given enzymatic reaction. Glucose and fructose are metabolized further following phosphorylation to the corresponding hexose-6-P, probably by separate enzymes for the two hexoses. Plant tissues contain several hexose kinases that have specificity towards either glucose or fructose. A substantial portion of the glucose kinase in plant tissues is associated with the outer surface of the outer mitochondrial membrane, while fructo-kinases appear to be soluble in the cytosol. Two molecules of ATP are required to metabolize the hexoses formed upon cleavage of sucrose by invertase. However, when sucrose is cleaved by sucrose synthase, part of the energy in the glycosidic bond is conserved in the UDP-glucose formed and only one molecule of ATP is required for the further metabolism of fructose. UDP-glucose may be converted to glucose-1-P by UDP-glucose pyrophosphorylase. Glucose-1-P is converted to glucose-6-P by phosphoglucomutase, and glucose-6-P to fructose-6-P by phosphohexose isomerase.

9.5.5 Increases in Cellulose

All functional cell wall components of asparagus spears increase in a closely temperature-dependent way when stored for a few days. The content of soluble glucose declines by a comparable degree, indicating major carbon flow of this storage sugar into cell walls (60%−70%). However, the contents of stored soluble fructose and sucrose remain more or less constant, irrespective of

temperature. At higher temperatures, secondary cell wall thickening results mainly from a large increase in cellulose content. The pronounced increase in the fractions of cellulose and especially lignin may stress the important role of lignin in cell wall strengthening. While the fraction of cell wall proteins decreases, those of hemicellulose and the pectic components are commonly not influenced.

9.5.6 Degradation of Cellulose to Glucose

Cellulose is a very stable molecule, but it can be degraded with strong acids and cellulase enzymes.

The degradation of cellulose and the activity of cellulases are not significantly related to the softening of most fruits. Ripening of mango fruit (*Mangifera indica* L.) is characterized by a gradual textural softening. In some cultivars, from the unripe to ripe stage, cellulose is reduced from about 2% to 0.9% and hemicelluloses from about 0.8% to 0.2%. Concomitantly, the total soluble solids increase from about 7% to up to 20%, whereas total soluble sugars increase from about 1% to 15%. The increase in activity of several of the carbohydrate-degrading enzymes, which results in solubilization of the various polysaccharide fractions, correlates with the fruit-softening phenomenon. Although cellulase activity increases very early during ripening of the fruit, it seems that, although the enzyme does not seem to contribute to early loss of firmness in the fruit, it is probably responsible for cellulose hydrolysis late in the ripening process.

9.5.7 Solubilization of Pectins

The solubilization of pectins is one of the main events that occur during the softening of fleshy fruits. The changes in pectin solubility are commonly determined by the extraction of the pectic polymers, firstly in water, secondly in chelating agents, and then in slightly alkaline solutions, in a sequential manner. The increase in pectin solubility is observed by a decline of chelator-soluble pectins, which are also known as calcium-bonded pectins, as well as a decrease in pectins soluble in alkalis or also called pectins linked by covalent bonds. Water-soluble pectins indicate little or no binding to cell wall components. It is assumed that homogalacturonans are bound together by calcium bridges, which is the basis for using chelating agents for their extraction. This type of pectin is found mainly in the middle lamina and in the tricellular junction region. The alkali-soluble pectins are covalently bound to hemicellulose and cellulose, therefore carbonate salts are used for their extraction. They interconnect the amorphous matrix with the fibrillar phase of the primary cell wall. The depolymerization is one of the distinctive processes of fruit ripening and is determined by estimating the molecular weight distribution of the polymers eluted through agarose gels with variable pore size. The changes in pectic polymers during fruit ripening in boysenberry have been shown after a first step of changes in cellulose and hemicellulose composition. Cell wall components during the maturation of boysenberry can be explained in stages, since the results suggest that temporary changes in cell wall degradation of

blackberry occur in at least three stages. This is a step where solubilization of the pectic polymers without depolymerization is presented, followed by a final step where a reduction of the galactose content in pectin is mainly present, and also a drastic increase in the depolymerization of the pectic polymers, with an increase in the activity of the enzymes polygalacturonase (PG), pectinmethylesterase (PME), and β-galactosidase. In raspberry, an increase in the solubilization of pectin, without depolymerization is associated with fruit softening in the intermediate stages of ripening where arabinose is the most abundant neutral noncellulose sugar of the cell wall, a drastic solubilization of arabinose along with a dramatic depolymerization of pectin was shown at the end of ripening. PG, PME, in addition to cellulase, seem to be responsible for softening in raspberry. In the case of starfruit (*Averrhoa carambola*) solubilization and depolymerization of the pectin and hemicellulose in the final stages of fruit ripening were noted, when the firmness of the tissue had decreased substantially, along with an increase in the activity of PG and β-(1−4)-glucanase. In cherry, the integrated action of the enzymes PG, PME, and β-galactosidase seem to be required for its softening, and in strawberry it was observed that the softer varieties had high activity of PG and PME.

9.5.7.1 POLYGALACTURONASES

PG is a hydrolytic enzyme, which acts on polygalacturonic acid (PGA), hydrolyzing α-1,4 glycosidic bonds of pectic acid. According to its mode of action, PG is classified as endo-PG (resulting in random degradation of the pectic chain, EC 3.2.1.15) or exo-PG (resulting in the degradation of nonreducing free ends of the pectic chain, EC 3.2.1.67). The exo-PG has not been found to have a great effect on the solubility of pectin, however, it was found that in tomatoes, the production of ethylene is increased, which in turn triggers the ripening process. On the other hand, endo-PG depolymerizes pectic acid in a random way, resulting in a rapid decrease in viscosity and therefore it contributes markedly to the ripening process. The hydrolysis rate by endo-PG decreases with the reduction in chain length. The extent and the rate of softening during fruit ripening is directly related to the composition of PG, that is, there is marked softening if endo- and exo-PGs are present and there is less softening if only exo-PG is present. Initially, it was generally accepted that PG is mainly responsible for the dissolution of the middle lamina during the ripening of the fruit. This is due to the ability of PG to dissolve middle lamina in apple fruit; however, in pear fruit the complementary action of the cellulase is required to increase the accessibility of PG to the substrate. It was confirmed recently that PG is not the only determining factor to explain softening and that this process is the result of the coordinated action of several enzymes.

9.5.7.2 PECTIN METHYL ESTERASES

This enzyme (PME, EC 3.1.1.11) catalyzes the de-esterification of pectins releasing protons, methanol, and pectates, thus exposing the pectate carboxyl groups. The enzyme acts preferentially on a methyl ester group of

galacturonate unit next to a nonesterified galacturonate unit. It acts before polygalacturonases, which need nonesterified substrates. The degree of methyl-esterification influences the physicochemical properties of the pectic polymers, affecting their charge density and gelation properties. The de-esterification of linear pectin polymers allows more calcium-mediated junction zones which can contribute to the cell wall rigidity and fruit tissue integrity. PME activity is present in cell walls of a wide range of organs and tissues of higher plants. In fruits, the activity of this enzyme was found to be high in immature stages and tends to decrease afterwards.

9.5.7.3 β-GALACTOSIDASES

Galactose is an important constituent of noncellulosic polysaccharides, and during fruit maturation it is mobilized from one pectic matrix to another and is significantly lost. β-Galactosidase (EC 3.2.1.23) catalyzes the enzymatic hydrolysis of the glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate molecule via general acid catalysis. This enzyme partially degrades the pectic and hemicellulosic components of the cell wall and is probably related to the breakdown of polysaccharides in over-ripe stages of fruits. The presence and increased activity of β-galactosidase during ripening were reported in several fruit species such as apples, prickly pear, noni fruit, and others.

9.5.7.4 GALACTANASES

The arabinogalactan endo-1,4-β-galactosidase or galactanase (EC 3.2.1.89) is an enzyme of the plant cell wall, which participates in the hydrolysis of the β-1,4-galactans bonds of the branched region of pectin, specifically hydrolyzing the (1→4)-β-D-galactosidic bonds in type-I arabinogalactans, which is the primary cell wall component of dicotydelons. Tomato, starfruit, and noni fruit are some of the fruits where the presence and action of galactanase have been reported.

9.5.7.5 RHAMNOGALACTURONASES

Rhamnogalacturonase or rhamnogalacturonan hydrolase (EC 3.2.1.171) performs an endohydrolysis of the glycosidic bond α-D-GalA-(1→2)-α-L-Rha in the structure of rhamnogalacturonan I with an initial inversion of the anomeric configuration, releasing oligosaccharides with β-D-GalA from the reducing end. The activity of this enzyme has been reported in apples, tomatoes, grapes, noni fruit, etc.

9.5.7.6 XYLANASES

Xylanase or endo-1,4-β-xylanase (EC 3.2.1.8) is an enzyme that carries out an endohydrolysis of (1→4)-β-D-xyloside bonds in xylan. It was found that xylanase activity was associated with the depolymerization of hemicelluloses during the ripening of papaya. In starfruit, an increase in xylanase activity was observed during ripening, whereas in noni fruit increased activity and a sudden fall were observed during ripening.

9.5.7.7 XILOGLUCANASES

Xyloglucan is one of the main components of the primary cell wall of higher plants and it is intimately associated with cellulose chains, forming the load-bearing network of the cell wall. Xylolucanase (EC 3.2.1.151), hydrolyzes 1,4-β-D-glycosidic bonds of xyloglucan, with retention of the β-configuration of the glycosyl residues. The enzymatic degradation of the xyloglucan polymer by xyloglucanase could allow the action of the cellulases to hydrolyze the cellulose polymer more efficiently.

9.6 CARBOHYDRATES IN FRUITS AND VEGETABLES, AND HEALTH ISSUES

9.6.1 The Glycemic Index and Glycemic Load

GI is a qualification system for foods that contain carbohydrates, and from a nutritional point of view it can achieve a deeper insight into the relationship between the physiological effects of carbohydrate-rich foods and human health. GI has become a more useful concept than the traditional classification of carbohydrates that group them as simple or complex, sugars or starches, or as available or not available. GI is the measure of the impact of consuming carbohydrate-containing foods on the blood sugar level of a subject, and shows how quickly the blood glucose level is affected after the food is eaten (Fig. 9.5). In practice, GI is the rise in blood sugar level measured 2 h after the consumption of a standard quantity of food. However, the rise depends on a number of factors such as the type of carbohydrate, the physical entrapment of the carbohydrates within the food, and the food composition such as its fat and protein contents. The GI value for a given food is determined giving on one occasion to a group of 10 or more volunteers, after a 12-h fast, a quantity of the tested food that contains 50 g of digestible carbohydrates (calculated as

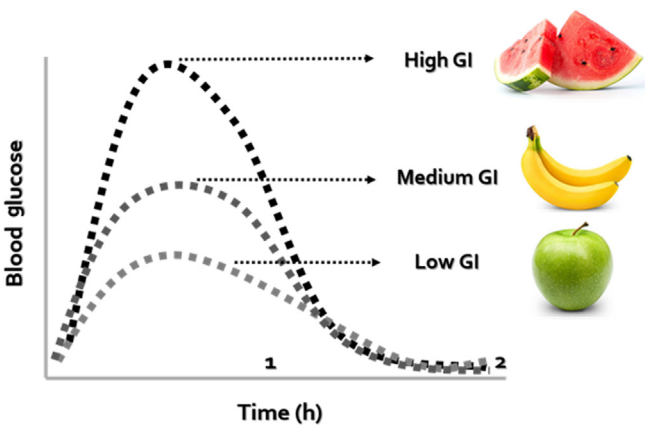

FIGURE 9.5
Glucose response after ingestion of some horticultural commodities.

the total carbohydrate minus fiber). The blood glucose level of all volunteers is monitored beginning before they eat the food and during the next 2 h. On another occasion, volunteers are given 50 g of glucose and their blood glucose levels are similarly monitored as previously mentioned. The averaged results from both occasions are used for calculations and compared. From both occasions, the area under the 2-h blood glucose response curve (AUC) is calculated and the test-food AUC is divided by the glucose AUC and multiplied by 100. A semi in vitro method was proposed to predict the glycemic index due to the problem associated with experimentation with humans where a security protocol is necessary. The semi in vitro test starts with a chewing step of the food by the volunteers, and after the hydrolysis continues in flasks with enzymes simulating the digestion in the gastrointestinal tract. The predicted glycemic values have shown a good correlation with in vivo tests. Different tested foods can be ranked on a percentage scale. GI values are commonly interpreted as low (55 or less), medium (56–69), or high (70 and above). A food ranked as low-GI will cause a slow rise in blood glucose level, whereas one ranked as high-GI will provoke a rapid rise in blood glucose. A lower glycemic response usually equates to a lower insulin demand and can improve long-term blood glucose control and blood lipids. However, in the case of insulin, a direct measure of the insulin response to a food is the insulin index.

Some fruits, although sweet, have shown low GI values, however, as fruits ripen they accumulate more sugars and their GI could increase, such as the case of bananas. Some ripe fruits such as mango, papaya, and pineapple exhibit a medium level of GI (Table 9.3). GI is a very useful tool; however, it

Table 9.3 Glycemic Index (GI) and glycemic Load (GL) for Some Raw Fruits (120 g of Serving Size)

Raw Fruits	GI	Level of GI	GL/Serving	Level of GL
Apple (mean of five studies)	36 ± 3	L	5	L
Apricots	34 ± 3	L	3	L
Banana (under-ripe)	30	L	6	L
Banana (slightly under-ripe, yellow with green sections)	42	L	11	M
Banana (ripe, all yellow)	51	L	13	M
Banana (over-ripe)	52	L	11	M
Cherries, sour	22	L	3	L
Grapes	49	L	9	L
Mango, ripe	60 ± 16	M	9	L
Oranges (mean of five studies)	45 ± 4	L	5	L
Papaya, ripe	60 ± 16	M	17	M
Pear (mean of four studies)	38 ± 2	L	4	L
Pineapple	66 ± 7	M	6	L
Strawberry	40 ± 7	L	1	L
Watermelon	80 ± 3	H	5	L

A GI of 55 or less is low (L), a GI of 56–69 is medium (M), and a GI of more than 70 is high (H).
A GL of 10 or less is low, a GL of 11–19 is medium, and a GL of 20 or more is high.

does not take into account the total amount of digestible carbohydrates that are contained in a given serving of food, and that is due to the fact that the test is standardized to only 50 g of digestible carbohydrates. In order to overcome such a restriction, a more complete indicator named the glycemic load (GL) was developed, which is based on the carbohydrate content per serving of the food consumed. Therefore, GL is a better indicator of how food carbohydrates will affect blood sugar. A food with a low glycemic index can contain a high carbohydrate amount per serving or vice versa. The GL takes into account this amount of digestible carbohydrates, because the GL is equal to GI in % multiplied by the grams of digestible carbohydrates per serving of food. Consuming food with a low GL will produce the most stable blood sugar levels.

9.6.2 Insulin Index

The insulin index of a food represents the elevation of the insulin concentration in the blood during the 2-h period after the food is ingested. The insulin index represents a comparison of food portions with equal overall caloric content (250 kcal or 1000 kJ). The insulin index can be more useful than either the glycemic index or the glycemic load, because certain foods, such as lean meats, cause an insulin response despite the fact that they contain very low amounts of carbohydrates. Table 9.4 shows data for glycemic and insulin scores for some fruits which were determined by feeding individuals with portions of fruits equal to 1000 KJ (239 kilocalories). Data were obtained recording the area under the glucose/insulin curve for 120 min after the fruits were consumed and dividing by the area under the glucose/insulin curve for white bread. The satiety provoked by the consumption of some fruits was determined by comparing how satiated the participants felt within 2 h after being fed a quantity of fruit equivalent to 240 calories while blindfolded to avoid food appearance biasing, then dividing that number by how satiated the participants felt after eating 240 kcal of white bread. The satiety level provoked by white bread receives a score of 100, so that fruits scoring higher than 100 are more satisfying than white bread and those under 100 are considered less satisfying.

9.6.3 Starch–Polyphenol Interactions

It is well known that horticultural commodities are good sources of phenolic compounds, and the consumption of these has been linked with a reduction in diverse chronic diseases, for example, cardiovascular, neurodegenerative,

Table 9.4 Glycemic, Insulin, and Satiety Levels for Some Fruits

Fruit	Glucose Score	Insulin Score	Satiety Score
Apples	50 ± 6 (L)	59 ± 4 (M)	197
Oranges	39 ± 7 (L)	60 ± 3 (M)	202
Bananas	79 ± 10 (H)	81 ± 5 (H)	118
Grapes	74 ± 9 (H)	82 ± 6 (H)	162

certain cancers, type II diabetes, etc., due to antioxidant, antiinflammatory, modulation of signal transduction, antimicrobial, and antiproliferation activities. The interactions between phenolic compounds and starch may modify, mainly, the nutritional characteristics of the polysaccharide, although the effect on the physicochemical properties is not clear due to the concentration of these compounds in plants. On the other hand, the phenolic compounds present in horticultural commodities can contribute to the sensory characteristics such as color, astringency, and bitter taste of the product.

The main issue regarding the interactions between starch and phenolic compounds is the effect on the hydrolysis of the polysaccharide by digestive enzymes with an impact on the glycemic and insulinemic responses. Noncovalent interactions between phenolic compounds, for example, polymeric or monomeric flavonoids, and starch, modify the starch structure that is not hydrolyzed by digestive enzymes, which means that resistant starch is produced and will be fermented in the colon by the microbiota (Fig. 9.6A). Also, phenolic compounds can inhibit digestive enzymes (such as α-amylase and amyloglucosidase) (Fig. 9.6B); and the third mechanism is related to the transport of glucose in the wall of intestinal brush, with a decrease in the level of glucose in the bloodstream (Fig. 9.6C).

9.6.4 Fruits and Vegetables as Sources of Fiber

9.6.4.1 DIETARY FIBER

Fibers are found in the structural part of all plant foods, including vegetables, fruits, grains, and legumes. The term dietary fiber was used for the first time in 1953, when Hipsley defined it as the nondigestible constituents making up the plant cell wall. Since then, many definitions of dietary fiber have been advanced. In 2001, the American Association of Cereal Chemists defined dietary fiber as: "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation." Definitions were later established for three types of fiber: (1) dietary fiber, (2) functional fiber, and (3) total fiber. The term "dietary fiber" describes nondigestible carbohydrates and lignin that are intrinsic and intact in plants when these or a part of these are eaten, whereas "functional fiber" is derived from nondigestible carbohydrates isolated from their natural source and is used as an ingredient in foods to increase the dietary fiber content; both have physiologically beneficial effects in humans. "Total fiber" is the sum of dietary fiber and functional fiber. These terms recognize the diversity of nondigested carbohydrates from plant sources, carbohydrates contributed by animal foods, and also those isolated carbohydrates of low molecular weight that occur naturally or have been synthesized or manufactured. Functional fiber may be isolated or

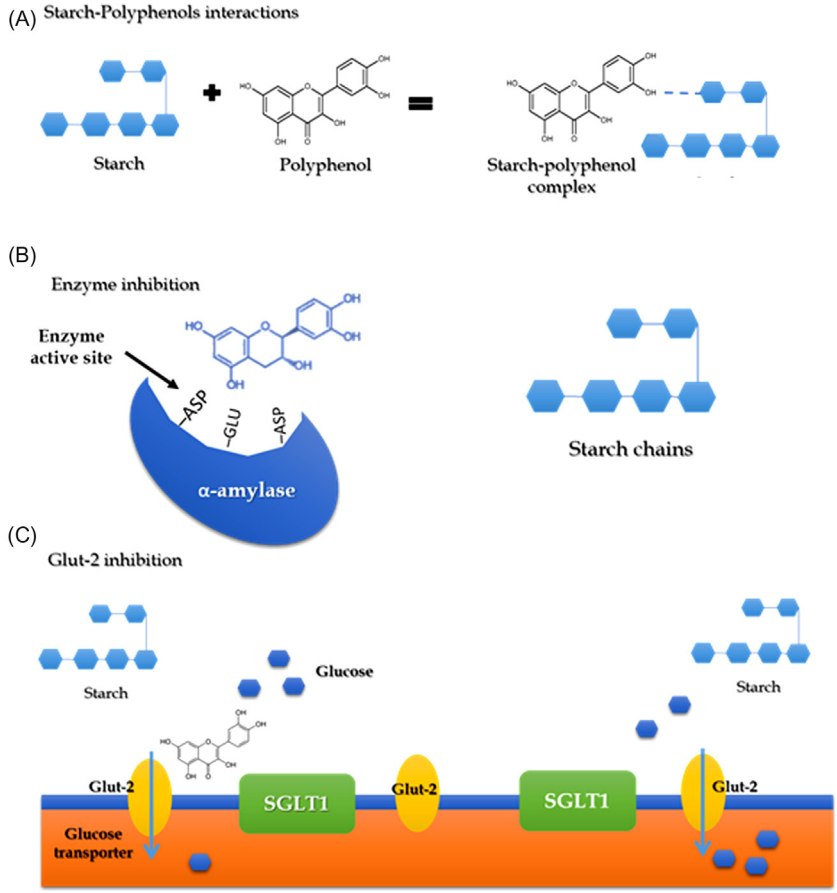

FIGURE 9.6

Mechanisms involved in the reduction and/or suppression of phenolic compounds on starch digestion. (A) Formation of starch—polyphenol complexes. The starch structure is modified and is not hydrolyzed by the digestive enzymes. (B) Polyphenol enzyme inhibition. The polyphenols are joined to the active site of the enzyme, and the substrate (starch) is not hydrolyzed. (C) Inhibition of glucose transporter (Glut-2) in the membrane. The polyphenol is joined by the glucose transporter (left side of C), blocking the site for the glucose. Glut-2 = glucose transporter; SGTL1 = sodium glucose linked transporter.

extracted using physical, chemical, or enzymatic procedures. The animal-derived nondigestible carbohydrates are included in the functional fiber category. For example, cellulose can be classified as dietary fiber or functional fiber, depending on whether it is naturally occurring in food (dietary fiber) or added to foods (functional fiber).

9.6.4.2 COMPOSITION OF DIETARY FIBER

Based on the terms previously described, dietary fiber consists primarily of plant nonstarch polysaccharides that are components of plant cell walls, including pectins, cellulose, and hemicellulose, as well as other

polysaccharides of plant or algal origin, such as gums and mucilages, and oligosaccharides such as inulin. Examples of oligosaccharides that fall under the category of dietary fiber are those that normally are constituents of a dietary fiber source, such as raffinose, stachyose, and verbacose in legumes, and FOS and inulin in foods such as Jerusalem artichoke, chicory, and onions. Resistant starch (RS) that is naturally occurring is now included in the definition of dietary fiber. There are different types of RS: RS1 is physically inaccessible, entrapped in cellular matrix; RS2 is the native (raw) starch granules with a particular organized structure; and RS3 is the retrograded starch after cooking and storage of the food. Two more types of RS are reported, but they are present in other foods and not in horticultural commodities. Common methods of analysis for fiber include AOAC 985.29 (total dietary fiber) and 991.43 (soluble and insoluble dietary fiber) and do not distinguish between naturally occurring fiber (dietary fiber) and added fiber (functional fiber). Both methods exclude low-molecular-weight oligosaccharides and resistant starch. Based on solubility, dietary fiber can be divided into soluble or insoluble dietary fiber. Soluble dietary fiber (SDF) consists mainly of noncellulosic polysaccharides (e.g., pectin, gums, mucilage), whereas insoluble dietary fiber (IDF) consists mainly of cell wall components (e.g., cellulose, lignin, and hemicellulose). Another method to determine total dietary fiber is an integrated method where RS and nondigestible oligosaccharides are included. This method is based on AOAC methods 2009.01 and 2011.25, and AACC methods 32.45.01 and 32.50.01. Later, a modification of this method was reported as a rapid integrated total dietary fiber assay procedure using 4 h of hydrolysis compared to the former that used 16 h of hydrolysis. Both integrated methods are recommended in products "as eaten" with no cooking of the samples before the analysis being necessary.

9.6.4.3 DIETARY FIBER IN FRUITS AND VEGETABLES

The main constituents of dietary fiber from fruits and vegetables are hemicelluloses, pectic polysaccharides, and cellulose. Hemicelluloses are largely composed of xylose and glucose in fruits and vegetables, xyloglucans and glucuronoxylans being the main constituents, whereas lignins appear in lignified tissues of fruits and vegetables. Although lignin represents only a small part of dietary fiber in most fruits and vegetables, it can be as high as 4% of dry matter in mature pears. Table 9.5 list some sources of soluble and insoluble fibers. Table 9.6 summarizes the total dietary fiber content (the sum of insoluble and soluble dietary fiber) for some climacteric and nonclimacteric fruits and for some types of vegetables. The content of total dietary fiber of fruits ranges from around 0.3−7 g/100 g of edible portion, depending on the species and variety. Blackcurrant and raspberry have a high content, while watermelon has low content. However, with the exception of blackcurrant, raspberry and avocado, the content in the rest of fruits ranges from 0.3 to 3.5 g/100 g of the edible portion. The total dietary fiber for vegetables ranges from 0.5 to 9.4 g/100 g of the edible portion. Artichoke and beetroot have high contents, whereas pumpkin and peeled potato have low contents. In general, vegetables provide two- to threefold more dietary fiber than fruits.

Table 9.5 Sources of Soluble and Insoluble Fibers

Type	Components	Sources
Soluble	Pectin	Whole grains, apple, legumes, cabbage, root vegetables
	Gum	Oatmeal, bean, legumes
	Mucilages	*Opuntia* cladodes
	Inulin	Asparagus, artichoke, garlic, agave
	Resistant starch (RS_2)	Raw potato, high-amylose maize
Insoluble	Cellulose	Whole grains, bran peas, root vegetables, beans, family of cruciferous, apple
	Hemicellulose	Bran, whole grains
	Lignin	Vegetables

Table 9.6 Total Dietary Fiber, Insoluble Dietary Fiber, and Soluble Dietary Fiber in Fruits and Vegetables (g/100 g of Edible Portion)

Fruit	Type of Fruit	Total Dietary Fiber	Insoluble Dietary Fiber	Soluble Dietary Fiber
Apple	Climacteric	1.0–2.3	1.5–1.8	0.2–0.7
Avocado	Climacteric	1.8–6.7	5.5	1.2
Banana	Climacteric	1.0–3.4	1.2	0.5–0.6
Mango	Climacteric	1.8–2.3	1.1	0.7
Nectarine	Climacteric	2.0	1.1	1.0
Peach	Climacteric	1.4–2.3	1.0–1.2	0.8–0.9
Pear	Climacteric	1.0–3.2	2.0–2.2	1
Blackcurrant	Nonclimacteric	7		
Grapefruit	Nonclimacteric	0.9	0.3	0.6
Grapes (white)	Nonclimacteric	0.7–2.2	0.3–0.7	0.5–0.6
Orange	Nonclimacteric	1.0–2.4	0.7–1	1.1–1.4
Pineapple	Nonclimacteric	0.6–1.5	1.1–1.4	0.04–0.2
Pomegranate	Nonclimacteric	0.6–3.5	0.5	0.1
Raspberry	Nonclimacteric	6.7		
Strawberry	Nonclimacteric	1.0–2.3	1.3–1.7	0.6–0.9
Tangerine	Nonclimacteric	1.8–1.9	1.4	0.4
Watermelon	Nonclimacteric	0.3–0.5	0.3	0.1–0.2
Vegetables	**Type of Vegetable**			
Artichoke	Flower bud	9.4		
Spinach	Leafy vegetable	2.6–6.3	2.1–2.4	0.5–0.8
Broccoli	Inflorescence	3.0–3.3	3.0	0.3
Brussels sprout	Axillary bud	2.3–4.3		
Cauliflower	Inflorescence	1.8–2.6	1.1–2.2	0.5–0.7
Celery	Petiole	1.5	1.0	0.5
Leek	Subterranean leaf base	2.8		
Lettuce	Leafy vegetable	1.0–1.5	0.9	0.1
Asparagus (green)	Stem	1.7		

(*Continued*)

Table 9.6 (Continued)

Vegetables	Type of Vegetable			
Cabbage (green)		2.2	1.8	0.5
Onion	Subterranean stem (bulb)	1.8–2.2	1.2–2.2	0.7
Potato (peeled)	Subterranean stem (tuber)	1.3–2	1.0	0.3
Beetroot	Root	7.8	5.4	2.4
Carrots	Root	2.5–2.9	2.3–2.4	0.2–0.5
Eggplant	Fruit-vegetable	2.4–6.6	5.3	1.3
Pepper (sweet, green)	Fruit-vegetable	1.5	1.0	0.5
Pumpkin	Fruit-vegetable	0.5–2.4		
Tomato	Fruit-vegetable	1.2–2.8	0.8–1.2	0.1–0.4

Nowadays, there is a trend to find new sources of dietary fiber that could be used as fiber-rich ingredients in the food industry. Typically, fibers derived from wheat, corn, and rice have been used in food processing in the past. However, recently, novel sources of fiber are those byproducts of the food industry resulting from the processing of fruits and vegetables (e.g., juices, fresh-cut fruits, and vegetables). These byproducts may include their peel, seeds, skins, stems, and cores, which were commonly discarded as waste. Some of the most recent documented sources of dietary fiber are byproducts of fruits such as apple (*Malus domestica*), grape (*Vitis vinifera*), lemon (*Citrus limon*), mango (*Mangifera indica*), orange (*Citrus sinensis*), peach (*Prunus persica*), and vegetables such as carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L. var botrytis), onion (*Allium cepa* L.), and potato (*Solanum tuberosum* L.).

9.6.4.4 PHYSIOLOGICAL EFFECTS ON HUMAN HEALTH

Fiber consumption may reduce the risk of cardiovascular diseases by delaying and blocking the absorption of cholesterol. In addition, when soluble fiber is digested, colon bacteria produce short-chain fatty acids, which decrease the production of low-density lipoproteins to healthy levels. It helps to lose weight, as diets rich in fiber satiate more. The fiber absorbs water and expands in the intestines and slows the movement of food in the upper part of the digestive tract. It reduces the risk of suffering from type II diabetes. By slowing digestion and absorption, the release of glucose into the blood is also slowed down, so that insulin production and blood glucose levels are regulated. In summary, fiber reduces the risk of colon cancer, prevents hemorrhoids, constipation, and diverticulosis, reduces the risk of heart disease, and helps in weight loss. Due to their fermentability, FOS and GOS have prebiotic properties, and when these are consumed, the undigested fraction serves as food for beneficial bacteria, such as *Bifidobacteria* and *Lactobacillus* species. The effect of dietary fiber on microbiota has been showed in recent years, where the prebiotic effect of dietary fiber to maintain "health" and beneficial gut microbiota

was reported. There is a relationship between the type of bacterium population and some health problems, such as obesity and insulin resistance. Studies shown that gut microbes can use the molecules present in foods that humans cannot digest, and the composition of the bacterium genus is different in lean persons and obese individuals. On the other hand, dietary fiber consumption has been used to prevent and treat obesity due to the increase in the satiety effect and the improvement of the intestinal transit of the food components. In general, the effects that link the dietary fiber, microbiota, and the host, are the products of the fermentation, such as short-chain fatty acids (SCFA). The total and relative molar concentrations of the main SCFA (acetate, propionate, and butyrate) depend on the components of dietary fiber (e.g., resistant starch, FOS, beta-glucans, etc.), the site of fermentation in the colon, and the bacterial species (e.g., *Bacteroides*, *Clostridium*, *Eubacterium*, *Enterobacter*, etc.).

Clinical studies have shown that administering FOS, GOS, and inulin can increase the number of these beneficial bacteria in the colon, while simultaneously reducing the population of harmful bacteria. Other benefits noted with FOS, GOS, or inulin supplementation include increased production of beneficial short-chain fatty acids like butyrate, increased absorption of calcium and magnesium, and improved elimination of toxic compounds. Three major mechanisms are believed to be responsible for the benefits of dietary fiber, including bulking, viscosity, and fermentation. Some fibers, generally insoluble dietary fiber, provide bulking effect, hence increasing stool mass, alleviating constipation, and improving regularity. Increased stool weight is due to the physical presence of dietary fiber as well as the water held inside the fiber matrix. Although the components of insoluble dietary fiber, such as cellulose and lignin, are mostly not fermentable in the colon, they can effectively increase fecal bulk by their particle formation and water-holding capacity. Insoluble dietary fiber has also been associated with a decrease in intestinal transit time that helps prevent and relieves constipation. On the other hand, soluble dietary fiber is readily fermented and may increase stool bulk by promoting the growth of intestinal and fecal microflora and their byproducts (e.g., gas and short-chain fatty acids). These properties might help normalize constipation and diarrhea problems.

9.6.4.5 DIETARY FIBER—PHENOLIC COMPOUND INTERACTIONS

The role of dietary fiber in the diet is widely recognized. Advances in dietary fiber, including quantification methods, have been reported in the last few decades. The indigestible fraction in foods was reported as an alternative method to determine the components present in foods that reach the colon. Those components include nonstarch polysaccharides, resistant starch, resistant protein, condensed tannins, lignin, etc. After the concept of antioxidant dietary fiber was proposed due to the role of dietary fiber and antioxidants in the prevention of chronic-degenerative diseases, it was postulated that an important amount of antioxidants, mainly polyphenols and some carotenoids,

transverse the small intestine in tandem with the components of dietary fiber. The antioxidant compounds that reach the colon are released after fermentation of the fiber by the microbiota, producing an antioxidant environment. The interactions between phenolic compounds and the components of dietary fiber (mainly nonstarch polysaccharides) are produced by hydrogen bonding (OH- groups of phenolics and oxygen atoms of glycosidic residues of polysaccharides), hydrophobic interactions, and covalent bonds. Those interactions can be produced during the gastrointestinal digestion of the food by the above-mentioned chemical interactions or physical entrapment due to the entanglement of polysaccharides. In the case of cell walls of horticultural commodities, nonstarch polysaccharides can bind to flavonoids and are released when the cell walls are degraded (e.g., during ripening). However, the interactions of phenolic compounds and dietary fiber components in both fresh and processed horticultural commodities deserve more research.

9.6.4.6 RECOMMENDED FIBER INTAKE

Fiber is a carbohydrate that is not digested or absorbed, so it contributes few, if any, calories. It is recommended that healthy adults consume daily: 25 g of fiber for women and 38 g of fiber for men. Fiber recommendations for children and the elderly are 14 g of fiber for every 1000 calories (kcal) consumed.

9.7 CONCLUSIONS

Horticultural commodities are rich in carbohydrates, are a very important source of energy in the diet, and have several other functions. They are classified, according to their chemistry, as monosaccharides, oligosaccharides, and polysaccharides (starch and nonstarch polysaccharides). However, this classification does not allow a simple translation to the nutritional effects since each class of carbohydrates has different physiological properties and health effects that overlap. Carbohydrates can also be classified according to their digestion and absorption in the human small intestine. Digestible carbohydrates are absorbed and digested in the small intestine. The nondigestible carbohydrates are resistant to hydrolysis in the small intestine and reach the large intestine where they are at least partially fermented by the bacteria present in the colon. There is no universal definition of the term "dietary fiber," but in general terms it refers to some or all of the nondigestible carbohydrate constituents and may also include other, quantitatively smaller components (e.g., lignin) that are associated with nondigestible carbohydrates in the walls of plant cells. Recently, resistant starch was included in a new definition, mainly in processed foods or foods analyzed "as eaten." The term functional fiber, proposed several years ago, implies physiologically beneficial effects in human health derived from fiber consumption, where antioxidant dietary fiber is included. Aspects, such as the effect of fruit/vegetable consumption as sources of carbohydrates on blood glucose and/or insulin levels need to be studied further.

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CHAPTER 10
Organic Acids

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10.1 INTRODUCTION

Organic acids are of fundamental importance in all plant species. They have been proposed to exhibit a multiplicity of functions, however, the evidence for some of these is much stronger than for others. Despite this, they have clearly been defined to have roles as important as photosynthate in crassulacean acid metabolism (CAM) and C_4 plants, energy production, carbon storage, stomatal conductance, the biosynthesis of amino acids, plant−microbe interactions, and mechanisms allowing plants to deal with excess cations, changing osmotic conditions, and soils low in nutrients, as well as those with high metal content. In addition to these varied roles, organic acids are important for taste, being responsible for sourness and contributing to the flavor. Acidity is also one of the main ripening indices that determines the harvest date of fruits. Therefore, understanding the factors that influence the concentration of these acids in plants and fruits cells is of primary importance. In this vein, the processes involved in the metabolism and accumulation of organic acids in mesocarp cells are under both genetic and environmental control. In this sense, high-throughput approaches such as transcriptomics, proteomics, metabolomics, and also quantitative trait loci (QTLs) studies have helped decipher, at the molecular level, some of the mechanisms that control acidity.

10.2 IMPORTANT ORGANIC ACIDS IN DIFFERENT FRUITS AND VEGETABLES

In fruits, the predominant organic acids are responsible for fruit acidity. In many fruits, malate and citrate are the major organic acids, however, other organic acids can also be relevant.

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The pattern of changes of organic acids during fruit development is very different when comparing different types of fruits and cannot be explained by the classification of species as climacteric or nonclimacteric, nor can they be attributed to changes in overall respiration rates. For instance, some climacteric fruits such as plum and tomato appear to use malate during the respiratory burst, while others such as banana continue to accumulate malate throughout ripening, even at the climacteric stage.

During the ripening of some fruits, such as grape and tomato, the amount of malate/citrate in term of both concentration (grams per gram fresh weight) and content per fruit (grams per fruit) decreases, and this shows that stored organic acids are dissimilated/metabolized. In other fruits such as strawberry and mango, the concentration of citrate/malate decreases during ripening. However, the amount of these acids per fruits increases up to commercial harvest. Thus, this means that there is not net dissimilation of these acids and the decrease is only a dilution effect due to an increase in the size of the fruit. In lemon both organic acid concentration and content per fruit increase throughout ripening. Therefore, during ripening there can be either a net dissimilation or synthesis of stored tricarboxylic acid (TCA) cycle, which occurs dependent on which fruit is considered.

Most of the citrate and malate content of fruit is found in the vacuole, which occupies 90% of most mature fruit cells. The main factor that is thought to determine this compartmentation is transport of malate and citrate across the membrane that separates the vacuole from the cytosol. In the cytosol, with a neutral or slightly alkaline pH, almost all malate is in the form of di-anion and almost all citrate is in the form of tri-anion. In the vacuole, with an acid pH, the main form is either protonated or the mono-anion. Only di-anion malate and tri-anion citrate can be transported into the vacuole. Once they have crossed the tonoplast and reached the acidic vacuole, they are immediately protonated. Efflux of the protonated forms of malate and citrate probably occurs through specific carriers (Etienne et al., 2013). Those involved in malate transport include several different malate transporters, and, in addition, there are malate channels. In *Arabidopsis*, one transporter is AttDT, which appears to be regulated by cytosolic pH, and two members of aluminum-activated malate transporter (ALMT) family, the AtALMT9 and AtALMT6 channels. Less is known about citrate transport across the tonoplast. Vacuolar tri-anion citrate uptake occurs by facilitated diffusion, possibly through the malate channel (Etienne et al., 2013). Thus, citrate appears to be easily transported into the vacuole as soon as its cytosolic concentration increases sufficiently. In citrus, several authors proposed that an ATP-dependent citrate pump may operate in addition to the malate channel. However, for both malate and citrate, the contributions of the different proteins involved in their transport across the tonoplast are poorly understood.

From their role in human health, we can highlight two groups of important organic acids present in fruits and vegetables, ascorbic acid (vitamin C) and

chlorogenic acids (CGAs). In plants, ascorbic acid functions as an antioxidant and enzymatic cofactor, and plays important roles in many physiological processes, including growth and development, photosynthesis, photo-protection, stress resistance, control of cell growth, and biosynthesis of hormones and cell wall constituents. Its content varies widely across plant species and tissues/organs. For instance, there is a high diversity in ascorbic acid content among crops, with values ranging from 5 mg/100 g fresh weight in apples and nectarines to 2.5 g/100 g fresh weight in camu camu. In general, in green tissues such as leaves, the maintenance of the redox state makes the regulation of ascorbic acid content tighter than in fruits, which, as sink organs, can accumulate ascorbic acid to higher levels (Cruz-Rus et al., 2011). Also, ascorbic acid content in plants varies in response to a wide variety of environmental and developmental cues. These include light and temperature, diurnal and seasonal fluctuations, fruit ripening, aging, plant tissue and cell compartment, and a wide range of stresses. CGAs, important for its antioxidant properties, are esters formed between certain *trans*-cinnamic acids (caffeic, coumaric, and ferulic acids) and quinic acid. CGAs have different subgroups that include caffeoylquinic, *p*-coumaroylquinic, and feruloyquinic acids and are major phenolic compounds in coffee, strawberries, pineapple, apple, sunflower, and blueberries.

10.3 METABOLIC PATHWAYS FOR ORGANIC ACIDS IN HORTICULTURAL COMMODITIES

Organic acids contain carboxylic groups negatively charged at neutral pH and to a lesser extent at acidic pH. Therefore, their functions can change depending on the pH of the solution, and their excretion can result in the release of protons and, therefore, in acidification of soil, apoplast, and vacuole. It is important to note that organic acids accumulate in plants mainly as a result of the incomplete oxidation of photosynthetic products and represent the stored pools of fixed carbon due to different transient times of conversion of carbon compounds in metabolic pathways.

10.3.1 Synthesis of Organic Acids

First, we focus on the synthesis of dicarboxylates, namely malate and oxaloacetate (OAA); considering that malate, in many plants, is the one of the most accumulated acids and can participate in the transfer of redox equivalents between cell compartments (Maurino and Engqvist, 2015). These require fixation of CO_2 on a carbon skeleton derived from hexose catabolism, which is achieved by phosphoenolpyruvate (PEP), catalyzed by PEP carboxylase (PEPC). In plants, cytosolic PEPC is necessary for the synthesis of TCA cycle acids from sugars. It catalyzes the conversion of PEP to OAA. If the fruit is accumulating malate, OAA is converted to malate by cytosolic NAD-dependent malate dehydrogenase (NAD-cytMDH), and malate is transported across the tonoplast into the vacuole in which it is stored (Fig. 10.1). Also,

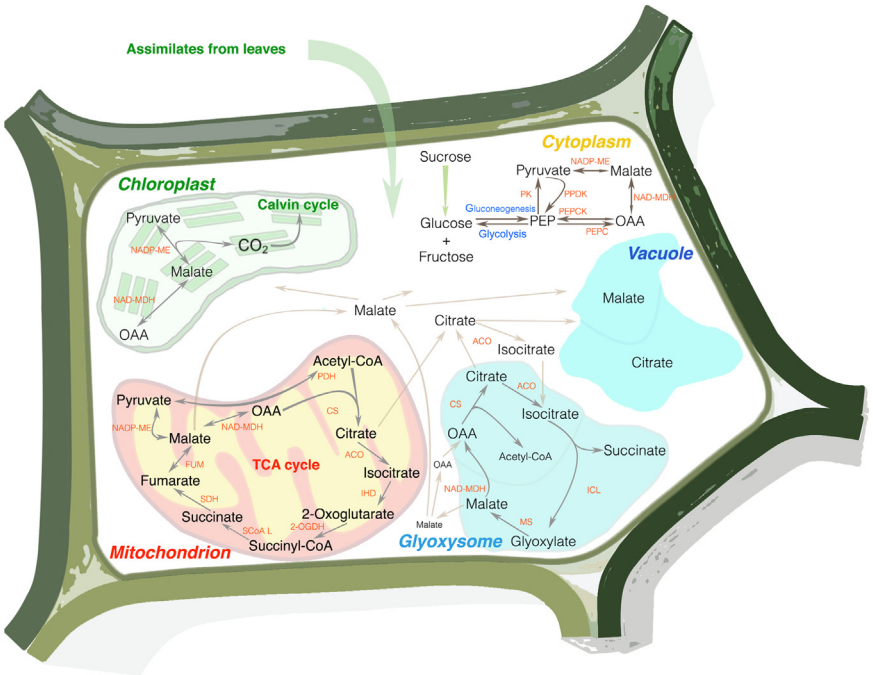

FIGURE 10.1

Scheme of organic acid metabolic pathways in plant cells. *CS*, citrate synthase; *ICL*, isocitrate lyase; *IDH*, isocitrate dehydrogenase; *MS*, malate synthase; *NAD-IDH*, NAD-isocitrate dehydrogenase; *NADP-IDH*, NADP-isocitrate dehydrogenase; *FUM*, fumarase; *2-OGDH*, 2-oxoglutarate dehydrogenase; *SCoAL*, succinyl-CoA ligase; *NAD-ME*, NAD-malic enzyme; *NADP-ME*, NADP-malic enzyme; *NAD-MDH*, NAD-malate dehydrogenase; *NADP-MDH*, NADP-malate dehydrogenase; *SDH*, succinate dehydrogenase; *PDH*, pyruvate decarboxylase; *PEPC*, phosphoenolpyruvate carboxylase; *PEPCK*, phosphoenolpyruvate carboxykinase; *PK*, pyruvate kinase; *PPDK*, pyruvate orthophosphate dikinase; *ACO*, aconitase.

OAA can supply to the TCA cycle if replenishment is necessary (Fig. 10.1). PEPC is controlled by both cytosolic pH and malate concentration in a way that stabilizes the cytosolic pH. PEPC isoforms can be found in multiple plant tissues, some of which are more specific to fruit. In grape berries, the expression of PEPC gene isoforms tends to correlate with malate levels at early developmental stages and the expression decreases during ripening, coinciding with a decrease in CO_2 dark-fixation, and glycolytic flux, which can indicate that PEPC is involved in photosynthesis and sugar breakdown in fruits. However, in other fruits such as peach, apple, or loquat, transcriptomic and enzymatic analyses suggest that PEPC is not responsible for the differences in malate content.

Malate dehydrogenase (MDH) catalyzes a reversible reaction between OAA and malate, and functions in the balance of these two metabolites, although the most likely direction is the synthesis of malate (Yao et al., 2011; Fig. 10.1). There are several isoforms of MDH in plants which require either NAD or NADP as a cofactor. It has been shown in several fruits that cytosolic

NAD-dependent MDH represents 70%–80% of the total NAD-dependent MDH, explaining why total NAD-dependent MDH activity correlates with malate levels. It seems, therefore, that MDH may be involved in net malate synthesis and net degradation, which could occur through differential regulation of individual isoforms (Martinez-Esteso et al., 2011). It is also known that while the MDH reaction is reversible, affinities of the mitochondrial (mMDH) and cytosolic (cytMDH) enzymes are higher for NADH and OAA than for NAD^+ and malate, therefore favoring the synthesis of malate in vitro. In this vein, Yao et al. (2011) showed that overexpression of the apple cytosolic *NAD-MDH* resulted in an increase in malate. Centeno et al. (2011) reported that silencing specifically the tomato fruit *mMDH*, also resulted in a decrease in malate, suggesting its direct involvement in malate synthesis. Also, due to its high concentration in mitochondria and other cell compartments, MDH has an important function of regulating the NADH/NAD^+ ratio and keeping NADH at a low level. This prevents the inhibition of several enzymes resulting from buildup of NADH and facilitates metabolic respiratory flux.

Malic enzyme (ME) catalyzes the reversible conversion between malate and pyruvate. Cytosolic and chloroplastic NADP-ME forms are involved in coordinating the levels of malate and pyruvate and play different roles, such as in defense, development, and stress responses, depending on the isoform present. Drincovich et al. (2001) suggested that the decarboxylation of malate to pyruvate by NADP-ME may release CO_2 to be taken up by Rubisco and the Calvin cycle (Fig. 10.1). However, NADP-ME activity is principally localized in the vasculature and core tissues; therefore, it has been suggested that NADP-ME is more likely to be involved in the metabolism of translocated assimilated or recapturing respired CO_2, rather than photosynthetic assimilation of CO_2 (Osorio et al., 2013; Saigo et al., 2013). Recently, it has been suggested that the conversion of malate to pyruvate via NADP-ME in the cytosol and NAD-ME in mitochondria is under the control of fumarate (Tronconi et al., 2015). When fumarate accumulates, the conversion of malate to pyruvate is facilitated.

10.3.2 Degradation of Organic Acids

Degradation of organic acids can occur through the conversion of tricarboxylates into dicarboxylates, but also through decarboxylation of the dicarboxylates, malate and OAA (Fig. 10.1).

Decarboxylation of OAA and malate allows the production of PEP and is linked to the activation of gluconeogenesis (Sweetman et al., 2009). Gluconeogenesis is a metabolic pathway that results in the generation of glucose from PEP. Evidence from radiolabeling works (Beriashvili and Beriashvili, 1996; Sweetman et al., 2009) suggests that gluconeogenesis does occur in fruits, particularly during ripening stages, when sugars are accumulating rapidly. Some studies through transcriptomics, proteomics, and metabolite analyses have provided evidence for a shift from the accumulation of organic acids

to sugar synthesis during the final stage of development in several fruit species (Carrari et al., 2006; Osorio et al., 2013).

PEP required for gluconeogenesis may originate from (1) malate through the activities of MDH and PEP carboxykinase (PEPCK) or (2) potentially ME and pyruvate orthophosphate dikinase (PPDK) (Fig. 10.1). Plant PEPC and PEPCK activities are differentially regulated by phosphorylation, whereby increased phosphorylation events can generally lead to higher PEPC and lower PEPCK activities, which will encourage OAA formation from PEP, thus allowing MDH to synthesize malate. Upregulation of PEPC kinase expression at an early stage of grape development suggests that this enzyme could activate PEPC while malate is accumulating within the fruit cells. Subsequently, a decrease in PEPC and PEPC kinase expression from veraison, suggests that flux from PEP to OAA may be slowed at this time, simultaneous to a decrease in grape respiration rates (Ollat and Gaudillere, 2000). PEP can also originate from the conversion of pyruvate through PPDK activity (Sweetman et al., 2009). Pyruvate required for PPDK activity may be supplied through the activity of NAD(P)-ME, which could use malate as it is released from the vacuole. The NADP-ME appears to be involved in the decrease in malate content during the ripening of several fruit species. Involvement of NADP-ME during the early stage of fruit growth differs between species. Also, several studies suggest that NADP-ME is regulated at the post-transcriptional level by cytosolic pH and the malate concentration. As yet, neither the expression of PPDK nor the activity has been identified in tomato and peach, nor in other fruits such as raspberry, blueberry, strawberry, and red currants. PPDK activity could be linked to a low oxygen stress response, presumably in providing pyruvate to fermentative metabolism, which can occur in ripening fruit if the cytosol becomes too acidic (Sweetman et al., 2009).

Another pathway that leads to degradation of organic acids is fermentation. TCA cycle acids can be converted to ethanol. There is evidence for the occurrence of aerobic fermentation to ethanol in the grape pericarp. During fermentation, malate is converted into pyruvate by either ME or the combined actions of MDH, PEPCK, and pyruvate kinase. This pyruvate is then converted to ethanol by the sequential actions of pyruvate decarboxylase and alcohol dehydrogenase.

10.3.3 Conversion Between Di- and Tricarboxylic Acids

Other organic acids associated with malate conversions are fumarate, succinate, and the derivatives of OAA. The conversion of malate or OAA into tricaboxylates can occur through two metabolic pathways: the TCA cycle and the glyoxylate cycle.

10.3.3.1 THE TRICARBOXYLIC ACID CYCLE IN THE MITOCHONDRIA

The TCA cycle results in the oxidation of pyruvate into CO_2 and a reduction in coenzymes through a series of conversions between organic acids, including

malate and citrate (Fig. 10.1). The cycle begins with the condensation of OAA and acetyl-CoA, the latter provided by the action of pyruvate dehydrogenase on mitochondrial pyruvate. In addition, both malate and citrate can enter the TCA cycle directly (Fig. 10.1). Then the TCA cycle either partially (open mode) or completely (closed mode) oxidizes these compounds to CO_2 (Sweetlove et al., 2010). The input of acetyl-CoA allows the TCA cycle to maintain a cycle flux mode under which it is not able to catalyze net synthesis of cycle intermediates. Therefore, the export of intermediates implies noncyclic flux modes, which are achieved due to the increase in redox level (Sweetlove et al., 2010).

In the noncyclic mode, one branch produces citrate, which can be transformed to isocitrate, 2-oxoglutarate, or their derivatives (including glutamate), while the other branch produces malate (or fumarate and even succinate), which can be exported from mitochondria and accumulate in vacuoles (Fig. 10.1). The enzymes that directly control citrate synthesis are the mitochondrial citrate synthase (CS), as shown in citrus and strawberry, and citrate degradation, the mitochondrial aconitase (ACO) which catalyzes the conversion of citrate into isocitrate (although it can also catalyze the reverse reaction) following by isocitrate dehydrogenase (IDH) that can catalyze the conversion of isocitrate into 2-oxoglutarate. There are two forms of isocitrate dehydrogenase, NADP-dependent and NAD-dependent forms. NAD-IDH is only found in the mitochondria, although its activity does not correlate with citrate accumulation. NADP-IDH can be found in the chloroplasts, cytosol, mitochondria, and peroxisomes. Once citrate has been produced by the TCA cycle, it can be degraded in the cytosol through (1) the GABA synthesis pathway, also called GABA shunt, or (2) cleavage into OAA and acetyl-CoA (Fig. 10.1).

1. It is likely that citrate is exported from mitochondria preferentially as compared to isocitrate and 2-oxoglutarate. The ACO equilibrium is strongly displaced toward citrate, and the mitochondrial ACO, as compared to the cytosolic form, has a lower affinity to citrate and a higher affinity to isocitrate that favors citrate accumulation (Eprintsev et al., 2015). The citrate carrier from pea is inactive with isocitrate, although the carrier from maize exhibited isocitrate transport capacity. Isocitrate and 2-oxoglutarate are mainly formed from the exported citrate in the cytosol through GABA shunt; cytosolic ACO which catalyzes the reversible conversion of citrate into isocitrate and NADP-ID which catalyzes the reversible conversion of isocitrate into 2-oxoglutarate. Both activities are high and usually exceed the activities of the mitochondrial isoforms of these enzymes (Eprintsev et al., 2015). Activation of GABA shunt appears to occur during postharvest of banana since an increase in 2-oxoglutarate level, NADP-IDH activity is mainly attributable to the cytosolic form and total *ACO* gene expression. Also, activation of GABA shunt can occur in sweet lemon since activation of genes involved in degradation of 2-oxoglutarate was observed (Aprile et al., 2011). In tomato ripe fruit, it has been demonstrated that the cytosolic ACO plays a key role in the control of citrate content (Morgan et al., 2013).

2. Citrate can also be degraded into OAA and acetyl-CoA through the activity of ATP-citrate lyase (ATP-CL). During mango fruit ripening, an increase in ATP-CL activity was correlated with a decrease in citrate level (Mattoo and Modi, 1970). Also, through proteomic analysis, an ATP-CL in ripe citrus fruit was identified (Katz et al., 2007). However, in contradiction to these results, Cercos et al. (2006) described a decrease at the transcriptome level of this enzyme. Thus, the role of this pathway in citrus fruit requires further investigation.

The coordinated operation of isoenzymes of the TCA cycle enzymes in mitochondria, cytosol, and other organelles balances the intercompartmental redox regulation, supplies intermediates for biosynthetic pathways, and provides the flexibility of the TCA cycle operation in open versus closed form. An important role in the coordination of regulation of the two branches of the TCA cycle may belong to the nonglyoxysomal isocitrate lyase, which operates in the cytosol at low pH and activated by manganese (Eprintsev et al., 2015). It may link the TCA cycle with glyoxylate/glycine metabolism, and, therefore, either supply glyoxylate for glycine and oxalate biosynthesis or utilize the photorespiratory glyoxylate in the reverse reaction.

10.3.3.2 THE GLYOXYLATE CYCLE

The glyoxylate cycle enables acetyl-CoA to be converted into malate. The glyoxylate cycle occurs in the peroxisomes and converts the acetyl-CoA produced by ß-oxidation of fatty acids into succinate (Fig. 10.1). Then, succinate is converted in malate through the TCA cycle. The enzymes involved in this metabolic pathway are localized in the glyoxysome, CS, isocitrate lyase, and malate synthase, and in the cytosol, ACO and NAD-MDH. This cycle has been suggested to function in malate synthesis in young grape and banana fruits and also to provide substrates for gluconeogenesis during postharvest ripening of banana (Liu et al., 2004; Terrier et al., 2005; Famiani et al., 2016). However, the involvement of this pathway in the accumulation of organic acids during fruit ripening could be specific to certain fruit since no isocitrate lyase proteins have been identified in several soft fruits (Famiani et al., 2005; 2014).

10.3.4 Metabolism of Other Organic Acids

Malonate is an important dicarboxylic acid in legume (Fabaceae) and Umbelliferae families described as essential for symbiotic nitrogen metabolism as well as a precursor of neutral lipids. Malonate can be synthesized either (1) via the malate branch of the TCA cycle, presumably in the reaction of OAA decarbylation and (2) via acetyl-CoA carboxylase from acetyl-CoA and further hydrolysis of malonyl-CoA. Malonate can be oxidized with the formation of CO_2, oxalate, glyoxylate, and formate (Fig. 10.2).

Oxalate is one of the most actively accumulating organic acids in many plants such as cereals, spinach, or beet. Oxalate can be found as a free form or stored

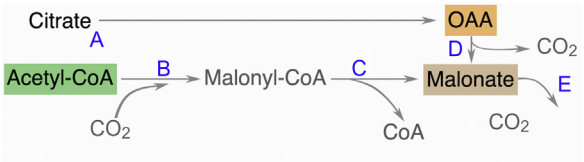

FIGURE 10.2

Malonate metabolism in plants. (A) ATP-citrate lyase; (B) acetyl-CoA carboxylase; (C) malonyl-CoA hydrolase reaction; (D) oxaloacetate decarboxylase reaction; (E) oxidation.

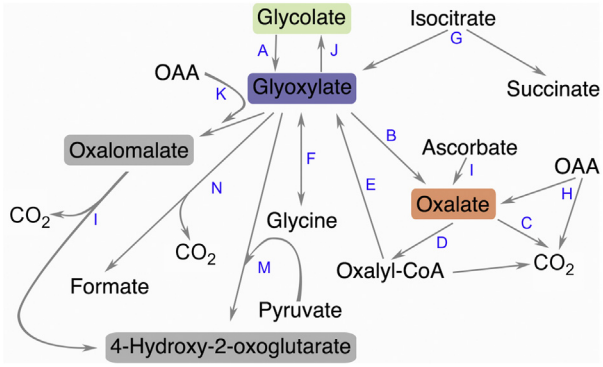

FIGURE 10.3

Oxalate and glyoxylate metabolisms in plants. (A) Glycolate oxidase; (B) glycolate oxidase (side reaction); (C) oxalate oxidase; (D) oxalyl-CoA synthetase; (E) oxalyl-CoA reductase; (F) aminotransferase; (G) isocitrate lyase; (H) oxaloacetate decarboxylase; (I) ascorbate metabolism; (J) glyoxylate reductase; (K) nonenzymatic condensation with oxaloacetate; (L) decarboxylation; (M) enzymatic condensation with pyruvate; (N) decarboxylation.

as a complex with Ca^{2+} in vacuole. Therefore, oxalate has a function of the storage of large amounts of Ca^{2+} and can regulate its balance between vacuole and cytosol. In plants, there are different pathways described leading to the biosynthesis of oxalate (Fig. 10.3): (1) via oxidation of glyoxylate by glycolate oxidase; (2) splitting of OAA to oxalate and acetate; and (3) degradation of ascorbate. Once oxalate is synthesized, it can be degraded to CO_2 by oxalate oxidase or efficiently incorporated in the metabolism. Its incorporation may take place through the transformation to oxalyl-CoA by oxalyl-CoA synthetase. Oxalyl-CoA can be incorporated in biosynthetic reactions via glyoxylate, therefore leading to other metabolites such as glycine, serine, as well as glycolate, alanine, glutamate, citrate, and malate as studied in pea leaves by Ivanov et al. (1989).

Formate is the product of CO_2 reduction by a single pair of electrons, making it one of the simplest organic acids that can provide cells with both carbon and reducing power. Formate potentially could be formed via the reduction of CO_2, the reaction which was considered for CO_2 fixation before the discovery of the Calvin–Benson cycle; however, this reaction can have only very minor, if any, contribution to CO_2 fixation. Formate is linked to the glycolate pathway and glyoxylate conversion. It can be produced in the reaction of

glyoxylate with hydrogen peroxide (Fig. 10.2). Condensation of two formate molecules can potentially yield glyoxylate, which is further converted to glycine and serine.

10.4 ORGANIC ACIDS IN C_4 AND CAM PLANTS

The finding that C_4 and CAM plants have four-carbon dicarboxylic acids as the first product of carbon fixation shows these organic acids play essential roles as photosynthetic intermediates, with OAA, malate, and aspartate being the formed substrates. In this cycle, OAA is the intermediate product of the initial CO_2 fixation step, which is then reduced to malate. Thus, malate becomes the central metabolite in C_4 and CAM photosynthesis. Via NAD- or NADP-ME, malate can be descarboxylated with the release of CO_2 used to make carbohydrate. In some plants, OAA is transaminated to aspartate, and the CO_2 is delivered via PEP carboxykinase.

Although OAA and malate are the primary products, this type of plant can accumulate organic acids as citrate and isocitrate. These plants maintain their metabolic balance by transforming a part of stored malate to citrate via TCA cycle (Herrera, 2013; Miszalski et al., 2013).

10.5 INFLUENCE OF PREHARVEST FACTORS ON ORGANIC ACID ACCUMULATION IN HORTICULTURAL COMMODITIES

The final organic acid content in a plant/tissue is determined by the net balance of acid synthesis, degradation, utilization, and compartmentation. There are different factors such as temperature, light, fertilization, water supply, and other plant management practices that affect the source:sink ratio, and therefore the organic acid content.

10.5.1 Temperature and Light

Most of the temperature effects on plants are mediated by their effects on plant biochemistry. The temperature at which plants are grown affects both their titratable acidity (TA) and content of stored organic acids. Most of the physiological processes going on normally in temperatures ranging from 0°C to 40°C. However, cardinal temperatures for the development of fruit and vegetable crops are much narrower and, depending on the species and ecological origin, it can be pushed toward 0°C for temperature species from cold regions such as lettuce and carrots. On the other hand, they can reach 40°C in species from tropical regions, such as many cucurbits and cactus species.

Higher than normal temperatures affect the photosynthetic and respiration processes through the modulation of enzyme activity as well as the electron transport chain. In general, increasing the temperature during fruit growth decreases TA as shown in tomato fruit in which the TA decreases 10% in those fruits exposed to direct sunlight, and grape. Also, it is important to note that

changes in organic acids in response to temperature also depend on the age of the plant or the fruit. Kiwifruit exposed to a higher temperature in early development had double the amount of malate at the peak, but when placed back under normal temperature conditions this decreased to meet the control level (Richardson et al., 2004). From the same experiment, kiwifruit exposed to high temperatures in later development contained malate levels that were reduced to almost half that of control fruit, even after the temperature was returned to normal. In grape, the reduction in malate by the effect of high temperature is only evident after veraison (this effect was enhanced by warmer nights), which suggested that the regulation of malate metabolism differs during day and night. Increased temperatures of fruits during ripening almost certainly decrease their content of TCA cycle acids by increasing the rate of many metabolic processes. In general, in all plant tissues, an increase in temperature increases the rate of their metabolism, and hence the consumption of compounds such as sugars and stored TCA cycle acids that serve as metabolic substrates. Modification in organic acid metabolism in response to temperature probably results from the impact of temperature on the reaction rates of glycolysis, TCA cycle respiration, fermentation, and gluconeogenesis by modifying enzyme activities and kinetic properties of the transport systems involved. However, all organic acids do not appear to be equally sensitive to temperature (Wang and Camp, 2000).

One of these effects of increasing temperature would be upregulation of respiration, which is consistent with respiration rates in ripe pears, which increased with increasing temperatures. Biale (1960) stated that the effect of temperature on fruit respiration is more to do with specific chemical responses, rather than speeding-up of reactions in the cells. Related to malate metabolism, for instance, temperature affects the metabolism fate of dissimilated malate in ripening grape. At higher temperatures, the ratio of the amount of malate utilized by respiration to that used by gluconeogenesis is greater than at lower temperatures. Also, it was shown that mMDH isoforms are shown to be more temperature-sensitive than cytMDH in grape; a rise in temperature from 25°C to 30°C may result in a switch from predominantly cytMDH to mMDH activity. This would support an increase in flux through the TCA cycle in response to higher temperatures. Additionally, in response to high temperature, malate could be more rapidly catabolized through the activity of NADP-ME in the cytosol. In vitro, NADP-ME from Carignane berries showed a specific optimum at 50°C and was more thermally recoverable than the malate-synthetic enzyme PEPC after heat-treatment of berries. However, Ruffner et al. (1976) showed that the NADP-ME activity was independent of temperature when the NADP−NADPH ratio remains steady. Furthermore, if the respiration is increased then the reductive power of the cell is likely to prevent and increase in NADP-ME conversion of malate to pyruvate.

Moreover, the temperature can affect the vacuolar storage of organic acids via several mechanisms: (1) the membrane fluidity can be modified by changes in lipid properties. Thus, high temperatures may change the tonoplastic

permeability of cells, which could increase leakage of solutes such as protonated forms of organic acids; and (2) temperature is a key variable in the thermodynamic equations that limit the operation of the proton pumps and the diffusion of organic anions through the tonoplast.

Light is an environmental factor that significantly affects plant growth and development. Therefore, manipulation of light quality is currently applied in horticulture via photo-selective netting or films to improve the yield, quality, and phytochemical composition of cultivated plants. It is very well known that light intensity impacts sugar accumulation in vegetables. Light quality and different wavelengths can affect fruit total soluble solids content (SSC; a parameter directly linked to sugar content). In tomato, under a black net, blue light transmittance is significantly higher than under other nets, whereas under red nets, red-light transmittance was higher and thus one would expect a higher production of photosynthates resulting in an increase of SSC. All tomato cultivars grown under black nets had the highest SSC.

However, what about TA? There is a contradictory literature regarding the effect of light on organic acid metabolism. One reason is that solar irradiance also increases plant temperature, and hence there is an interaction between light and temperature. A second reason is that, in general, shade can delay fruit ripening, and this can cause higher organic acid levels. However, there is contradicting literature regarding shading effect on TA levels. According to El-Gizawy et al. (1992), increasing shading levels from 35% to 63% increased the TA in tomato fruits, while Riga et al. (2008) reported that shading tomato plants by 50% did not affect the concentration of TA. The increase of TA content of pepper fruits under black nets may be associated with fruit senescence. Fruits grown under pearl and yellow nets showed moderate SSC/TA ratios after postharvest storage. Decay incidence during postharvest storage of pepper fruits obtained from black nets may have increased the SSC/TA ratio. The SSC/TA ratio was higher in lettuce produced under yellow and pearl nets. In contrast, the SSC/TA level was lower in lettuce produced under red and black nets. No significant difference was observed among the varieties (Ntsoane, 2015).

10.5.2 Mineral Nutrition

In the leaves of many plants, nitrogen metabolism is the major factor affecting the content of TCA cycle acids and a large amount of these can accumulate. This is because the assimilation of nitrate or ammonium into amino acids is not a proton-neutral process. For instance, the assimilation of nitrate into amino acids in leaves consumes protons. Malic acid is synthesized in leaf and malate is transported to root. In contrast to leaves, fruits import amides such as glutamine and asparagine as their main source of nitrogen as the main source of nitrogen, and the assimilation of these into compounds is proton-neutral.

Most studies of the effect of nitrogen nutrition on organic acid levels describe contradictory effects. Some authors found a negative correlation between

nitrogen nutrition and TA and organic acids content (Spironello et al., 2004), others found a positive correlation (Radi et al., 2003), and still others that it had no significant effect (Cummings and Reeves, 1971). These contradictory effects can be explained because nitrogen fertilization affects plant vigor and the leaf/fruit ratio, which can then affect both fruit ripening and humidity and sunlight penetration inside the canopy. Also, the form in which nitrogen (nitrate or ammonium) is supplied affects organic acid levels. Nitrate fertilization is likely to have a positive impact on the concentration of organic anions in the phloem sap since nitrate assimilation in the leaves requires the coordinated synthesis of organic acids, which are then transported in the phloem sap together with potassium. Conversely, ammonium fertilization does not cause the synthesis of organic anions, and may affect cation uptake by root such as potassium.

Potassium supply has an impact on fruit acidity, but contradictory effects have also been reported. In some cases, an increased potassium supply led to a reduction of fruit TA, others that potassium fertilization increased fruit TA, and still others had no affect on TA and organic acid levels (Etienne et al., 2014). At the cellular level, different mechanisms allow potassium to affect the metabolism and stored organic acids. As mentioned before, organic anions are synthesized in the leaves to buffer the excess of organic cations absorbed from the soil. As a result, the potassium supplied to the fruit by the sap is accompanied by an equivalent number of organic anions, mostly malate. This mechanism can explain the pH change in the fruit. However, in this case, TA would not be affected, since no protonated forms would be added to the fruit. Thus, a modification of fruit TA in response to the supply of potassium implies that potassium affects the synthesis or the vacuolar storage of the organic acids within the fruit itself. The regulation of tonoplastic transport may be an essential contributor to the effect of potassium.

The effect of other mineral elements has not been extensively studied. For instance, magnesium and phosphorous nutrition appear to have little or no effect on fruit TA.

10.5.3 Water Availability

The effect of water supply on fruit acidity is complex, and this is because of the interactions with other factors, such as climate conditions and time during fruit development when water stress occurs. In most cases, water supply was shown to be negatively correlated with fruit TA and organic acid content such as in orange, clementine, tomato, and apple (Kallsen et al., 2011). Other authors reported a positive correlation between water supply and fruit TA, such as in grape (de la Hera-Orts et al., 2005). Taken together, these data suggest that different mechanisms can occur. Water availability can affect the volume of fruits, so a simple dilution effect needs to be considered. Also, water stress influences osmotic status/adjustment. Under water stress, all plant tissues accumulate sugars and organic acids to lower their osmotic potential. Therefore, this mechanism can affect the import of organic acids into the

fruits. In addition, this situation is further complicated because water supply can also increase vegetative growth, which can have an effect by increasing yield and delaying fruit ripening.

10.5.4 Source:Sink Ratio

Management practices such as fruit thinning and pruning (including defoliation) change the source:sink ratio of the plant, which usually leads to changes in sugars and other nutrients imported into a fruit. These practices also change fruit composition indirectly by delaying fruit ripening. In peach and mango, a different behavior of citric and malic acids was observed. A high leaf/fruit ratio leads to an increase in the citric acid content at the beginning of growth and decreases near maturity. The opposite effect was shown in malic acid content. It can be hypothesized that during early developmental stages there is a high respiration rate in which a large amount of photoassimilates are available for the production of malate via glycolysis and its conversion to citrate via the TCA cycle. In contrast, during ripening, sugars may no longer be available for respiration since they are stored in the vacuole, therefore there is a shift from sugars to organic acids as respiration substrate. On the other hand, fruit load had no effects on the accumulation of organic acids in banana fruits (Etienne et al., 2014) but a negative correlation with TA was found at harvest in kiwifruit (Famiani et al., 2012).

10.6 INFLUENCE OF POSTHARVEST FACTORS ON ORGANIC ACID ACCUMULATION IN HORTICULTURAL COMMODITIES

Harvest of fruits and vegetables occurs at different times of the year depending on cultivar, water availability, climate conditions, management practices, pest control, and maturity index, among other important factors.

After harvesting, the fruits and vegetables have undesirable changes in quality parameters. The major pathway responsible for these changes is the respiration process. It would be best to interfere with this process to extend the shelf-life of the fruit and vegetables; however, there are not many options since they are dependent on the product-specific characteristics. Alternatively, growers can adopt different techniques to extend the quality of fruits and vegetables. The postharvest technology comprises different methods of harvesting, packaging, rapid cooling, storage under cool temperatures, as well as modified and controlled atmospheres, and transportation under controlled conditions, among other important technologies. Additionally, irradiation with ionizing radiation from radioisotopes (gamma rays), electron beam, or X-rays represents an alternative method to disrupt the genetic material of the pest or microorganism infesting fruits and vegetables, causing either the sterilization or death of the target organisms. Depending on the storage conditions, the fruit and

vegetable nutritional composition changes differently, which also affects the organic acid content, which is the focus of this chapter.

10.6.1 Irradiation

The effect of irradiation depends on the variety or cultivar of fruit, irradiation dose, ripening stage of the fruit, harvest season, storage time, and posttreatment storage conditions of fruit such as temperature. For pummelos, gamma irradiation decreases the major organic acids which was slightly reflected by lower TA (Jain et al., 2017). Similar treatment on blueberry and raspberry also had the same effect on individual organic acids, although it was not reflected in the overall effect of TA. The decrease in organic acids can be explained by an increase in the respiration rate in irradiated fruits. Moreover, gamma irradiation did not have the same effect in other fruits. In mandarins and bananas, although the total TA was not affected, a decrease in the major organic acids was observed (Ornelas-Paz et al., 2017). This can demonstrate that gamma irradiation shifted the glycolytic pathway to the pentose–phosphate pathway, causing a reduction in the production of energy and an increased usage of proteins to enhance the gluconeogenic flux. Thus, these results suggest that irradiation modified the activity of several enzymes (i.e., PEP carboxykinase, isocitrate lyase, fructose diphosphatase, and those of glyoxylate cycle) that caused an increase in organic acid content after irradiation. A few studies are available in this regard, with limitations in the range of irradiation doses, storage conditions, and fruit type.

Ascorbic acid is massively affected after irradiation. The irradiation-mediated loss of ascorbic acid in vegetable foods has been attributed to the direct oxidation of ascorbic acid through the action of free radicals generated by water radiolysis, as well as by the involvement of ascorbic acid in the protection of other compounds against oxidative damage.

10.6.2 Modified Atmospheres

Storage in a modified atmosphere positively increases the shelf-life of many plant products by slowing down respiration. The two main modified atmospheres used in postharvest are enriched atmospheres with CO_2 and O_3. As described for irradiation, the effect of these modified atmospheres depends upon the variety or cultivar of fruit, ripening stage of the fruit, harvest season, storage time, and posttreatment storage conditions of fruit such as temperature. Strawberries cv. Camarosa stored under an ozone-enriched atmosphere and cool temperature showed a threefold increase in ascorbic acid content when compared to berries stored at the same temperature under a normal atmosphere (Perez et al., 1999). However, Kute et al. (1995) reported that strawberries stored in atmospheres with an ozone-enriched atmosphere showed no effect on ascorbic acid levels after 7 days of storage under refrigerated conditions. Similarly, tomato fruits cv. Carousel after ozone exposure at 13°C did not modify the organic acid levels when compared to fruits kept

under ozone-free air. Furthermore, in mulberry fruit, a decrease in the TA was observed during storage. However, O_3 treatment delayed the loss of TA, which might be associated with the metabolic activity and respiratory rates of fruits. Moreover, in apple after storage under normal air for several months the malic acid concentration is lower than at harvest. However, this decrease can be reduced and also delayed if a CO_2-enriched atmosphere surrounds the fruits.

10.7 CONCLUSIONS

The aim of this chapter was to give an overview of the metabolism and functions of organic acids in plants. It also showed that agro-environmental factors affect the balance of organic acids in all plants by acting on a cellular level. Interestingly, in different plants/tissues, it appears that different mechanisms (such as metabolic pathways or transport processes at the tonoplast) are responsible for these differences. Further studies using integrative approaches like physiological, biochemical, molecular, and modeling studies, are necessary to further understand the mechanism responsible for these differences. An understanding of these mechanisms would be of assistance in both genetic improvement programs and in the optimization of cultural practices.

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CHAPTER 11
Pigments

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11.1 INTRODUCTION

Pigments are among the most important natural chemical compounds, which yield various colors and perform diverse functions. The colors in flowers and fruits attract animals to pollinate flowers and disperse seeds. The functions include light-harvesting in photosynthesis, protection from photooxidative damage, and regulation in development and defense. Whereas photosynthesis is of primary importance in the assimilatory organs and tissues, which augments proper development and maintenance of the overall carbon economics of plants, the attractive coloration seems to be crucial in fruit.

The major classes of pigments are chlorophylls (Chl), carotenoids, flavonoids, and the less commonly seen betalains. Each class has a unique basic chemical structure and consists of subgroups of derivatives that have substitutional modifications of the basic structure for different colors and biological functions. Most of the pigments are essential nutrients and some are provitamins for humans and animals. Some pigments are known to have additional nutraceutical and health benefits, including the prevention and treatment of some diseases.

As a main pigment-rich diet source, botanical fruit of all types (including those designated as vegetables) appear to be primary pigment research subjects. Pigments in fruit undergo directed and specific transformations during ripening, and hence their content and composition represent a visual marker of fruit maturation, and ripening and quality conditions. Understanding pigment compositional and metabolic changes in fruit before and after harvest is

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important for optimization of the storage conditions and extending postharvest life. Likewise, much knowledge is also gained from leafy, stem, and root vegetables and other plants rich in certain pigments.

This chapter focuses on the diversity, functions, and practical significance of pigments, with an emphasis on characteristic patterns of pigment composition during off-tree ripening and postharvest storage and handling of horticultural commodities. The changes to the pigment composition during fruit processing are also briefly considered.

11.2 CHLOROPHYLLS

Chl are primary photosynthetic pigments in plants and other photoautotrophs. The term chlorophyll is derived from the Greek *chloros* meaning green and *phyllon* meaning leaf. The characteristic green color of Chl results from its ability to absorb the light in the blue and red regions, whereas Chl attenuates the light in the green region to a much less extent. The main function of Chl is to capture light to drive photosynthesis and to convert the absorbed light energy into chemical energy, which is ultimately stored in the form of sugars. There are several types of Chl in photosynthetic organisms. Higher plants, including horticultural commodities, contain Chl *a* and Chl *b*, with a typical ratio of about 3:1 − 4:1.

The backbone of Chl is composed of a cyclic tetrapyrrole ring, which is a large planar structure of a symmetric arrangement in which the four pyrrole rings are joined together by methine (−C =) bridges, and four nitrogen atoms are coordinated with a central metal atom, magnesium (Mg). A long hydrophobic phytol (C_{20}, an esterified isoprenoid alcohol) group that anchors the molecule to the pigment-binding proteins within the thylakoid membrane is attached to the ring (Fig. 11.1A and B). There are different catabolism pathways of Chl in horticultural commodities (Fig. 11.1C). The resulting structure contains a chromophore absorbing light in the red (peak at 670−680 nm) and blue (peak at 435−455 nm) spectrum (Fig. 11.2).

Fresh fruits and green vegetables are rich in Chl, where its content reaches 1000−2000 ppm. It is registered as a food additive (green colorant) used in a variety of foods and beverages. Generally, Chl comprises 0.6%−1.6% on a dry weight basis; however, large variations exist in plants.

Both natural- and commercial-grade artificial derivatives as copper Chl have been extensively used as beneficial biological activities, which include wound healing, control of calcium oxalate crystal, antiinflammatory properties, cardioprotective, and even anticancer effects. Chl have been used for a long time as a traditional medicine for therapeutic functions. Humans have no potential to synthesize Chl, but are able to uptake it dietary as absorbed or with minor alteration of Chl structure. Early research suggests that Chl are not only responsible for green colors but can also prevent lung and skin cancers by

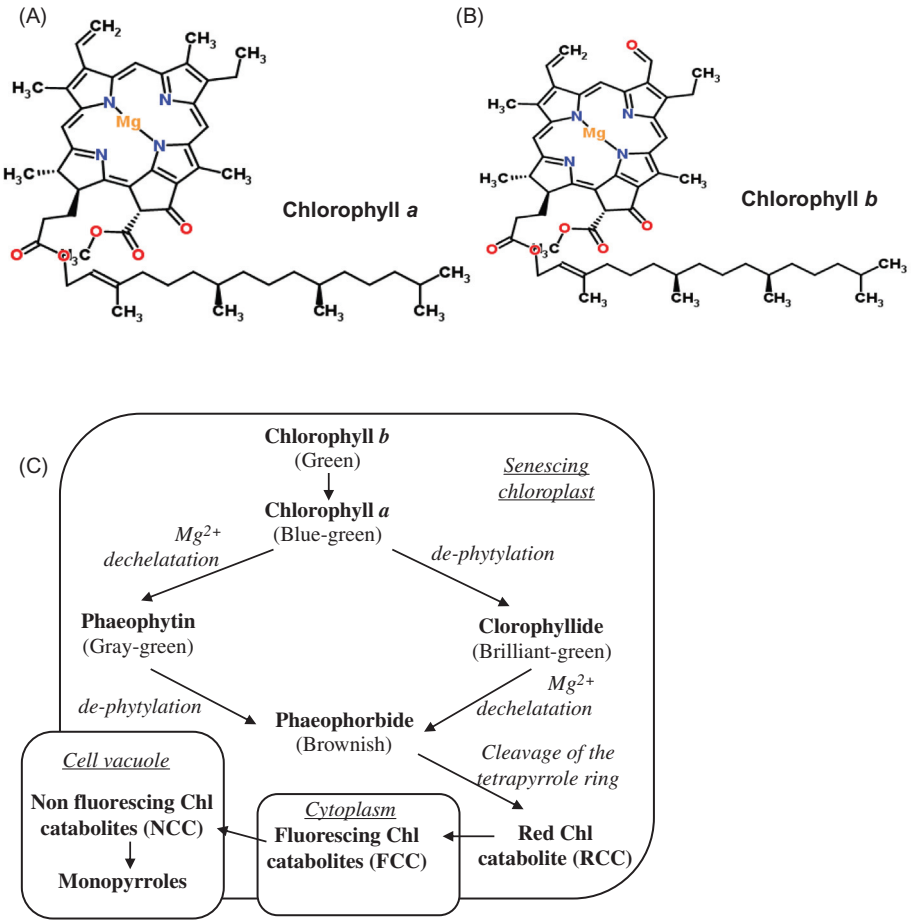


FIGURE 11.1
Structures (A, B) and (C) catabolism pathways of the Chl contained in horticultural commodities (according to Hörtensteiner, 2006).

injecting intravenously along with the drug Talaporfin, followed by laser photodynamic therapy in the early stages of cancers.

The transformation of Chl is of special interest since Chl is one of the most abundant compounds on Earth. The amounts of Chl annually synthesized and degraded are found to be roughly equal. Senescence of plant leaf and ripening and senescence of fruits and vegetables involves Chl degradation. At the same time, the mechanism of Chl degradation remained enigmatic for a long time. Briefly, it is an enzymatic process comprising removal of phytol by chlorophyllase, removal of Mg by magnesium dechelatase, and oxidative cleavage of Chl macrocycle by pheophorbide oxygenase. The last step rapidly yields colorless catabolites of Chl, which is important for protection of assimilatory tissues from photodamage during senescence when their photosynthetic capacity declines but the Chl content is still significant (Fig. 11.1C).

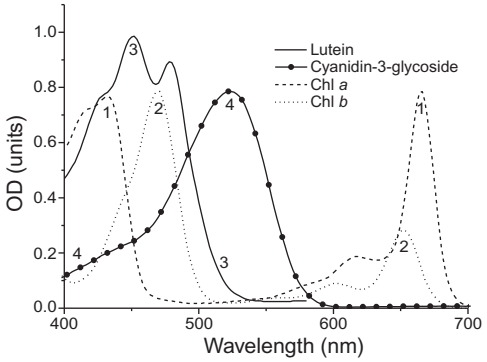


FIGURE 11.2

Absorption spectra (in acetone) of Chl *a* (1), Chl *b* (2), lutein (3), and cyanidin-3-galactoside (4). Lutein is a carotenoid in the xanthophyll subgroup, and cyanidin-3-galactoside is a commonly seen anthocyanin. In the anthocyanin absorption spectrum, a long-wave maximum in the green region ($\lambda_{max} = 535$ nm) is shown, the short-wave part below 400 nm is not presented.

Another potential pathway of Chl degradation involves lipoxygenases, which are widely distributed in plants, and involved in off-flavor development and color losses. The oxidative degradation of Chl involves hydroperoxide and radical formation by oxidation of lipids, which can destroy Chl and carotenoids during frozen storage. Lipid oxidation is related to Chl degradation in spinach. Peroxidases are assumed to play an important role in Chl degradation, a process accompanying ripening and senescence in most fruits and vegetables. Peroxidase is responsible for off-flavor development during storage of canned products, especially in nonacidic vegetables, which often exhibit high levels of activity. They impair the sensory properties, the product marketability, and the nutritive value as well. Apple fruit, as an example, possesses fully functional photosynthetic apparatus similar to senescing leaves, the disassembly of photosynthetic apparatus of ripening fruit involves a gradual decrease in Chl and accumulation of significant amounts of carotenoids. This process results in changes of coloration toward the appearance characteristics of ripe fruit.

The rate of maturation and ripening in horticultural commodities, and hence the rate of pigment transformation, are affected by many climatic and agronomic factors such as solar radiation, water supply, temperature, sink-source relations, fertilizing, pruning, etc., and strongly accelerated by fruit detachment from the tree. The knowledge of color changes during fruit maturation and ripeness is essential for the estimation of the "harvesting window" (maturity and harvesting indices), selection of homogeneous sets of fruits suitable for short- or long-term storage, prediction of the onset of senescence breakdown, etc. Therefore, efforts have been undertaken to use the specificity of pigment changes for estimation of fruit ripeness using skin Chl and a characteristic pattern of changes of the individual xanthophylls, whereas the use of total carotenoids for this purpose was still inefficient (the postharvest transformation of fruit carotenoids is discussed in the next section).

The stability of Chl during processing of fruits and vegetables is vital for color retention, an important sensory characteristic crucial for the acceptance of food by customers. Chl *a* imparts a blue-green color whereas Chl *b* imparts a yellow-green color. Chl are highly susceptible to degradation during processing, resulting in food color changes. Degradation of Chl in processed fruits and vegetables occurs via chemical and biochemical reactions. Chemical reactions involve the formation of pheophytin from the Chl via loss of magnesium ions from the porphyrin ring by an acidic substitution, heat treatment, or after the action of Mg chelatase. Chl degradation has been shown to follow different pathways depending on the commodity. However, the mechanisms and kinetics of those reactions have only been partially characterized. In food products, Chl degradation studies revealed that Chl degrades to pheophorbide via pheophytins or chlorophyllide.

Diverse physical factors are responsible for degradation of Chl during processing, for example, acids, heat, and oxygen. Under thermal processing conditions, Chl structure undergoes chemical and structural alterations deleterious to its color. Demetalation and epimerization were observed during heat treatment, and prolonged heating leads to additional demethoxycarbonylation of the molecule. Decarbomethoxylation may occur during a strong heat treatment, leading to the conversion of pheophytin to pyropheophytin, for example, during canning of vegetables. Dephytylation of Chl is achieved enzymatically during fermentation and storage and is often observed together with demetalation.

Chl also differ in their thermal stability. Chl *b* was reported to be thermally more stable, whereas Chl *a* was found to be thermally unstable. At the temperature range of 70−100°C, green peas showed degradation of Chl *a* and *b* following a first-order kinetics model. The degradation of Chl *a* was reported to be 12−18 times faster than that of Chl *b* and was found to be primarily dependent upon the temperature regime during the processing. The higher thermal stability of Chl *b* has been attributed to the electron-withdrawing effect of its C-3 formyl group.

The conversion of Chl to corresponding pheophytins, thus the color of Chl, is also affected by pH. Chl appear to have higher stability at alkaline pH conditions. Olive brown pheophytins are synthesized under acidic conditions, which interchanged the magnesium in the Chl by the two hydrogen ions. During the processing of peas for puree, formation of the three Chl-linked degraded products was reported at a high pH, under short-time heating, and after the storage of the puree at room temperature. The use of magnesium carbonate in combination with high-temperature (at 150°C) and short-time processing initially improved retention of Chl in pureed spinach. However, the effect was not stable during storage. Magnesium compounds resulted in the formation of hard white crystals of magnesium-ammonium-phosphate. Color degradation accelerated when broccoli was submerged in Mellvaine's buffer at decreasing pH. Pheophytinization followed first-order reaction kinetics. The

logarithmic values of reaction rate constants were linearly correlated to the ambient pH up to pH 7. Acids containing a benzene ring were found to cause a faster color change than acids with simple chains due to their hydrophobicity.

The degradation of green color of broccoli (*Brassica oleracea*) after blanching and storage at 7°C, under pH of 3−8, resulted in an increased Chl degradation as the pH decreased. The thermal degradation of Chl and chlorophyllides in the puree of spinach at 100−145°C (for 2−25 min) and 80−115°C (for 2.5−39 min), respectively, led to the formation of the derivatives, pheophorbides, pyropheophorbides, pheophytins, and pyropheophytins.

Chl degradation and yellowing of peel in limes (*Citrus latifolia* Tan.) is one of the dominant problems worldwide. Application of UV-B treatment at 8.8 kJ/m^2 effectively delays the reduction of Chl content, and the pheophrobide content decreases, followed by an increase in the pheophytin *a* content during later stages of storage.

Pistachio (*Pistacia vera* L.) kernels are green in color due to the presence of Chl. The degradation of the green color in pistachio is one of the unacceptable features. Heat treatments applied to pistachio during roasting lead to degradation of Chl *a* and *b* to pheophytins and pyropheophytins, which ultimately influences the quality and market value of pistachio kernel.

Postharvest hot-water treatment delays Chl degradation and maintains quality of Thai lime (*Citrus aurantifolia* Swingle cv. paan) during storage. The quantitative determination of Chl *a*, *b* and the corresponding pheophytins under refrigerated storage conditions followed by industrial processing of spinach showed that pheophytins were the prominent Chl derivatives formed under refrigerated conditions at 8°C for 3 weeks. The heating of spinach leaves showed degradation of Chl, and the effect was found more under microwave cooking or blanching in comparison to steaming or baking. Pheophytins were formed under steamed conditions, whereas under the effect of microwave cooking, pyrochlorophyll *a* and *b* were formed.

11.3 CAROTENOIDS

Carotenoids are accessory pigments ubiquitous in plants and other photoautotrophs, displaying yellow, orange, and red hues. More than 800 carotenoids have been discovered in plants. Carotenoids are lipophilic tetraterpenoids that share a 40-carbon (C_{40}) polyene hydrocarbon chain with alternating double and single bonds between the carbons. The striking structure determines the molecular shapes, chemical reactivity, and light properties of various carotenoids. It forms a conjugated system that effectively delocalizes the π-electrons over the entire polyene chain and serves as the light-absorbing chromophore responsible for the characteristic yellow, orange, or red colors of carotenoids. Carotenoids, according to their composition, are divided into two subgroups: carotenes and xanthophylls (Fig. 11.3). The former has a simple hydrocarbon

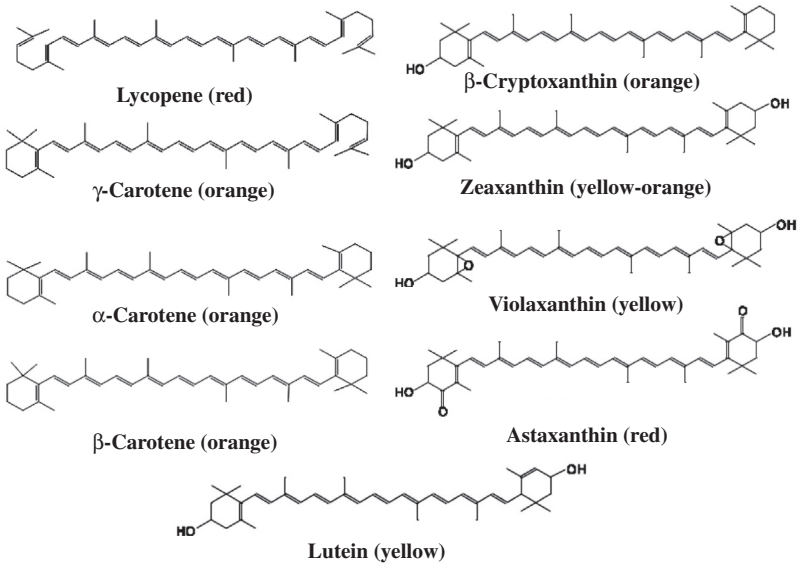


FIGURE 11.3
Structure of some important carotenes and xanthophylls.

compound with a molecular formula of $C_{40}H_{56}$, such as α-carotene, β-carotene, and lycopene. The latter, with a molecular formula of $C_{40}H_{56}O_2$ or $C_{40}H_{56}O$, is oxygenated derivatives of carotenes and contains one or two oxygen atoms within hydroxy-, epoxy-, or keto- groups, such as lutein, zeaxanthin, and violaxanthin.

Traditionally, carotenoids have been given trivial names derived usually from the isolated biological source, but a semisystematic scheme, using two Greek letters to designate the two end groups, has been devised to define their structure unambiguously. For example, α-carotene is referred to as β,ε-carotene, and β-carotene as β,β-carotene. Carotenoids also participate in light-harvesting, fulfill a photoprotective function, provide antioxidant protection in lipid structures, and play roles in signaling and regulation via apocarotenoid hormones (abscisic acid and strigolactone; see Fig. 11.4).

Carotenoids, commonly with at least one β-ionone ring in the tetraterpenoid structure, including α-carotene, β-carotene, and β-cryptoxanthin, can be converted to vitamin A, which is essential for normal vision, development, and immunity of humans and animals. These so-called provitamin A carotenoids are also known as potent antioxidants. A carotenoid-rich diet also correlates with a lower risk for certain cancers and chronic diseases. Carotenoids are among dietary bioactive compounds providing protection against degenerative conditions including cardiovascular diseases, chronic liver disease, diabetes, cancer, and macular degeneration. Numerous mechanisms, that is, quenching of singlet oxygen, scavenging of peroxyl radicals, modulation of gene

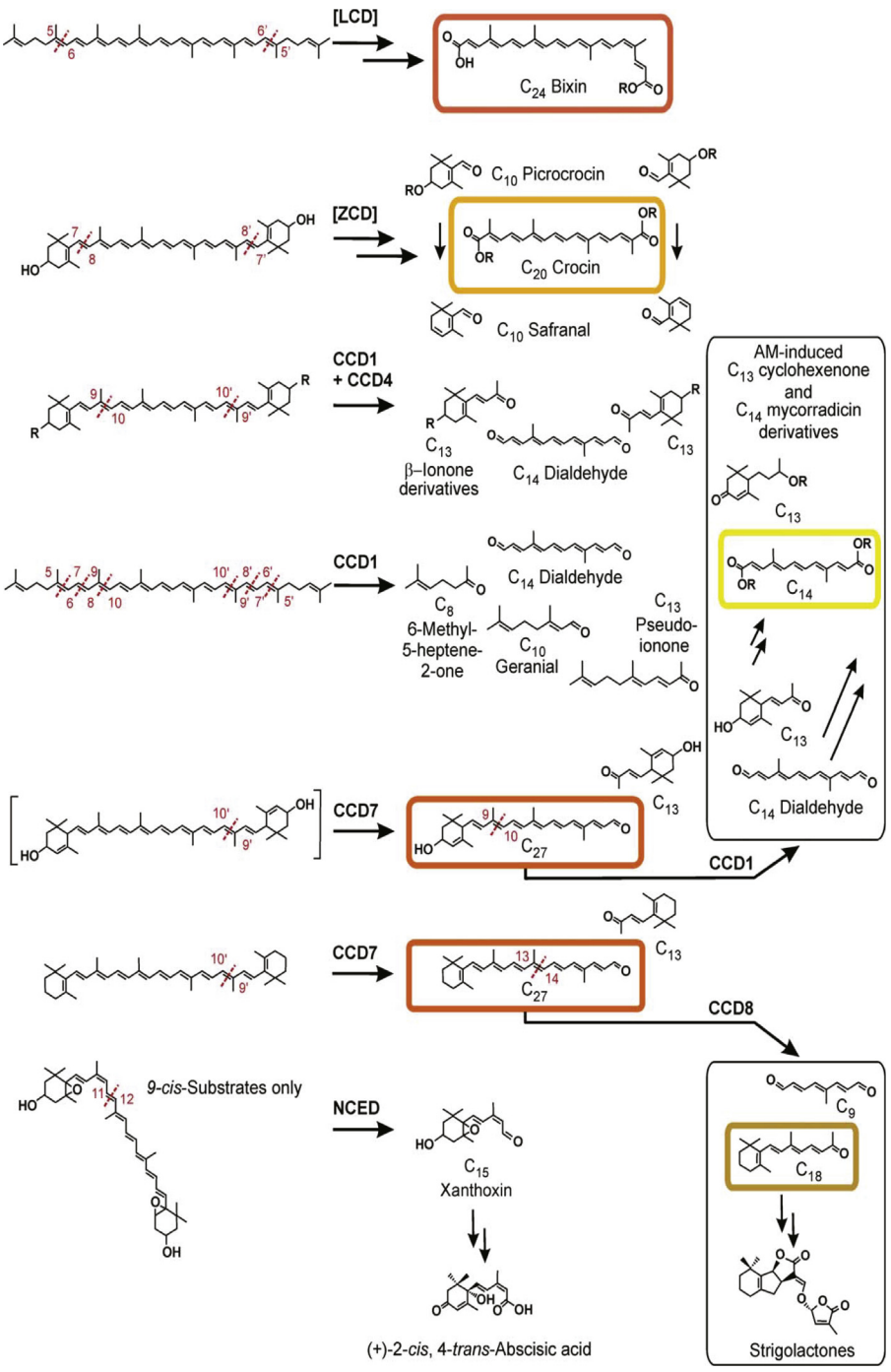


FIGURE 11.4
Specific enzymatic cleavage reactions of carotenoids or apocarotenoids catalyzed by various carotenoid cleavage dioxygenases from plants. Cleavage sites in substrates are indicated by dotted red lines (dotted black lines in print version). Known chromophores in cleavage products are boxed in color. Two cases of sequential cleavage are highlighted, and their end products are boxed in black. Square brackets indicate predicted structures or limited characterization. *Reproduced from Nat. Prod. Rep., 2011, 28, 663, with permission from the Royal Society of Chemistry.*

expression and protein translation, inhibition of cell proliferation, enhancement of cell differentiation via retinoides, stimulation of cell-to-cell communication, enhancement of the immune response, filtering of blue light, and modulation of carcinogen metabolism and adipocyte biology were proposed for the health-promoting effects of carotenoids.

Some of the unique carotenoids include capsanthin and capsorubin predominant in red peppers, bixin as the major pigment of the food colorant annatto, and crocetin as the main coloring component of saffron. Other unique carotenoids have been found in some algae, namely astaxanthin, fucoxanthin, and echinenone. Although green leaves contain unesterified hydroxycarotenoids, most carotenols in ripe fruits are esterified with fatty acids. However, the carotenols of a few fruits, particularly those that remain green when ripe, such as kiwifruit, undergo limited or no esterification.

Lipophilic carotenoids have little or no solubility in water and good solubility in organic solvents, although there are a few exceptions. Therefore, they are expected to be restricted to the hydrophobic areas of the cell, such as the inner core of the membranes, except when association with protein allows them access to an aqueous environment. Polar functional groups alter the polarity of carotenoids and affect their interactions with other molecules. The overall size and shape of the molecule are important in relation to properties and function of carotenoids.

Carotenoids are synthesized via the condensation of eight isoprenoid monomers and stepwise desaturation of the resulting colorless precursors (Fig. 11.4). Upon attaining certain levels of unsaturation, the cyclization of the end groups takes place, yielding one or two ionone rings. Cyclization and other modifications, such as hydrogenation, dehydrogenation, double-bond migration, chain shortening or extension, rearrangement, isomerization, introduction of oxygen functions, or combinations of these processes, result in a myriad of structures. Carotenoids exist primarily in the more stable all-*trans* isomeric form, but small amounts of *cis*-isomers are also present naturally or are transformed from the all-*trans* forms during processing. Some carotenoids have structures containing fewer than 40 carbon atoms and are referred to as apocarotenoids (carbon atoms lost from the C_{40} ends) or as norcarotenoids (carbon atoms lost within the C_{40} chain). These modifications are caused by oxidative degradation at the level of the terminal rings either by nonspecific mechanisms (lipoxygenase, photo-oxidation) or by specific mechanisms (dioxygenases; Fig. 11.4). These oxygenated carotenoids have significant roles in developmental and environmental response signaling. They also make important contributions to the flavor and nutritional quality of several types of foods such as fruits, tea, and wine. Two well-known natural apocarotenoids, bixin and crocetin, have economic importance as pigments and aroma in food. Apocarotenoids also act as visual or volatile signals to attract pollinating agents, and also play important roles in plant defense mechanisms. Studies have shown that the loss of cleavage enzymes induces the development of

axillary branches, indicating that apocarotenoids convey signals that regulate plant architecture. Another apocarotenal, *trans*-β-apo-8'-carotenal, which has a low provitamin A activity, is found in spinach and citrus fruits, used in pharmaceuticals and cosmetic products, and also used as an additive (E160e) legalized by the European Commission for human food. Otherwise, a wide range of apocarotenals are produced by oxidative reactions during food processing and are intermediates in the formation of even smaller molecules of significance in food color and flavor.

Most carotenoids absorb maximally at three wavelengths between 400 and 480 nm, the blue part of the spectrum (Fig. 11.2C), resulting in three-peak absorption maxima. At least seven conjugated double bonds are needed for a carotenoid to have perceptible color. The greater the number of conjugated double bonds, the higher the λ_{max} values. For example, the acyclic lycopene, with 11 conjugated double bonds, absorbs at the longest wavelengths and displays red colors. The ζ-carotene is also acyclic but its three well-defined absorption peak wavelengths are much lower, compared to those from lycopene. Hydroxyl groups exert a great influence on light absorption, while methylation, acetylation, and sialylation markedly reduce this effect.

About 70%–90% of consumed carotenoids originate from fruits and vegetables. More than 100 carotenoids have been reported in fruits and vegetables alongside with Chl in leaves. A composition of these colored carotenoids at different concentrations generates a range of characteristic yellow, orange, and red colors in many vegetables and fruits, such as carrot (*Daucus carota*), tomato (*Solanum lycopersicum*), green apple (*Malus domestica*), watermelon (*Citrullus lanatus*), banana (*Musa paradisiaca*), citrus (*Citrus* spp.), and papaya (*Carica papaya*), to name a few. Carotenoids and their biosynthetic precursors can be used as biomarkers of fruit product quality and adulteration of one product with another, for example, making fraudulent apricot jams and spreads with pumpkin extracts, and differentiation of pumpkins and squashes.

Based on their carotenoid composition, foods of plant origin can be divided into three groups: (1) green vegetables such as broccoli, spinach, and green beans, that contain a high diversity of xanthophylls and carotenes; (2) yellow and red fruits and vegetables (plums, carrots, melons, and tomatoes), with a more special carotenoid distribution than the first category (fruits and vegetables belonging to this category contain primarily carotenes); and (3) yellow or orange fruits, including pumpkins, oranges, and peaches that primarily contain xanthophyll esters.

Generally, carotenoids in ripe fruits are significantly more diverse than in green leaves and unripe fruits. Most of this diversity is represented by secondary carotenoids. The diversity of carotenoid species in ripe fruits is further increased by esterification of xanthophylls with long-chain fatty acids, as is the case in apple skin. However, those of a few fruits, particularly those that remain green when ripe, such as kiwifruit, undergo limited or no esterification. In some green plants and plant parts, generally the darker the color the higher

will be the carotenoid content. For example, carotenoid content in pale green cabbage is less than 1% of that in dark green cabbage, since carotenoid content in green fruits and vegetables displays a strong direct relationship with that of Chl. In nongreen tissues, including fruit at advanced stages of ripening, carotenoids are localized in chromoplasts (Fig. 11.5). The major "photosynthetic" (or primary) carotenoids of higher plants include β-carotene and a number of xanthophylls. During senescence and under stress, plants accumulate secondary carotenoids that are located outside the thylakoid membrane (Fig. 11.5) and do not participate in photosynthesis. These carotenoids may be chemically the same as or different from the photosynthetic carotenoids. Accumulation of secondary carotenoids, especially in the form of fatty acid esters, is typical in ripening fruit.

Carotenoids can exist as protein–carotenoid complexes (as in the case of green leafy vegetables), crystals (as in carrots or tomatoes), or oil-dissolved (as in mango and papaya). Carotenoids commonly found in human blood are lutein, zeaxanthin, β-cryptoxanthin, lycopene, β-carotene, and α-carotene.

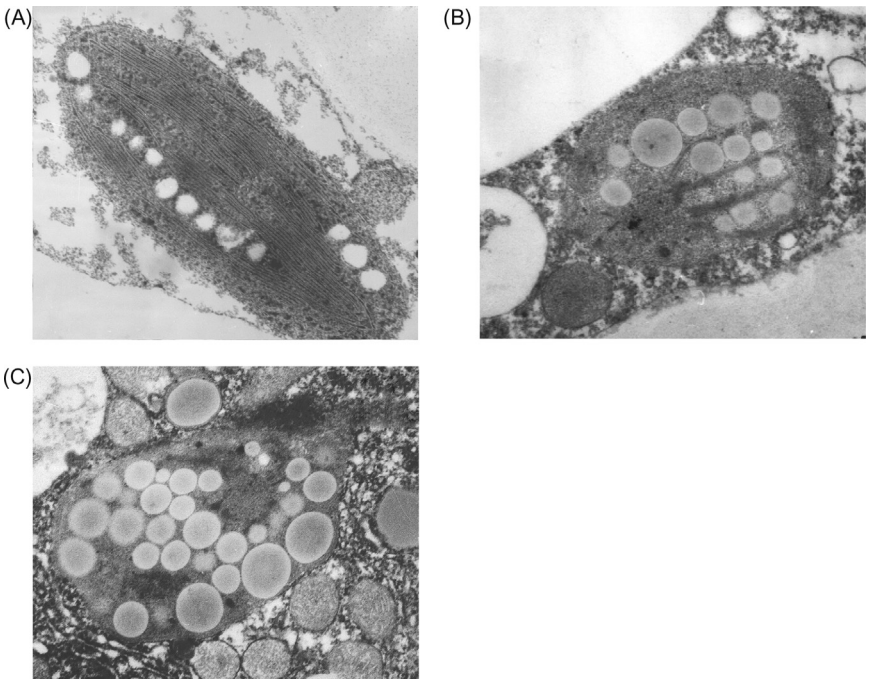


FIGURE 11.5

Accumulation of plastoglobuli containing carotenoids during chloroplast–chromoplast (from A to C) transformation in the peel of ripening "Antonovka" apples. Top micrograph: fixation in glutaraldehyde + $KMnO_4$, magnification: × 15,000; others: fixation in p-formaldehyde + glutaraldehyde + OsO_4, magnification: × 30,000. *Reprinted from Merzlyak, M.N., Solovchenko, A.E. Photostability of pigments in ripening apple fruit: a possible photoprotective role of carotenoids during plant senescence. Plant Sci. 163, 886, with permission from Elsevier.*

Leaves have a strikingly constant carotenoid profile comprised mostly by photosynthetic (primary) carotenoids. The leaf carotenoid profile is dominated by lutein (about 45%), β-carotene (usually 25%−30%), violaxanthin (15%), and neoxanthin (15%). α-Carotene, β-cryptoxanthin, α-cryptoxanthin, zeaxanthin, antheraxanthin, and lutein 5,6-epoxide are also reported as minor carotenoids. Lactucaxanthin is a major xanthophyll in a few species, such as lettuce. Other green vegetables, such as broccoli, follow the same pattern as green leafy vegetables. In contrast to leafy and other green vegetables, all botanical fruits, including those designated as vegetables, are known for their complex and variable carotenoid composition. Some palm fruits are especially rich in carotenoids, particularly provitamin A carotenes. Carotenoid mono- and di-esters were identified in mandarin essential oil, in various vegetables and fruits, in shrimps and microalga *Haematococcus pluvialis*, and in the Antarctic krill *Euphausia superba*. Of the acyclic carotenes, lycopene and ζ-carotene are the most common. Lycopene is the principal pigment in very few red-fleshed fruits and fruit vegetables, such as tomato, watermelon, red-fleshed papaya and guava, and red or pink grapefruit. ζ-Carotene is more ubiquitous but it is usually present at low levels except in Brazilian passion fruit and in carambola, in which it occurs as a major pigment.

Neurosporene has limited occurrence and is normally found in small amounts. The bicyclic α-carotene is the most widespread of all carotenoids in foods, either as a minor or as the major constituent. The bicyclic α-carotene and the monocyclic γ-carotene sometimes accompany β-carotene, generally at much lower concentrations. Substantial amounts of α-carotene are found in carrot and some varieties of squash and pumpkin and substantial amounts of γ-carotene are found in rose hips and *Eugenia uniflora*. Although less frequently encountered, δ-carotene is the principal carotenoid of the high delta strain of tomato and the peach palm fruit. The hydroxy derivatives of lycopene, lycoxanthin, and lycophyll, are rarely encountered; they are found in trace amounts in tomato. The xanthophyll cryptoxanthin can be present as three isomers: β-cryptoxanthin, α-cryptoxanthin, and zeinoxanthin. α-Cryptoxanthin is rarely found in fruits, whereas zeinoxanthin is widely distributed in citrus fruits and maize, but generally at low levels. The xanthophylls α-cryptoxanthin and zeinoxanthin are widely distributed, but generally at low levels. β-Cryptoxanthin is the main pigment of many orange-fleshed fruits such as peach, nectarine, orange-fleshed papaya, persimmon, fruit of the tree tomato, and *Spondias lutea*, but occurs rarely as a secondary pigment. In contrast to the relative abundance of the parent carotenes, α- and β-carotene, respectively, lutein is normally present in plant tissues at considerably higher levels than is zeaxanthin. Lutein is the predominant carotenoid in leaves, green vegetables, and yellow flowers (e.g., marigold, *Tagetes erecta*). Except for yellow corn, the Brazilian fruit *Cariocar villosium*, sweet orange-fleshed pepper, and *Lycium barbarum* (wolfberry), in which it is the major pigment, zeaxanthin is a minor food carotenoid. The epoxycarotenoid violaxanthin may be underestimated in foods because it is easily degraded.

Pumpkins are excellent sources of carotenoids and vitamin A. The content of β-carotene and α-carotene in different species (*Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata*) was found to be 60 − 7400 and 0 − 7500 μg/100 g, respectively. Several other fruits are important sources of carotenoids, although there is great diversity between them. The most important carotenoids with vitamin A activity found in fruits include β-carotene and β-cryptoxanthin. Tropical and subtropical fruits are thought to contain more carotenoids compared to temperate fruits, which contain more anthocyanins. Mango and papaya are among the tropical fruits rich in carotenoids. β-Carotene in mango has been reported as 60 μg/100 g in Thailand, 2900 μg/100 g in India, and 6700 μg/100 g in "Keitt" and 5800 μg/100 g in "Tommy Atkins" mangoes from Brazil. β-Cryptoxanthin was reported as half the content of β-carotene in mango, and 288−1034 μg/100 g in papaya. The content of carotenoids in banana is low, but the high consumption of this fruit makes it an important carotenoid source. Several carotenoids have been identified and quantified in the fruit of *Lycium barbarum*, a traditional Chinese herb containing functional components such as carotenoids, flavonoids, and polysaccharides, widely used in the health food industry because of its possible role in the prevention of chronic disease like age-related macular degeneration.

One of the main factors affecting carotenoid content in fruits and vegetables is the ripening process. The content of some carotenoids can increase from zero to high levels in few days as a consequence of maturation and ripening. This increase in carotenoid content is triggered by the metabolic activity of ethylene. Carotenoid content in mangoes increase steadily during ripening. Similar findings have been reported in several fruits and vegetables such as apricots, acerola, and tomatoes. Immature pepper (*Capsicum* spp.) fruit generally contains lower levels of lutein and xeaxanthin than mature, colored fruit. In tomatoes, carotenoids, especially lycopene, significantly increase during maturation and ripening on or off the plant, and the magnitude of carotenoid accumulation depends on different factors, such as temperature and light intensity. However, there are exceptions. For example, the carotenoid content in three date cultivars decreased during ripening, and was highest in "Khalal" (immature) stage and lowest in "Rutab" (ripe) stage.

The induction of carotenoid synthesis in some fruits such as apples occurs at the terminal stages of on-tree ripening but it normally does not reach the same extent as in postharvest. The detachment of fruits triggers a shift in hormonal balance resulting from cessation of the influx of hormones, mainly ethylene antagonists such as auxins and gibberellins. Detachment of fruit accelerates breakdown of Chl and synthesis of carotenoids due to shifting of the hormonal balance in favor of ethylene, resulting in an increase of carotenoids/Chl ratio in postharvest. In many fruits, including apples, ripening is coupled with chloroplast-to-chromoplast transition accompanied by thylakoid degradation and formation of plastoglobuli with concomitant conversion of xanthophylls into their fatty acid esters (a typical example is shown in Fig. 11.3). Generally, the latter was the harvesting date and the lower was on-

tree Chl content, the higher is the rate of carotenoid accumulation in postharvest. This pattern is moderated by climatic conditions such as temperature, solar irradiation, precipitation, etc. Thus, sun-exposed surfaces of apple fruit (in the outer part of the canopy) pigment transformation associated with ripening occurred more rapidly than in shaded skin of the same fruits, both on-tree and in postharvest. Accordingly, the pigment changes in postharvest are determined by the physiological state which fruit has attained by the date of harvest but not the harvest date per se. The carotenoids/Chl ratio can be exploited as an internal marker of fruit physiological state, indicative of both ripeness and quality. The effect of accelerated ripening on sun-exposed surfaces of fruit might be considerable in fruits growing under contrasting illumination conditions, for example, in the outer part of the canopy, and harvesting indices should be taken into account, and for selection of proper storage conditions.

Green, unripe fruits commonly contain chloroplast carotenoids, and when the fruit ripen chromoplasts develop and carotenoids are produced on a large scale, usually not the same ones as those found in the chloroplast. During ripening, many genes are "switched on," including those related to Chl degradation and carotenoid biosynthesis, thus leading to the disappearance of Chl and accumulation/pigmentation of carotenoids. Several enzymes have been characterized, such as a ripening-specific phytoene synthase in tomato. Molecular genetic manipulation has been employed in fruits, such as in the case of tomato, where transgenic tomato plants have been developed containing increased amounts of phytoene synthase through the introduction of multiple copies of the phytoene synthase gene, thus substantially increasing lycopene content.

Several examples of an increase in carotenoids during ripening and maturation have been reported, such as in apple (*Malus* × *domestica*), *Momordica charantia*, yellow Lauffener gooseberry, red pepper, badami mango, and leafy vegetables. In some anthocyanins and Chl colored ripe fruits, the carotenoid concentrations decrease with ripening. The same trend is seen with some fruits that undergo yellowing simply by unmasking the carotenoids through Chl degradation. Senescence in green vegetables decreases the carotenoid content. Ripening conditions can also affect the carotenoid content. For example, carotenoid biosynthesis in the flesh of ripening Alphonso mango was highest at tropical ambient temperature (28−32°C). "Keitt" and "Tommy Atkins" mangoes harvested at the mature-green stage showed a marked increase in all-*trans*-β-carotene, all-*trans*-violaxanthin, and 9-*cis*-violaxanthin during ripening. Great variations in the stability of carotenoids in fruits and vegetables have been reported during storage. Generally, losses or changes in carotenoids in mature fruits and vegetables that can be stored for long periods of time are small and occur slowly compared to ripe fruits and vegetables.

Carotenoid accumulation can occur after harvest, during maturation and ripening in storage, or during transport. Rapid losses in carotenoids and changes

in their composition can occur due to inappropriate storage conditions. Carotenoid esters are generally more stable than free carotenoids. Carotenoid losses during postharvest storage were reported in some vegetables, particularly leaves, especially under conditions favorable to wilting, high temperature, and light exposure. The effect of different postharvest treatments (such as film wrapping in conventional and biodegradable materials, foodtainer, surface coating) were evaluated with respect to their suitability to prevent losses of bioactive compounds in highly perishable fruits and vegetables. In lettuce, film packaging did not show any beneficial effects on pectic substances and pigments, but in pepino dulce fruits the use of foodtainer maintained the β-carotene and Chl contents.

The β-carotene content of tomatoes increased during storage when fruit were still in the ripening process and this increase was more pronounced at higher temperatures of up to 25°C; whereas in some sweet potato cultivars no increase in β-carotene during storage occurred as the synthesis of carotenoids was already completed by the time of harvest. This maturity–temperature interaction was also observed in pepino. Premature and mature pepino fruit stored at 18°C showed a stronger increase in β-carotene compared to those stored at 5°C; however, in ripe pepinos, β-carotene were unaffected by temperature. Storage at 7–20°C for 16–43 days caused a substantial decrease in total carotenoid content, even when fruits were subsequently ripened at optimal conditions. Losses in α-carotene, β-carotene, and lutein increased in carrots as storage temperature increased above 4°C and as illumination increased. Lycopene content in several fruit and vegetables has been reported to increase at a temperature of about 25°C, but no synthesis occurs above 30°C. In chilling sensitive crops such as tomato, lycopene was reported to decrease at low (chilling) temperatures. However, this effect was not reported in hydroponically grown tomato fruit. Contents of lycopene and β-carotene precursors, phytoene, phytofluene, and ζ-carotene, were highest in tomatoes stored at 20°C when compared with those stored at 30°C. This indicates that the activity of the key enzymes catalyzing the synthesis of β-carotene and lycopene, phytoene synthase and phytoene desaturase, is mainly triggered at the maturation process and can be additionally influenced by temperature. For example, temperatures above 30°C limit carotenoid production. This phenomenon may be due to inhibition of ethylene biosynthesis, and/or to reduced levels of phytoene synthase as reported for tomatoes. However, peaches held at 41°C for 24 h prior to postharvest ripening at 27°C had a higher carotenoid content than tree-ripened fruit.

Adequate modified atmospheres (MAs) and controlled atmospheres (CAs), especially atmospheres with low concentrations of oxygen, are known to maintain carotenoids and reduce their losses. Very high concentrations of carbon dioxide have been shown to result in some losses in carotenoids. The use of MA storage for 6 days at 5°C preserved carotenoid content in broccoli compared to those stored in normal air at the same temperature, which lost about half of the carotenoid content. CA storage of mature pepino fruit for a total of

21 days with varying high CO_2 atmospheres (15 kPa for 2 days followed by 5 kPa for 19 days) maintained β-carotene content, but high CO_2 atmosphere (15 kPa) caused a strong reduction within 14 days. High CO_2 levels also reduced β-carotene content in spinach, melon, and kale. "Golden Delicious" apples held in 15 kPa CO_2 retained their green color due to inhibition of carotenoid production. A CA atmosphere of 3 kPa O_2 and 20 kPa CO_2 inhibited lycopene accumulation in tomato fruit, but it increased once the fruit were replaced in air, although at a reduced rate. In addition, modified atmosphere packaging (MAP) with 10 kPa CO_2 and 10 kPa O_2 resulted in a decline in lycopene content in watermelon stored at 2°C for 7 or 10 days.

Low oxygen atmospheres enhanced the retention of carotenes in carrots, air + 5 kPa CO_2 caused a loss, while air + 7.5 kPa CO_2 or higher appeared to cause de novo synthesis of carotene. MAP retains carotenoids in broccoli florets. The retention of β-carotene in jalapeno pepper rings after 12 days at 4.4°C plus 3 days at 13°C was 87% in MAP (5 kPa O_2 + 4 kPa CO_2) and 68% in air, and retention of α-carotene was 92% in MAP and 52% in air after 15 days. Peach slices kept in air + 12 kPa CO_2 had a lower content of β-carotene and β-cryptoxanthin (retinol equivalent) than slices kept in air, 2 kPa O_2, or 2 kPa O_2 + 12 kPa CO_2 for 8 days at 5°C. Storage of persimmon slices in 2 kPa O_2 or air + 12 kPa CO_2 resulted in slightly lower retinol equivalent after 8 days at 5°C, but the loss was insignificant in slices kept under 2 kPa O_2 + 12 kPa CO_2. For sliced peaches and persimmons, the limit of shelf-life based on sensory quality was reached before major losses of carotenoids occurred.

Ethylene treatment was reported to be associated with carotenogenic gene expression and the corresponding carotenoid accumulation in apricot. Generally, postharvest ethylene applications accelerate tomato fruit ripening and the accumulation of lycopene, while ethylene inhibitors inhibit lycopene accumulation. However, this effect can be influenced by the stage of ripening. For example, tomato fruit harvested at the breaker stage accumulated more lycopene at the same temperature than those harvested green and treated with ethylene. Apple fruit harvested on a later date (e.g., at a more ripe stage) also tended to accumulate more carotenoids at a higher rate (Fig. 11.6).

The use of the ethylene antagonist 1-methylcyclopropene (1-MCP) to extend the postharvest life of cherry tomato (*Lycopersicon esculentum* var. Cerasiforme) delayed the ethylene-induced climacteric peak in mature green and breaker fruit, Chl degradation, and accumulation of lycopene and carotenoids. Higher 1-MCP concentrations inhibited the accumulation of lycopene and carotene such that the color of the fruit did not reach that of the control fruit.

Lower doses (at or below 200 Grays) of irradiation coupled with 35 days of storage at 10°C were not harmful in the retention of lycopene and other health-promoting compounds in early-season grapefruit, but higher doses (400 and 700 Grays) and 35 days of storage had detrimental effects on early-season grapefruit, however, no significant effect was observed in late-season fruit.

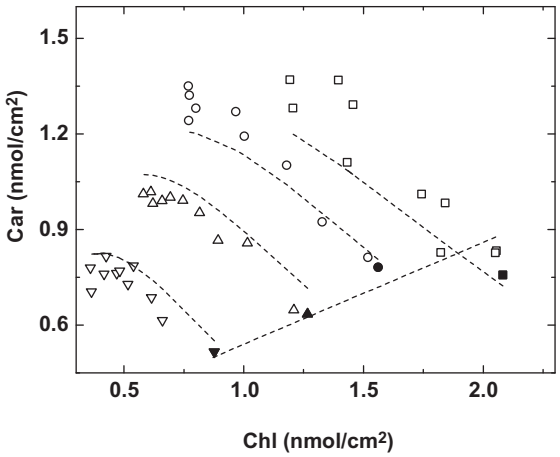


FIGURE 11.6

Relationships of the carotenoid-to-chlorophyll ratio versus Chl content in Antonovka apple fruit (harvested in 1996, Moscow) during on- and off-tree ripening (closed and open symbols, respectively). *Reprinted from Solovchenko, A.E., Chivkunova, O.B., Merzlyak, M.N., Gudkovsky, V.A., 2005. Relationships between chlorophyll and carotenoid pigments during on- and off-tree ripening of apple fruit as revealed non-destructively with reflectance spectroscopy. Postharvest Biol. Technol. 38, 9–17, with permission from Elsevier.*

Hot-water treatment was reported to delay carotenoid synthesis and thus yellowing of broccoli florets (at 40°C for 60 min) and kale (at 45°C for 30 min), but did not affect Brussels sprouts. Hot air treatment (38°C at 95% RH for 24 h) slightly decreased lycopene and β-carotene content in tomato fruit, however fruit heated at 34°C for 24 h and stored at 20°C developed higher lycopene and β-carotene than nonheated fruit. Moist (100% RH) hot air (at 48.5°C or 50°C) for 4 h caused injury to papaya and losses in lycopene and β-carotene, but similar treatment with dry air (50% RH), alone or in combination with thiabendazole, had no effect on lycopene and β-carotene. High-temperature treatment also suppressed 1-aminocyclopropane-1-carboxylic acid oxidase activity and thus indirectly prevented carotenoid synthesis.

Degreening with ethylene is a common postharvest practice in citrus fruit. The ethylene-induced fruit coloration and carotenoid content in the flavedo increased with the ripening stage of the fruit. Ethylene stimulated an increase in phytoene, phytofluene, and (9Z)-violaxanthin, which is the main carotenoid in fully ripened orange peel, and the apocarotenoid β-citraurin, and decreased the concentration of chloroplastic carotenoids. These changes are consistent with the effect of ethylene on the expression of carotenoid biosynthetic genes, since it upregulates the expression of phytoene synthase, ζ-carotene desaturase, and β-carotene hydroxylase genes, sustained or transiently increased accumulation of phytoene desaturase, plastid terminal oxidase, β-lycopene cyclase, and zeaxanthin epoxidase mRNAs, and decreased the expression of the ε-lycopene cyclase gene. These data indicate that exogenous ethylene reproduces and accelerates the physiological and molecular changes in the carotenoid biosynthesis naturally occurring during maturation of citrus

fruit. On the other hand, gibberellic acid, which delays fruit degreening, reduces the ethylene-induced expression of early carotenoid biosynthetic genes and the accumulation of phytoene, phytofluene, and β-citraurin.

Being highly unsaturated, carotenoids are prone to isomerization and oxidation. Heat, light, acids, and adsorption on an active surface, such as alumina, promote isomerization of *trans* carotenoids, their usual configuration, to the *cis* forms. This results in some losses in color and provitamin A activity. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by light, enzymes, metals, and co-oxidation by lipid hydroperoxides. Carotenoids appear to have different susceptibilities to oxidation; lutein and violaxanthin are thought to be more labile than others. Formation of epoxides and apocarotenoids, that is, with shortened carbon skeleton, appears to be the initial step. Subsequent fragmentations yield a series of low-molecular-weight compounds similar to those produced in fatty acid oxidation. Thus, total loss of color and changes in the biological activities are the final consequences. Therefore, retention of naturally occurring or added carotenoids in prepared, processed, and stored foods is an important consideration. Unfortunately, the same structural attributes of carotenoids that are thought to impart health and sensory benefits also make these compounds easily degraded by different chemical reactions. Understanding the mechanisms of carotenoid degradation is essential for developing technologies for the incorporation of these compounds into functional foods. By identifying the predominant carotenoid degradation pathway likely to occur in a particular food product, delivery systems could be engineered for optimal stability.

In view of carotenoid instability, processing of fruits and vegetables exerts profound and diverse effects on carotenoids, depending on the type and conditions of processing methods. Tissue disruption, such as by chopping or homogenizing, can lead to substantial losses in carotenoids, especially through the action of oxidation processes. For example, up to 30% of β,β-carotene can be lost within a few minutes when green leaves are macerated. Carotenoids are generally stable during heat processing and cooking of vegetables and fruits, but can increase the amounts of Z-isomers. A significant increase in *cis*-lycopene and a slight decrease in *trans*-lycopene are observed in guava juice after processing. Carotenoids are commonly retained during the processing of mango slices. The only significant change is the increase in luteoxanthin, compatible with the conversion of 5,6- to 5,8-epoxide. More evident changes occur on processing mango puree, where the principal pigment β-carotene decreases, auroxanthin appears, and violaxanthin and luteoxanthin decrease. During storage of mango slices in lacquered or plain tin-plate cans, no appreciable loss of β-carotene is noted for 10 months, but between the 10th and 14th months a 50% reduction occur. Violaxanthin tends to decrease and auroxanthin to increase during storage. β-Carotene shows greater susceptibility to degrade in bottled mango puree (about 18% loss after 10 months) than in the canned product. As with the mango slices, however, both bottled and canned puree suffer up to 50% loss of β-carotene after the 14th month. Violaxanthin and

luteoxanthin tend to decrease, whereas auroxanthin maintains a comparatively high level throughout storage. Substantial differences are commonly noted in commercially processed mango juice. Violaxanthin, the principal carotenoid of mango fresh, was not commonly detected; auroxanthin appears in an appreciable level; and β-carotene becomes the principal carotenoid. Both lycopene (the major pigment) and β-carotene show no significant changes during the processing of papaya puree. *Cis*-lycopene increase up to sevenfold and β-cryptoxanthin decreases to up to 34%. During 14 months of storage, β-carotene, lycopene, and *cis*-lycopene remained practically constant. β-Cryptoxanthin did not change significantly during the first 10 months but decreased 27% after 14 months. Auroxanthin and flavoxanthin appeared during storage. In olives, only β-carotene and lutein resist the fermentation and brine storage. Phytofluene and ζ-carotene disappear. Violaxanthin, luteoxanthin, and neoxanthin give rise to auroxanthin and neochrome, the total pigment content does not usually change. In carrot juice, canning (121°C for 30 min) usually results in the greatest loss of carotenoids, followed by high-temperature short-time heating at 120°C for 30 s, 110°C for 30 s, acidification plus 105°C heating for 25 s, and acidification. Heating increases *cis*-isomers, 13-*cis*-β-carotene being formed in the largest amount, followed by 13-*cis*-lutein and 15-*cis*-α-carotene. Canning increases the percentage of total *cis*-isomers of provitamin A carotenoids in several fruits and vegetables. Canning of sweet potatoes causes the largest increase, followed by processing of carrots, tomato juice, collards, tomatoes, spinach, and peaches.

Heat induces the formation of 13-*cis*-β-carotene in sweet potatoes, and the quantity formed is related to the severity and length of the heat treatment. However, carotene content is reduced by canning, by dehydration, by microwaving, and by baking. *Cis*-isomers have also been reported to increase during heating of carrot juice, 13-*cis*-β-carotene being formed in the largest amount, followed by 13-*cis*-β-lutein and 15-*cis*-α-carotene. However, canning (121°C, 30 min) commonly results in the greatest loss of carotenoids, followed by high-temperature short-time heating (120°C for 30 s, 110°C for 30 s, acidification plus 105°C for 25 s, and acidification). Carrot juice color turns from orange to yellow with intensive treatment. When this processed carrot juice is stored, the concentrations of lutein, α-carotene, and β-carotene decrease with increasing storage temperature and illumination. The formation of 13-*cis*-isomers increases under light and of 9-*cis*-isomers increases under dark storage. Storage of tomato and carrot juices under light (230 ft-c at 4°C) cause a 75% reduction in α- and β-carotene and a 25% reduction in lycopene, but storage in darkness causes no negligible losses.

Dehydrataion (hot air drying at 65°C), freezing (at −30°C) and freeze-drying of spinach previously immersed in salt and bicarbonate solution, lost 12% β-carotene, but no significant losses of lutein, violaxanthin, and zeaxanthin. Losses in all-*trans*-β-carotene were 33% in freeze-dried Italian spinach, and 43%−38% in solar dried Italian Spanish, spring cabbage, and cowpea leaves. In winter squash, no losses in carotenoids were reported during blanching and

freeze-drying, lutein decreased slightly, and β-carotene was stable during freezing. However, losses in β-carotene were 15%, 20%, and 53% in freeze-dried squash stored at 30°C for 1, 2, and 3 months, respectively. Storage at lower temperature (3°C) for 3 months causes only 10% losses in β-carotene.

Thermal processing is common for fruits and vegetables. The thermal sensitivity of carotenoids is commonly assumed, however, the effect of thermal treatment on fruits and vegetables is not clear. Boiling (for 20 min), frying (for 10 min), or drying (at 57°C for 10 h) of sweet potatoes reduce the content of all-*trans*-β-carotene by 77%−88%. These types of processing induce β-carotene isomerization (from all-*trans* to 9-*cis*). Similarly, the carotenoid content in bell peppers decreases by 55%−80% as the temperature of the osmotic dehydration process increases from 25°C to 55°C. Conventional drying of mango fruit induces isomerization of all-*trans*-β-carotene to 13-*cis*-β-carotene, whereas solar drying favors the formation of 9-*cis*-β-carotene. In contrast, thermal processing (drying) of paprika increases the content of red carotenoids by 40%. Similarly, zeaxanthin and β-carotene contents in fruits increase significantly; 2−22 times that of fresh fruits of *Lycium barbarum* at the beginning of the drying period. Heat pasteurization enhanced the content of some carotenoids (lycopene, β-carotene, and phytofluene) and the red color of juices. On the other hand, freezing can decrease, increase, or maintain carotenoid levels of fruits and vegetables. Minimal processing and milling of fruits and vegetables induce carotenoid losses. In contrast, pulsed electric fields, osmotic dehydration, radiation, and high-pressure processing induce small or no losses of carotenoids in intact fruits and vegetables and their juices and purees.

The evaluation of different cooking forms on the content of α- and β-carotene in carrots has shown that lower temperature and less cooking time resulted in the most retention. Scalding of carrots in water at different temperatures (50°C, 70°C, 90°C) for 15 min did not result in significant losses of lycopene, and only slight losses of β-carotene at 90°C. Sun drying has been reported to result in significant losses in carotenoids. However, fresh mangoes dried using a solar dryer to a final moisture content of 10%−12%, had 4000 ± 500 μg/100 g and 3680 ± 150 μg/100 g of β-carotene after 2 and 6 months of storage, respectively.

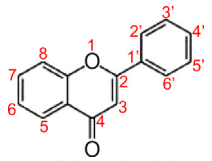
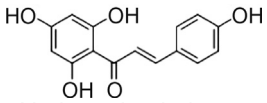
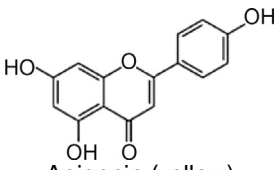
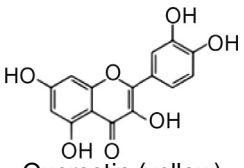
Therefore, in most processing methods, carotenoid retention seems to be reduced and losses seem to be increased at higher temperature and longer exposure/processing time. Reducing temperature, reducing processing time, reducing the presence of oxygen, and incorporating antioxidants can greatly reduce the losses in carotenoids.

11.4 FLAVONOIDS

Flavonoids are water-soluble phenolic compound pigments with very diverse structures. The basic structure of a phenolic compound is one or more aromatic rings with hydroxyl substituents. The key phenolic compound groups in plants include simple phenols and phenolic acids with C6−C1 carbon

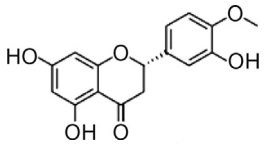
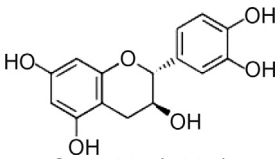
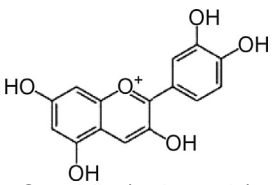
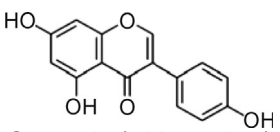
backbone, hydroxycinnamate, and other phenylpropanoid derivatives with C6−C3, and flavonoids with C6−C3−C6. Phenolic compounds are found in every plant species. Among them, over 9000 polyphenolic derivatives belong to flavonoids that share the 15-carbon benzo-γ-pyrone (C6−C3−C6) backbone but differ in substituent combinations. The substitutions result in at least nine subgroups: anthocyanins (anthocyanidins), condensed tannins (proanthocyanidins), flavonols, flavones, flavandiols, isoflavonoids, chalcones, aurones, and phlobaphenes (Table 11.1). They play more diverse biological roles and display a wider spectrum of colors, compared to carotenoids. The colors are not only determined by the combinations of substituents, but also changed with alteration of the pH values and metal ions. Flavonoids in the diet may benefit humans and animals against allergens, carcinogens, pathogens, and other inflammatory agents. Many flavonoid pigments, including anthocyanins, chlorogenic acid, and quercetin glycosides, exert a strong antiradical activity in vitro.

Table 11.1 Monomeric Flavonoids: Basic Structure, Edible Sources, and Representative Aglycones (Strack and Wray, 1989; Harborne, 2013)

Subclass	Edible Sources	Aglycone (Color)
Basic structure		 2-Phenyl-4*H*-chromene-4-one (white)
Chalcone	Tomato skin, soybean	 Naringenin chalcone (yellow)
Flavone	Grapefruit, lemon, orange, red/green pepper, celery, garlic, herbal tea	 Apigenin (yellow)
Flavonol (3-hydroxyl-flavone)	Red onion, apple, grape, cilantro, fennel, berries, garlic, kale, spinach, tea, broccoli, red wine, cherry tomato	 Quercetin (yellow)

(*Continued*)

Table 11.1 (Continued)

Subclass	Edible Sources	Aglycone (Color)
Flavanone	Grapefruit, lemon, orange, tomato, citrus fruits and juices, herbal tea	 Hesperetin
Flavan-3-ol	Cocoa, prune, açai, black tea, peach, apple, black tea, blueberry, red wine	 Catechin (white)
Anthocyanidin	Grape, blueberry, cherry, cranberry, apple, plum, red cabbage, red onion, red wine, strawberry, blood orange	 Cyanidin (red-purple)
Isoflavone	Currant, raisin, soybean, legumes, peanut	 Genistein (white-yellow)

Flavonoids are synthesized in chloroplasts or cytoplasm (Fig. 11.7). Briefly, starting with general phenylpropanoid metabolism, malonyl CoA and 4-coumaroyl CoA are the first substrates catalyzed by chalcone synthase (CHS). Various end products are then formed through terminal modification by the addition of sugars, methyl, ferulate, and other substituent groups, which eventually lead to the nine main subgroups of colored and colorless flavonoids. After glycosylation, they are transported to, and accumulated within, the vacuoles or excreted into apoplast where they remain within the cell wall or incorporated in cuticle. Flavonoids serve a plethora of protective functions in plants including defense against phytopathogens and herbivores and photodamage by UV and visible light.

Anthocyanins appear to be the most studied and colorful flavonoids that are able to display almost all nongreen visible colors in plants, including these characteristic purple and violet colors in anthocyanin-rich fruits and vegetables, such as apple (*Malus pumila*), blackberry (*Rubus* spp.), blueberry (*Vaccinium* spp.), and eggplant (*Solanum melongena*), to name a few. Apple

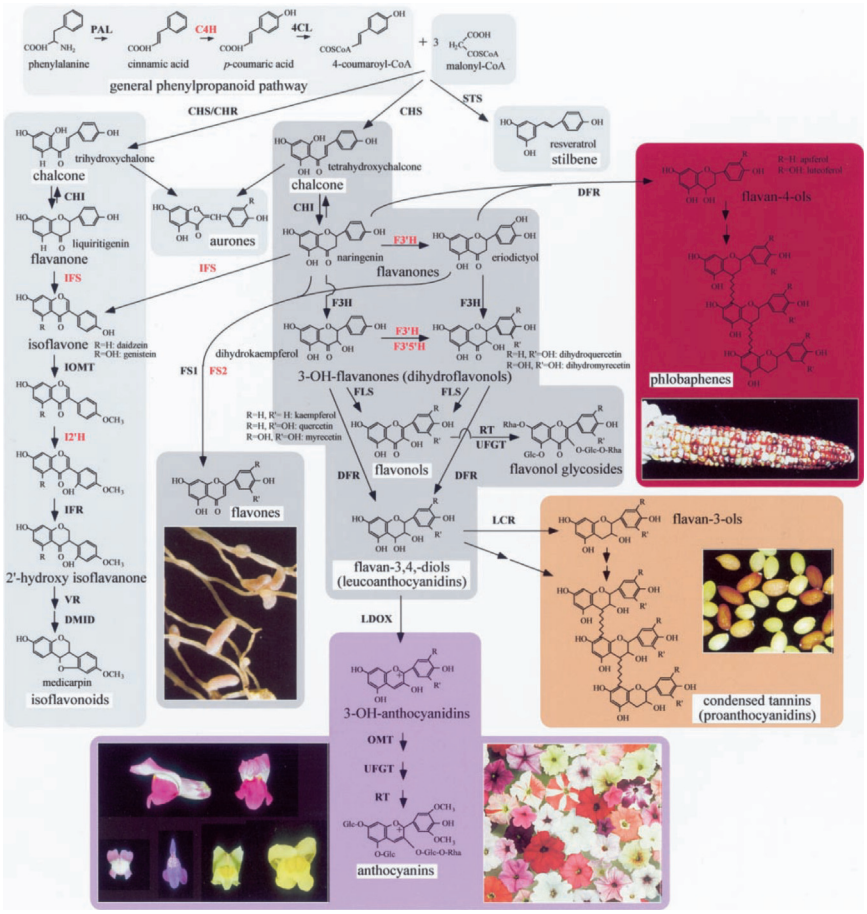


FIGURE 11.7

Illustration of flavonoid biosynthesis pathways. Enzyme names are abbreviated as follows: cinnamate-4-hydroxylase (C4H), chalcone isomerase (CHI), chalcone reductase (CHR), chalcone synthase (CHS), 4-coumaroyl:CoA-ligase (4CL), dihydroflavonol 4-reductase (DFR), 7,29-dihydroxy, 49-methoxyisoflavanol dehydratase (DMID), flavanone 3-hydroxylase (F3H), flavone synthase (FSI and FSII), flavonoid 39 hydroxylase (F39H) or flavonoid 3959 hydroxylase (F3959H), isoflavone O-methyltransferase (IOMT), isoflavone reductase (IFR), isoflavone 29-hydroxylase (I29H), isoflavone synthase (IFS), leucoanthocyanidin dioxygenase (LDOX), leucoanthocyanidin reductase (LCR), O-methyltransferase (OMT), Phe ammonia-lyase (PAL), rhamnosyl transferase (RT), stilbene synthase (STS), UDPGflavonoid glucosyl transferase (UFGT), and vestitone reductase (VR). *Reprinted from Plant Physiol. 126, 486 (Winkel-Shirley 2001), with permission from the American Society of Plant Biologists.*

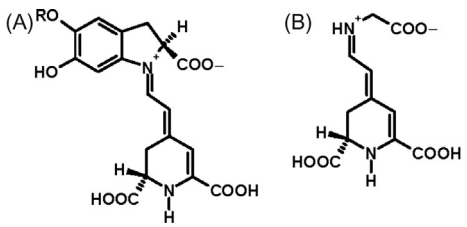
fruit contains high amounts of flavonoids, comprised mainly by quercetin glycosides. Remarkably, in sunlight (but not when shaded) apple skin flavonoids content could achieve as high as 3.50 mmol/m^2 that exceeds Chl and carotenoid content by more than 10-fold. Apple flavonoids, the potent reactive oxygen species scavengers, exert multifaceted beneficial effects on human health, for example, provide protection from cardiovascular and oncology diseases. It was reported that the skin flavonoid content tends to change considerably in the course of apple fruit storage.

Characteristic absorption spectrum of screening-relevant phenolic compounds in the UV usually contains two bands. The first band peaking around 280 nm appears due to the presence of aromatic ring(s), and is detected in the spectra of all flavonoids. The second, long-wave band is situated in the 300–360 nm range, the exact position of its maximum varies for different classes of phenolics. In anthocyanidins and their glycosylated forms known as anthocyanins, the maxima of the second absorption band are located in the blue-green part of the visible spectrum. In particular, the long-wave absorption band of cyanidin, the predominant aglycone of anthocyanins responsible for the reddish coloration of leaves and fruit in many species, is centered at 525 nm.

In solutions, flavonols and anthocyanins often undergo inter- and intramolecular copigmentation. As a result, the increase of absorption coefficients, bathochromic shifts of maxima, and peak flattening are observed to significantly affect the efficiency of absorption of light by these compounds localized within the cells and tissues. In the case of common flavonols in plants (such as quercetin and kaempferol glycosides), their tautomerization occurring in planta induces more profound bathochromic shifts of the long-wave absorption maxima.

11.5 BETALAINS

Betalains are a small group of indole-derived glycoside pigments, which contain nitrogen in their molecules and are found in plants of the Caryophyllales order and some mushrooms in the Basidiomycota phylum. Betalains are structurally (Fig. 11.8) different from anthocyanins and never coexist in plants with anthocyanins, but both share some similar chemical properties, biological functions, and color spectrums. Betalains are also water-soluble and accumulate in cell vacuoles, mainly in epidermal and subepidermal tissues. Betalains are also free-radical scavengers, more efficient at alkaline and neutral pH. The similarity of spectral properties and subcellular localization between betalains and anthocyanins suggests that betalains fulfill the function of anthocyanins in these plant species lacking anthocyanins.



Betacyanin Portulaxanthin III

FIGURE 11.8

Typical representatives of two important betalain groups: (A) betacyanins and (B) betaxanthins (Strack et al., 2003).

Betalains are derived from tyrosine via the immonium conjugates of betalamic acid with cyclo-DOPA and amino acids or amines. They also exist in plants as glycosides, acylglycosides, or other complex forms of compounds (e.g., esters with ferulic acid and flavonol conjugates synthesized as a result of UV irradiation). Their core structure is represented by the protonated 1,2,4,4,7-pentasubstituted 1,7-diazaheptamethin system that is responsible for their chroma. Betalains are structurally divided into betacyanins and betaxanthins (Fig. 11.8). The former display reddish to violet colors and the latter show yellow to orange colors in different plant parts, such as flowers, fruits, roots, bracts, seeds, leaves, and stems, where they play important physiological roles. Absorption spectra of betacyanins are characterized by a broad band with the maximum near 593 − 543 nm; a bathochromic shift to 550 nm is possible as a result of intramolecular copigmentation. The spectra of betaxanthins feature three main bands with the maxima near 217, 262, 546 − 471 nm.

The betalain biosynthesis pathway has not been elucidated as well as the carotenoid and flavonoid biosynthesis pathways. Betacyanin synthesis in red beet (*Beta vulgaris*) leaves is induced by wounding and bacterial infiltration as a response of plant protection against damage caused by mechanical injury or UV radiation. From betalamic acid, various spontaneous cyclisation, molecule condensations, and glycosylation steps are carried out in order to complete the formation of betalains. The first step in betalain synthesis is the simultaneous hydroxylation and oxidation of tyrosine by tyrosine hydroxylase to form DOPA (L-3,4-dihydroxyphenylalanine) and the subsequently oxidation and cleavage of the aromatic ring of DOPA by DOPA-dioxygenase producing 4,5-seco-DOPA, which exhibits a spontaneous cyclization and becomes betalamic acid. Tyrosine hydroxylase, being a bifunctional enzyme, hydrolyzes and oxidizes tyrosine, whereas DOPA-dioxygenase performs an O_2-dependent cleavage of the aromatic ring of DOPA.

About 75 betalains have been structurally identified from plants of about 17 families out of 34 families under the order Caryophyllales, such as Aizoacea, Amaranthaceae, Basellaceae, Cactaceae, Chenopodiaceae, Didieraceae, Holophytaceae, Nyctaginaceae, Phytolactaceae, and Portulacaceae families, to name a few. Betalains have also been found in mushrooms in the genera Amanita, Hygrocybe, and Hygrosporus. Only a few fruits and vegetables contain betalains and the best known is beet roots (*Beta vulgaris*) that produces betanin ($C_{24}H_{27}N_2O_{13}$), an important natural food colorant. The other edible sources known to contain betalains are fruits of *Opuntia* spp. (e.g., *O. ficus-indica*, commonly known as prickly pear), Swiss chard (*B. vulgaris* subsp. *cicla*), grains or leaves of amaranth (*Amaranthus* spp.), pitahaya (*Hylocereus* spp., also known as dragon fruit), ulluco tubers (*Ullucus tuberosus*), djulis (*Chenopodium fromosanum*, a native cereal in Taiwan), and garambullo (*Myrtillocactus geometrizans*, an endemic plant locally consumed in Mexico), which contains 233 mg/kg of total betalains in its fruit pulp.

Betalains can be extracted from macerated raw material with pure water (cold or at room temperature), but methanol and ethanol solutions at 20%–50% v/v improved the extraction. The addition of ascorbic acid (c.50 mM) to aqueous methanol as extraction medium has been recommended in order to stabilize betacyanin molecules by a slightly acidic pH, but also to inhibit the potential betacyanin oxidation by polyphenoloxidases and to avoid high tyrosinase activities when betaxanthins are present. In the case of materials rich in free sugars, aerobic juice fermentation with *Saccharomyces cerevisiae* is needed in order to deplete free sugars and to obtain higher yields in the extraction process. The purification of betalains can be done using conventional anion-exchange column chromatography or size-exclusion chromatography prior to NMR identification. Dark conditions and low temperature in the laboratory are recommended during the isolation process of betalains in order to avoid pigment degradation.

Stability of betalains is affected by several factors including pH, temperature, light, oxygen, and water activity. The hue color parameter from betalains is unaffected in the range of pH 3.5–7. This is an acidic range at which most foods are included. Particularly, betanin showed that its stability is pH-dependent and the pH for maximum betanin stability ranges from 5.5 to 5.8 when oxygen is present. On the other hand, red beet solutions showed their maximum stability at pH 5.5, which is the normal pH for beets. Also, vulgaxanthin-I was most stable between pH 5.0 and 6.0, being more stable in juice than in purified extracts, whereas reconstituted powders showed optimal pigment stability at pH 5.7.

Temperature has a clear effect on betalain stability. Heating of betanin solutions produces a gradual reduction of red color, and eventually the appearance of a light-brown color. The rate of betanin degradation increased 15.6% after the exposure of pigment to daylight at 15°C, following a first-order kinetic. Furthermore, the degradation was higher at pH 3.0 ($k = 0.35\ \text{day}^{-1}$) than at pH 5.0 ($k = 0.11\ \text{day}^{-1}$) when betacyanins were exposed to fluorescent light. However, betacyanins were most stable ($k = 0.07\ \text{day}^{-1}$) in dark conditions.

Betanin reacts with molecular oxygen, producing pigment degradation in air-saturated solutions. It was observed that color degradation increases up to 15% due to air conditions when betanin-buffered solutions at pH were stored under an atmosphere of air and nitrogen for 6 days at 15°C. It has been established that water activity (a_w) has a pronounced exponential effect on pigment stability, which decreases in one order of magnitude when a_w increases from 0.32 to 0.75. Water activity has been included among the primary factors affecting betalain stability and/or the color of a food product containing these pigments. The greatest stability of betalains has been reported in foods or model systems of low moisture and a_w. The increase in stability of betanin with decreasing a_w may be attributed to reduced mobility of reactants or limited oxygen solubility.

11.6 CONCLUDING REMARKS

Horticultural commodities contain all key groups of plant pigments including Chl, carotenoids, flavonoids, and betalains. At early stages of development, their pigment composition is more or less similar to that of green leaves but changes dramatically in the course of maturation and ripening. The rate of pigment transformation depends on the species, environmental conditions, hormonal status of the plant, and the process tends to accelerate upon detachment of the fruit as an effect of the ripening hormone, ethylene build-up within the fruit. Typical patterns of fruit pigment transformation include degradation of Chl on the background of retention or even accumulation of secondary carotenoids. In many cases ripening is accompanied by an increase in the phenolic compounds, such as anthocyanins, which is promoted by environmental stimuli (light and temperatures). Many fruit pigments exert extensively documented beneficial effects on human health, being powerful vitamins and antioxidants, among other effects. In addition, pigments are important components of the visual and sensory aspects of horticultural commodities. Therefore, high pigment content is considered as an added value of these commodities. Special care is required to ensure maximal retention of these compounds during handling, storage, and processing. Insights into the chemistry, biochemistry, and biology of pigments in horticultural commodities obtained to date are outlined in this chapter, and form the essential basis for achieving this goal in the horticulture crop industry.

ACKNOWLEDGMENT

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CHAPTER 12
Phenolic Compounds

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12.1 INTRODUCTION

Several epidemiological studies have shown an inverse correlation between a high intake of fruits and vegetables and the incidence of nontransmissible chronic diseases (NTCDs) such as cardiovascular diseases, cancer, obesity, diabetes, etc. It has been suggested that these beneficial effects of the consumption of fruits and vegetables are due to the presence of different antioxidant phytochemicals (which are plant secondary metabolites), that scavenge reactive oxygen species (ROS), partially responsible for the incidence of these NTCDs, neutralizing their negative effects. Among them, phenolic compounds (PCs) play an important role in these potential health benefits. PCs represent the second most abundant group of organic compounds in the plant kingdom (just behind cellulose), and show different activities in the plant such as structural support, and protection against ultraviolet (UV) solar radiation, biotic or abiotic stress, pathogens, herbivores, etc. From the consumers' point of view, PCs provide protection against NTCDs not only by means of their antioxidant activity, but also by regulating many cellular processes at different levels, including enzyme inhibition, modification of gene expression, protein phosphorylation, etc. Moreover, they also have an impact on commonly accepted quality attributes of fruits and vegetables, such as bitterness, color, and flavor. It has been described that different preharvest and postharvest treatments can induce the synthesis of PCs and increase the shelf-life of fruits and vegetables, by the activation of their antioxidant defense system. This increment in PCs can modify the beneficial health effects, as well as consumers' acceptability of the fruits and vegetables.

Postharvest Physiology and Biochemistry of Fruits and Vegetables. DOI: https://doi.org/10.1016/B978-0-12-813278-4.00012-9

In this chapter, a systematic description of the main PCs found in fruits and vegetables will be given and some PCs important in other plant foods will be mentioned. PCs can be classified as flavonoids and nonflavonoids, and according to their complexity, as monomeric and polymeric compounds. The biosynthetic pathways of the main PCs described in the first section will be described in the second section, and finally, the effect of different postharvest treatments on the PC content will be described.

12.2 STRUCTURE, CLASSIFICATION, AND OCCURRENCE OF PHENOLIC COMPOUNDS

PCs are ubiquitously distributed in most plant tissues, including edible parts such as fruits, seeds, leaves, stems, roots, etc. The occurrence of PCs in foods (raw or processed) can be consulted online in: Phenol Explorer (http://phenol-explorer.eu/) and the USDA Database for the Flavonoid Content of Selected Foods (https://www.ars.usda.gov/). All PCs contain at least one aromatic ring with one hydroxyl group in their structure. There are more than 8000 individual plant PCs, with great structural variability; they can be classified into two main groups: flavonoids and nonflavonoids. Flavonoids are the most abundant PCs in fruits and vegetables, they account for nearly two-thirds of dietary PCs; and, as a group, they are the most bioactive. They contain a phenyl benzopyran skeleton: two phenyl rings (A and B) joined through a heterocyclic pyran ring (ring C; Fig. 12.1). Flavonoids can be divided into six groups or families, according to differences in the pyran ring. In each family, individual compounds differ in their pattern of hydroxylation and methylation of rings A and B. The basic skeleton and numbering of C atoms in flavonoids are shown in Fig. 12.1.

Flavones are the most basic structure of flavonoids. They contain a keto group in C4, a double bond between C2 and C3, and a B ring is attached to C2. The most abundant flavones in fruits and vegetables include apigenin, luteolin, and their glycosides (Fig. 12.2), in which a carbohydrate (mono- or disaccharide) is linked to the flavonoid moiety (named aglycone) through an OH. Flavones are abundant in herbs and spices like celery, parsley, thyme, and others; they are also present in some fruits, especially cantaloupe and watermelon, and vegetables, especially sweet and hot peppers, Chinese cabbage, and artichokes. Special mention is deserved for polymethoxylated flavones

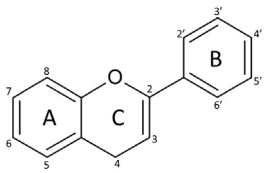
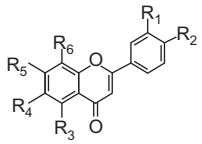


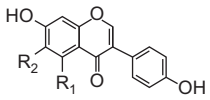
FIGURE 12.1
Phenylbenzopyrane skeleton, basic structure of flavonoids.

Flavones



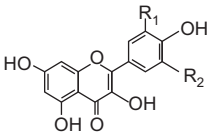
	R1	R2	R3	R4	R5	R6
Apigenin	H	OH	OH	H	OH	H
Luteolin	OH	OH	OH	H	OH	H
Tangeretin	H	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
Nobiletin	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3

Isoflavones



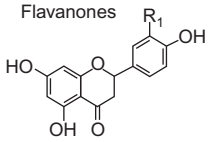
	R1	R2
Daidzein	H	H
Genistein	OH	H
Glycitein	H	OCH_3

Flavonols



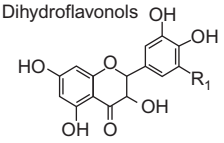
	R1	R2
Kaempferol	H	H
Quercetin	OH	H
Myricetin	OH	OH

Flavanones



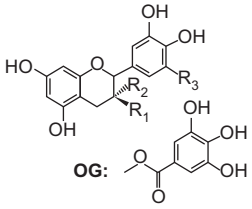
	R1
Naringenin	H
Eriodictyol	OH
Hesperitin	OCH_3

Dihydroflavonols



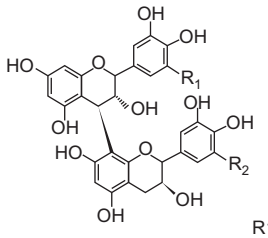
	R1
Taxifolin	H
Ampelopsin	OH

Flavan-3-ols (monomers)



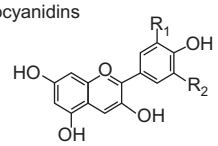
	R1	R2	R3
(+) catechin	OH	H	H
(–) epicatechin	H	OH	H
(+) gallocatechin	OH	H	OH
(–) epigallocatechin	H	OH	OH
(+) catechin gallate	OG	H	H
(–) epicatechin gallate	H	OG	H
(+) gallocatechin gallate	OG	H	OH
(–) epigallocatechin gallate	H	OG	OH

Proanthocyanidins (oligomeric flavan-3-ols)



	R1	R2
Procyanidin B1 EC-(4b-8)-c	H	H
Prodelphinidin B1 EGC-(4b-8)-GC	OH	OH

Anthocyanidins



	R1	R2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

FIGURE 12.2
Flavonoid families.

(e.g., tangeretin and nobiletin; Fig. 12.2), which are abundant in citrus peels and present in some citrus juices and have been shown to have powerful bioactivities. Isoflavones are flavones in which B ring is attached to C3 instead of C2. This structural feature makes isoflavones resemble estrogens

(female sex hormones) so they possess mild estrogenic activity and are known as phytoestrogens. Isoflavones are abundant in soy beans and in many soy products; they are also present in low amounts in other legumes such as common beans and peanuts. The main isoflavones are daidzein, genistein, glycitein (Fig. 12.2), and their 7-O glycosides (daidzin, genistin, and glycitin, respectively). Flavonols are flavones hydroxylated in C3. They are some of the best antioxidant flavonoids due to their pattern of hydroxylation, where the OH in C3 is thought to increase the stability of the flavonoid radical formed once the compound has acted as a radical scavenger. They are also the most abundant flavonoids in fruits and vegetables, the major flavonols are kaempferol, quercetin, and myricetin (Fig. 12.2), and their glycosides. Kaempferol is mostly found in vegetables (kale and various leaf vegetables) and herbs (dill and tarragon), it is also present in common beans and berries. Quercetin is widespread in fruits and vegetables: in fruits, it is abundant in berries, including grapes, and is also present in cherries, apples, and others; in vegetables, artichokes, Chinese cabbage, hot peppers, lettuce, and onion are good sources of quercetin. Herbs from the apiaceae family are also rich in this flavonol. Myricetin is mostly found in berries and walnut.

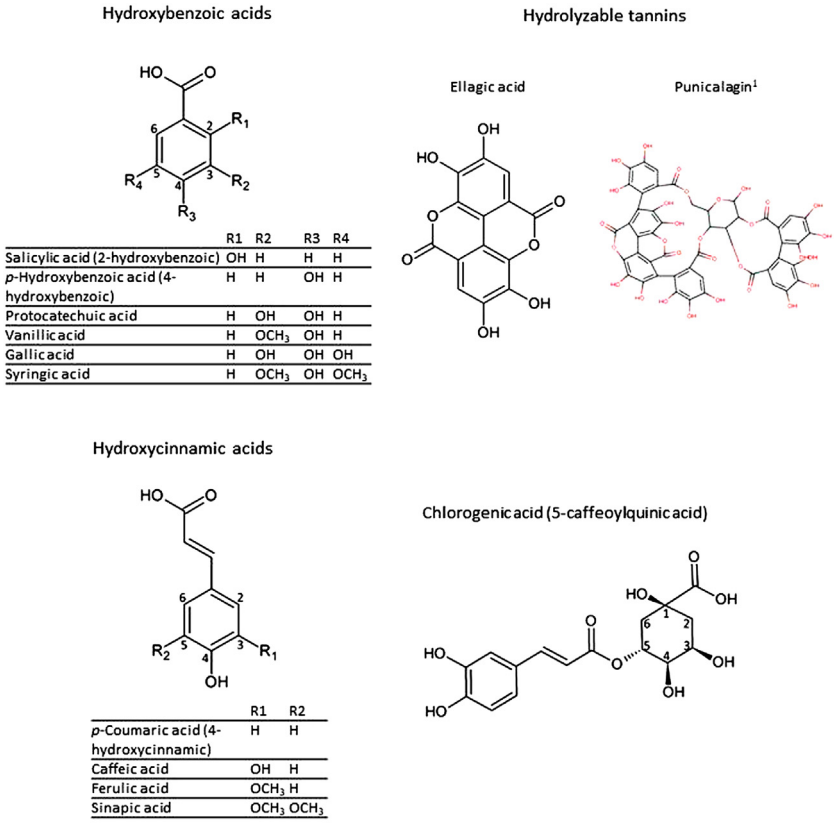
In flavanones and dihydroflavonols (or flavanonols) ring C presents a saturated pyrane group (no double bond between C2 and C3), they also possess the keto group in C4 and dihydroflavonols contain OH in C3. Major flavanones are naringenin, eriodictyol, and hesperetin (Fig. 12.2). They are found in citrus fruits and juices and in some herbs like Mexican oregano and peppermint. Taxifolin (or dihydroquercetin; Fig. 12.2) is the most important dihydroflavonoid in foods; it is abundant in Mexican oregano. Taxifolin and ampelopsin (dihydromyricetin) glycosides have also been reported in wines. Flavanones and dihydroflavonols, together with other nonconventional flavonoids (chalcones and aurones), are considered minor flavonoids because they are not abundant in nature.

Flavanols or flavan-3-ols lack the double bond between C2 and C3 and the oxo group in C4, and contain an OH in C3. This structural characteristics become to C2 and C3 at ring C in chiral centers since four different groups are attached to each of these carbon atoms, therefore flavan-3-ols possess several different configurations. The major flavan-3-ols are catechin, epicatechin, gallocatechin, epigallocatechin, their 3-O-gallates, polymers, and oligomers (Fig. 12.2). Flavan-3-ols are rarely found as glycosides. Catechins and epicatechins are stereoisomers in C3 (as for gallocatechins and epigallocatechins); while gallates are esterified with gallic acid (a nonflavonoid PC) in C3. Oligomeric flavan-3-ols are called pronathocyanidins (because acid degradation in the presence of alcohols yields free anthocyanidins), while polymeric forms are named condensed tannins, although the terms are usually interchangeable. B-type proanthocyanidins are constituted by flavan-3-ols joined by one C−C bond, while in A-type compounds the monomeric units are joined by two bonds; both types can be made up of only catechin/epicatechin

(procyanidins) or contain also units of gallocatechin/epigallocatechin (prodelphinidins). Flavan-3-ols are the most abundant flavonoids in nature. Their polymeric and oligomeric forms are the most abundant plant-derived PCs; they are also good antioxidants, especially those containing galloyl moieties ([epi]gallocatechins and all the 3-O-gallates). In food products, they are especially abundant in tea, but also in chocolate, red wine, nuts, and several fruits. Epicatechin and catechin are the most widely distributed flavan-3-ols and can be found in many fruits like grape, strawberry, blackberry, peach, nectarine, apple, and fruit juices. Oligomeric flavan-3-ols or proanthocyanidins are one of the most widespread compounds present in food ingested daily; they can be found in tea, wine, cereals, chocolate, nuts, and fruits. Procyanidins B2 (epicatechin−epicatechin dimer) and B1 (Fig. 12.2) are the most common proanthocyanidins, they are abundant in fruits such as peach, nectarine, plum, and apple, and in vegetables such as broad beans.

Anthocyanidins, like flavan-3-ols, do not present a keto group in C4 and possess an OH in C3, and two double bonds in ring C; due to these structural characteristics, they are the only ionic flavonoids. The basic skeleton of anthocyanidins is the flavilium cation, which endows these flavonoids with unique properties: they constitute the largest and probably the most important group of water-soluble plant pigments and their color changes in relation to matrix pH. For instance, at low pH, they appear pink; purple in neutral conditions, and greenish-yellow in basic pH, making them natural pH indicators. If pH is very alkaline, they are colorless. In nature, anthocyanidins are invariably glycosilated, forming anthocyanins, most glycosilations occur in C3, although they are also common in C7 and C5. Anthocyanins are abundant in berries, but they can also be found in colored fruits like grapes, cherries, plum, nectarine, peach, and vegetables like black beans, red lettuce, and red onion. Red wine is also a good source of anthocyanins. Almost all anthocyanins are glycosylated derivatives of six anthocyanidins: pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Fig. 12.2).

The group of nonflavonoids includes PCs with very diverse chemical structures, most of them smaller and simpler than flavonoids, but there are also some compounds with complex structures and high molecular weights. The most important group of nonflavonoids in fruits and vegetables are phenolic acids, which contain a single phenyl group substituted by one carboxylic group and one or more OH groups. Phenolic acids can be further divided into hydroxybenzoic acids, hydroxycinnamic acids, and other hydroxyphenyl acids (acetic, propanoic, and pentaenoic), differing among them in the length of the chain containing the carboxylic group. Fig. 12.3 shows the most common hydroxybenzoic (basic skeleton C6−C1) acids, which can be found in many fruits, vegetables, and other edible products. Berries, nuts, tea, chicory, and some spices are good sources of these compounds. Hydroxybenzoic acids are rarely found in their free form, they commonly appear glycosylated, linked to small organic acids (quinic, maleic, or tartaric) or bound to structural components of the plant cells (cellulose, proteins, or lignin). Other common



	R1	R2	R3	R4
Salicylic acid (2-hydroxybenzoic)	OH	H	H	H
p-Hydroxybenzoic acid (4-hydroxybenzoic)	H	H	OH	H
Protocatechuic acid	H	OH	OH	H
Vanillic acid	H	OCH_3	OH	H
Gallic acid	H	OH	OH	OH
Syringic acid	H	OCH_3	OH	OCH_3

	R1	R2
p-Coumaric acid (4-hydroxycinnamic)	H	H
Caffeic acid	OH	H
Ferulic acid	OCH_3	H
Sinapic acid	OCH_3	OCH_3

FIGURE 12.3
Phenolic acids. *From Phenol-Explorer, URL: http://phenol-explorer.eu/compounds/449.*

derivatives of hydroxybenzoic acids are hydroxybenzoic aldehydes like vanillin (derived from vanillic acid) and syringealdehide (from syringic acid). Sometimes gallic acid forms complex structures with carbohydrates, called hydrolyzable tannins, which can be divided into gallotannins, which provide sugar and gallic acid on hydrolysis, and ellagitannins, which also produce ellagic acid (a gallic acid dimer; Fig. 12.3). Punicalagin (Fig. 12.3) is one ellagitannin abundant in pomegranate husk, and is also found in its juice. Hydrolyzable tannins are also found in berries, mango, and nuts.

Hydroxycinnamic acids possess a C6–C3 (phenylpropanoid) basic skeleton (Fig. 12.3). The major hydroxycinnamic acids are *p*-coumaric acid, caffeic acid, and the methylated forms ferulic and sinapic acids; however, as for other phenolic acids, hydroxycinnamic acids are almost always bound with small or large molecules. The most abundant hydroxycinnamic acid derivative in plant foods is an ester of caffeic and quinic acids, named chlorogenic acid (5-caffeoylquinic acid, Fig. 12.3), some of its isomers, such as *neo*-chlorogenic (3-caffeoylquinic acid) and *crypto*-chlorogenic (4-caffeoylquinic acid) acids are also common.

Chlorogenic acid and its isomers are the major PCs in coffee beans and coffee drinks; they are also found in many fruits (including plum, berries, nectarine, peach, apple, pear, etc.) and vegetables (broccoli, tomato, chicory, lettuce, artichoke, etc.), with the five isomers being the most abundant. *p*-Coumaric and caffeic acids have also been reported in many fruits, vegetables, and other foods. *p*-Coumaric is abundant in cranberry, common beans, peanuts, maize, and clove; caffeic acid is abundant in coffee (mostly as chlorogenic derivatives, but it can be detected as free acid after hydrolysis of the samples) and in some berries, herbs, and vegetables like olives, swiss chard leaves, and carrot. Ferulic acid is known to be present in high quantity in cereals, mainly wheat, maize, and rye, it is also abundant in common beans and present in fruits, herbs, and some vegetables. Sinapic acid can be found mainly in olives, but is also present in fruits, vegetables, cereal grains, oilseed crops, and some spices, and some derivatives of sinapic acid are characteristic of the *Brassicaceae* family.

Overall, phenolic acids account for almost one third of dietary PCs, so flavonoids plus phenolic acids comprise the great majority of PCs in edible products. However, there are certain PCs that are not included in these groups but are characteristic and even major components of some fruits, vegetables, or other food products. Lignans are nonflavonoids formed by two phenylpropanoid units (C6−C3−C3−C6), they are widely distributed but present at low concentrations in cereals, fruits, nuts, vegetables, etc. Secoisolariciresinol (Fig. 12.4), matairesinol, lariciresinol, and pinoresinol are the most common lignans; they are abundant in flaxseed. Chalcones and dihydrochalcones can be considered as flavonoids due to their basic C6−C3−C6 structure; however, the C3 chain is not closed, forming a pyran ring. They are found in just a few foods, the most common dihydrochalcones (C3 chain is saturated) are phloretin (Fig. 12.4) and its glucoside phloridzin, which are characteristic of apple and some apple products. Stilbenes have a C6−C2−C6 skeleton; they are nonflavonoids typical of grape and wine. *Trans*-resveratrol (Fig. 12.4) is the most important polyphenol with a stilbene skeleton; it is found mostly in red wine and in the skins of red wine grapes, but also, in low quantities, in other grapes and berries, peanuts, and pistachio. Furanocoumarins (furano benzopyran skeleton) are a class of coumarins that can be found in celery, parsley, and members of the *Apiaceae* family, where the major furanocoumarins are bergapten and psoralen (Fig. 12.4). Bergapten and some prenilated furanocoumarins (such as bergamottin) are also present in grapefruit juice where they are known to possess undesirable interactions with several medications.

Herbs and spices are rich in some unique PCs with important bioactive properties. Many of these are volatile PCs, such as eugenol (a hydroxyphenyl propene; Fig. 12.4) the main PC of cloves, or carvacrol (phenolic terpene; Fig. 12.4), a typical component of oregano. Curcumin (diferuloylmethane; Fig. 12.4) and curcuminoids are PCs found only in the rhizomes of *Curcuma longa*, from which turmeric, a main ingredient of curry, is obtained. Curcumin is known to have an important role in prevention and treatment of various illnesses, from cancer to autoimmune, neurological, cardiovascular, and diabetic

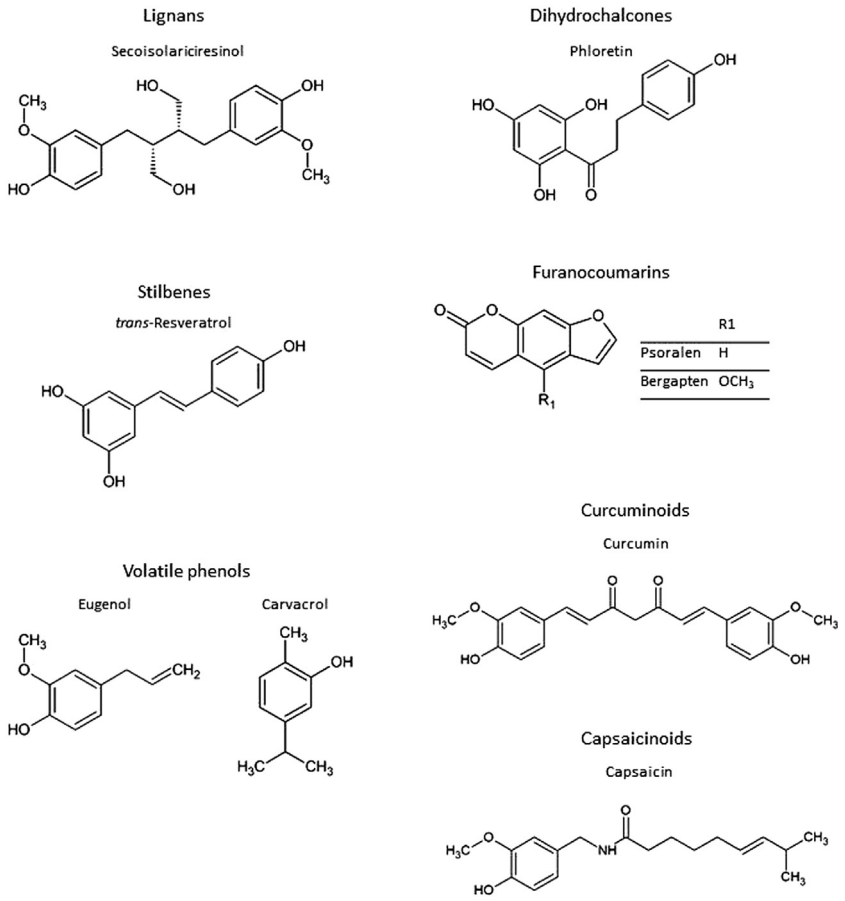


FIGURE 12.4
Other nonflavonoid PCs.

diseases. Capsaicinoids are only found in the fruits of plants from the genus *Capsicum*, they contain a vanillyl group joined to a fatty acid chain through an amide bond and capsaicin (Fig. 12.4) is the main representative of this group. Capsaicinoids have numerous biological actions but they are mostly recognized for being the pungent ingredient of hot peppers.

12.3 METABOLISM OF PHENOLIC COMPOUNDS

Plant metabolism can be separated into primary pathways, that are found in all cells and deal with the production and storage of energy and the major biomolecules common to all living forms; and secondary pathways, that are responsible for the production of a wide variety of compounds that possess specific activities such as protection. The primary pathways deal with the metabolism of carbohydrates, lipids, proteins, and nucleic acids. In contrast,

the secondary metabolites (e.g., terpenes, alkaloids, PC, and related compounds) are produced through the shikimic, malonic, and mevalonic acid pathways, as well as the methylerythritol phosphate pathway.

The chemical diversity of PCs, discussed in the previous section, is responsible for their varied roles in the plant. Depending on their structure, they possess different activities such as mechanical support; protection against harmful UV solar radiation and excessive water loss; attraction of pollinators and seed dispersers; signals that induce defensive reactions against biotic or abiotic stresses, etc. Some of these compounds can suppress the growth of nearby competing plants (i.e., allelopathy), or provide protection against herbivores and pathogens. For all these reasons, they are recognized as valuable plant molecules with very important biological functions.

PCs are synthesized mainly through the phenylpropanoid metabolism, which involves the presence of precursors that are synthesized by two main pathways: the shikimic acid pathway and the malonic acid pathway. Even though both pathways participate in the biosynthesis of PCs in plants, the shikimic acid pathway is the main one responsible for the production of the precursors of most plant PCs. Then, the phenylpropanoid metabolism leads to the production of numerous molecules such as hydroxycinnamic acids, hydroxybenzoic acids, flavonoids, and lignin. Other hydroxybenzoic acids are synthesized directly from an intermediate of the shikimic acid pathway [3-dehydroshikimic acid (3-DHS)]. Therefore, in this section we are going to describe the main pathways of the phenylpropanoid metabolism, beginning with the shikimic acid pathway.

12.3.1 Shikimic Acid Pathway for Phenolic Compounds Biosynthesis

The production of shikimic acid, the first step in the phenylpropanoid metabolism, is shown in Fig. 12.5. The first step is the synthesis of 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) from the condensation of erythrose-4-phosphate (E4P) and phosphoenolpyruvate (PEP), through the enzyme DAHP synthase. Next, phosphate and water are removed in two successive enzymatic reactions, to form 3-dehydroshikimic acid (3-DHS), the precursor of gallic acid, gallotannins, and ellagitannins. Shikimic acid is then obtained by a hydrogenation reaction catalyzed by shikimate dehydrogenase. Chorismate is the next key intermediate in the shikimic acid pathway, the rearrangement of its aliphatic chain, a transamination and a dehydration, leading to the production of phenylalanine and tyrosine, which are the main substrates for the synthesis of PCs through the phenylpropanoid pathway.

12.3.2 Hydroxycinnamic and Hydroxybenzoic Acid Biosynthesis

Phenylalanine is the primary substrate for the synthesis of PC through the phenylpropanoid pathway in most plants, while tyrosine is used to a lesser

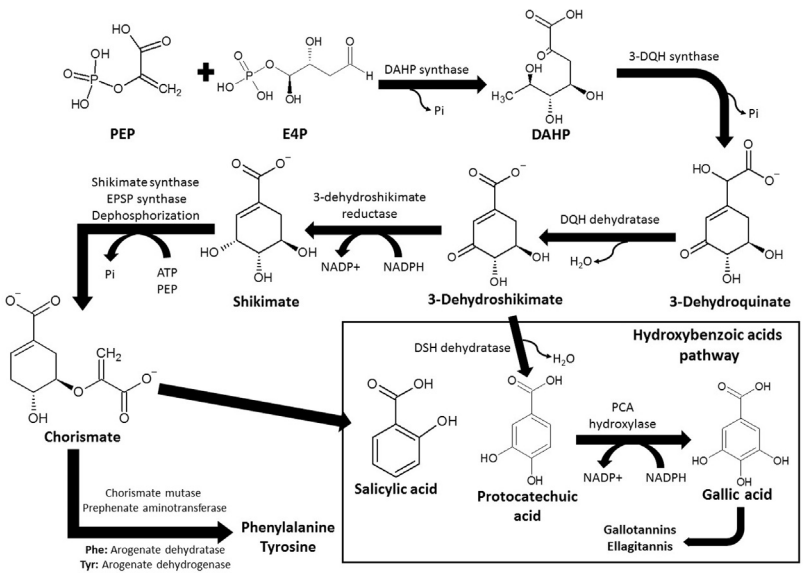


FIGURE 12.5

Shikimic acid and hydroxybenzoic acid biosynthesis. *DAHP*, 3-deoxy-D-arabinoheptulosonate-7-phosphate; *DHS*, 3,5-didehydroshikimate; *DQH*, dehydroquinate; *PEP*, phosphoenolpyruvate; *E4P*, eristrose-4-phosphate; *PCA*, protocatechuic acid.

extent in some plants. PCs are divided into numerous classifications (see Section 12.2); however, most are derived from hydroxycinnamic acids. The first reaction involved in the synthesis of PCs from L-phenylalanine is the loss of an amino group to form *trans*-cinnamic acid, through the activation of the enzyme phenylalanine ammonia lyase (PAL) (Fig. 12.6). Subsequently, *trans*-cinnamic acid is hydroxylated by the enzyme cinnamate-4-hydroxylase (C4H) adding a hydroxyl group in position 4 to produce *p*-coumaric acid. *p*-Coumaric acid can also be obtained from the deamination of tyrosine by the enzyme tyrosine ammonia lyase (TAL), this being the only different step between the phenylalanine and tyrosine pathways. Caffeic acid is synthesized by the addition of a hydroxyl group in position 3 by the enzyme coumaryl 3-hydroxylase (C3H); this is an essential step in the production of hydroxycinnamic acids. Ferulic acid is synthesized by the 3-O-methylation of caffeic acid by the enzyme caffeic acid O-methyltransferase (COMT). Caffeic acid is combined with one coenzyme A (CoA) through the enzyme 4-coumarate CoA ligase, triggering the production of chlorogenic acid, which is produced by the esterification of caffeoyl-CoA with quinic acid, through the enzyme hydroxycinnamoyl-coenzyme A quinate transferase (HCQT). Sinapic acid is obtained from ferulic acid by its hydroxylation at position 5 and later O-methylation through the action of ferulic 5-hydroxylase (F5H) and COMT, respectively, this being the last step in hydroxycinnamic acid biosynthesis. Coumaric, ferulic, and sinapic acids can be directed to lignin production.

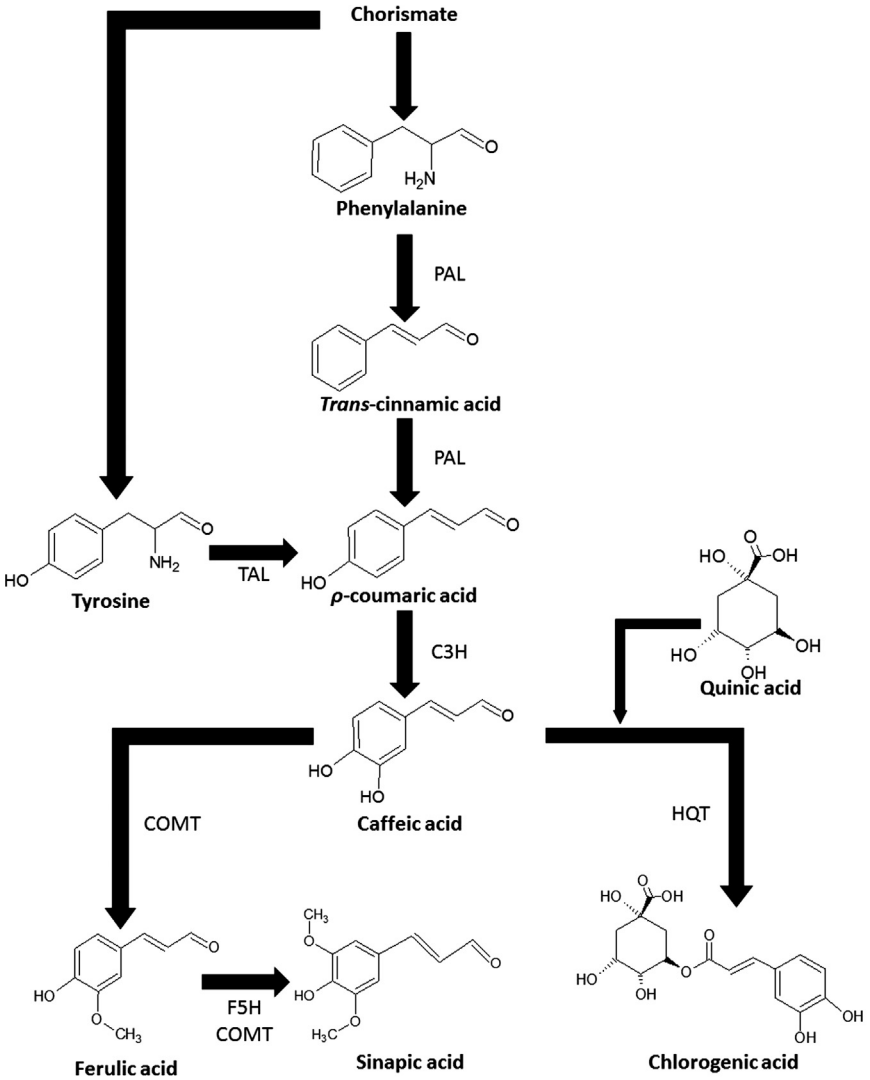


FIGURE 12.6

Hydroxycinnamic acids pathway of synthesis. *C4H*, cinnamate-4-hydroxylase; *C3H*, coumaryl 3-hydroxylase; *COMT*, caffeic acid o-methyltransferase; *F5H*, ferulic 5-hydroxylase; *HCQT*, hydroxycinnamoyl-coenzyme A quinate transferase; *PAL*, phenylalanine ammonia lyase; *TAL*, tyrosine ammonia lyase.

Hydroxybenzoic acids, unlike hydroxycinnamic acids, can be synthesized directly from the shikimic acid pathway (Fig. 12.5), they are not phenylpropanoids and, consequently, they can be produced even if PAL is not active. Gallic and salicylic acids are two of the most relevant hydroxybenzoic acids. Studies suggest that gallic acid is synthesized during the shikimic pathway from 3-DHS by the action of the enzyme shikimate dehydrogenase to produce

3,5-didehydroshikimate. This latter compound tautomerizes to form the redox equivalent gallic acid. Gallic acid can lead to the production of polymeric compounds such as gallo and ellagitannis. Salicylic acid can be synthesized in plastids by the shikimic acid pathway (Fig. 12.5).

12.3.3 Flavonoid Biosynthesis

Flavonoids are synthesized in the final steps of the phenylpropanoid pathway through the activation of a multienzyme complex found in the cytosol. The initial step in the biosynthesis of flavonoids (Fig. 12.7) consists of the formation of the core or basic flavonoid skeleton by the interaction of three molecules of malonyl-CoA and one molecule of p-coumaroyl-CoA, by the activation of the enzymes chalcone synthase (CHS) and chalcone isomerase (CHI), through a two-step condensation that yields the flavanone naringenin. Naringenin can be redirected to flavone production by the action of the enzyme flavone synthase (FNS), which forms apigenin, followed by the addition of a hydroxyl group at positions 3′ or 5′ of ring B (to produce luteolin or tricetin). Flavanones can also be oxidized by the enzyme flavanone-3-hydroxylase (F3H) to synthesize dihydroflavonols, by the addition of a hydroxyl group at position C-3 in ring C. Dihydroflavonols can participate in two pathways to produce flavonols or to continue with the leucocyanidins and flavan-3-ols biosynthesis. If the flavonol route is activated, the enzyme flavonol synthase (FLS) catalyzes the desaturation between C-2 and C-3 in ring C. Leucocyanidin synthesis requires the action of two enzymes: flavonoid 3′-hydroxylase (F3′H) and dihydroflavonol 4′-reductase (DFR), which catalyzes the addition of a hydroxyl group on C-3′ in ring B, and the reduction of the oxo group at C-4 in ring C, respectively. Leucocyanidins can produce flavan-3-ols or anthocyanidins. Flavan-3-ols are produced by the action of the enzyme leucocyanidin reductase (LAR) that reduces the hydroxyl group at C-4 in ring C. Anthocyanidin formation requires the activation of the enzyme anthocyanidin synthase (ANS) that dehydrates leucocyanidins, removing the hydroxyl group at C-4 and forming a double bond in ring C. The last step in the flavonoid and anthocyanin pathway is anthocyanin biosynthesis, which consists of the anthocyanidin glycosylation by UDP-glucose:flavonoid 3-O-glucosyl transferase (UFGT).

12.3.4 Phenolic Compounds Transformation and Degradation

Polymeric PC can be synthesized from monomeric PC. Hydroxycinnamic acids are precursors in the biosynthesis of lignin. *p*-Coumaryl (*p*-hydroxyphenol), coniferyl (guaiacol), and sinapyl alcohols are the main monomers involved in the synthesis of lignin, and they are derived from *p*-coumaric, ferulic, and sinapic acids, respectively. Hydrolyzable tannins are synthesized from hydroxybenzoic acids (gallic and ellagic) which are esterified between them and with carbohydrate moieties, while proanthocyanindins are synthesized by condensation of flavan-3-ols.

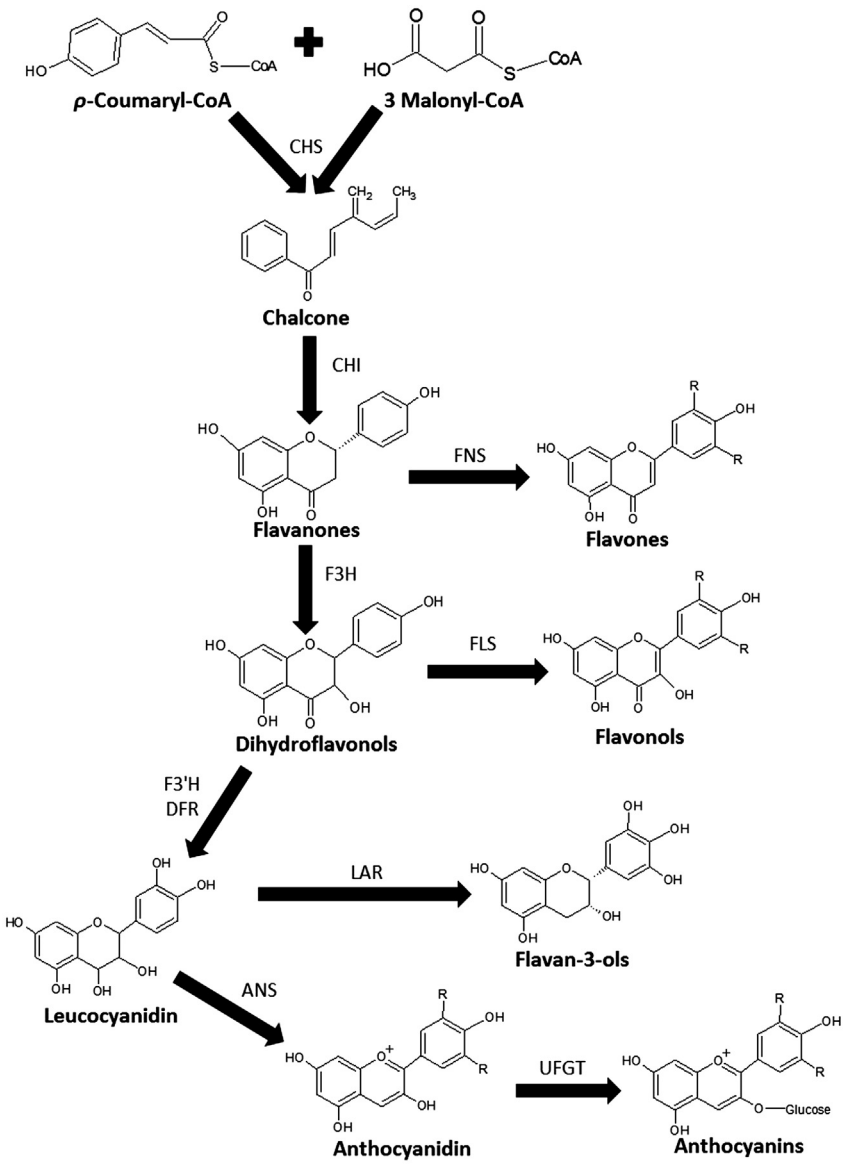


FIGURE 12.7

Biosynthesis pathway for flavonoid production in plants. *CHS*, chalcone synthase; *CHI*, chalcone isomerase; *F3H*, flavonone 3-hydroxylase; *F3'H*, flavonoid 3'-hydroxylase; *FNS*, flavone synthase; *FLS*, flavonol synthase; *DFR*, dihydroflavonol 4-reductase; *LAR*, leucoanthocyanidin reductase; *ANS*, anthocyanidin synthase; *UFGT*, UDP-glucose: flavonoid 3-O-glucosyltransferase.

PCs also suffer catabolic or degradation processes through enzymatic and non-enzymatic pathways. There are several enzymes, such as polyphenol oxidase (PPO), peroxidase (POD), laccase, and lipoxygenase that participate in their degradation. For example, PPO has two catalytic activities: the transformation

of monophenols into *o*-diphenols and the oxidation to the corresponding *o*-quinones, which then follows nonenzymatic reactions to produce melanin products. Nonenzymatic degradation reactions of PCs include the Maillard reaction between PCs and amino and carbohydrate groups. Other processes such as fermentation, may affect the PCs in fruits and vegetables.

12.4 INFLUENCE OF POSTHARVEST TREATMENTS ON PHENOLIC COMPOUNDS CONTENT IN FRUITS AND VEGETABLES

Since PCs are secondary metabolites with diverse protective roles, their synthesis is finely regulated by several stress signals and environmental factors. Therefore, different preharvest and postharvest practices can be critical to the accumulation or degradation of PCs in fruits and vegetables. Among preharvest conditions, temperature, soil properties, light irradiation, irrigation, fertilizers, harvest stage, among others, play an important role in the PC content of fruits and vegetables. During postharvest handling, wounding, temperature, modified atmosphere, light irradiation, and elicitor treatments are known to be used to regulate the content of PCs in fruits and vegetables. These technologies induce oxidative stress (formation of ROS in fruits and vegetables); this triggers the plant defense system, which involves the synthesis of antioxidant secondary metabolites such as PCs, and the activation of antioxidant enzymes (Fig. 12.8). The effect of the different postharvest technologies will be different for processed and nonprocessed fruits and vegetables. Normally, PCs are more bioavailable in processed products because they are released from the food matrix, but for the same reason they are degraded more rapidly. It is important to study the effect of postharvest treatments in the PC content of fruit and vegetable products, because even though an increase in PCs is desirable for their health-related benefits, an overproduction could result in a loss of sensorial quality. In this section, the effect of temperature, modified atmosphere, and irradiation on nonprocessed fruit and vegetable products will be discussed.

12.4.1 Effect of Temperature

Controlling the temperature of the products after collection and before their consumption is vital for the preservation of their overall quality. Depending on the fruit or vegetable, they can be stored at room temperature, refrigerated (4°C) or frozen (−20°C or −80°C), with or without bleaching (thermal treatment prior storage). When applying cold temperature, it is important to avoid temperatures that induce chilling injuries in the different crops. The effect of temperature on PC content will depend on the degree of tissue damage. All plant foods undergo some damage when being harvested (wounding stress); this triggers the production of reactive oxygen species, which activate the production of PCs through the phenylpropanoid pathway (activation of PAL). Normally, storage at higher temperatures is better to induce the accumulation

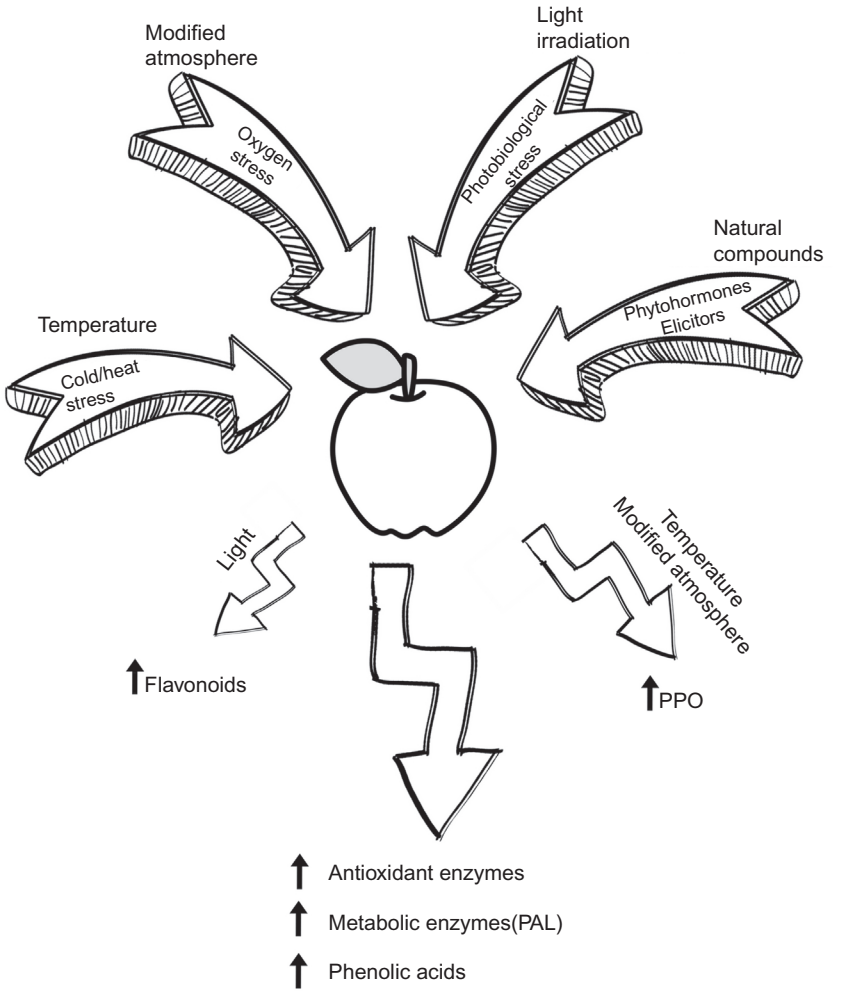


FIGURE 12.8
Increase in PCs by stress-inducing postharvest treatments in fruits and vegetables.

of these compounds, but their degree of accumulation will depend on the product and storage conditions, as well as the type of PC. This is because temperature can activate different defense mechanisms in addition to the phenylpropanoid pathway, including the activation of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APx)], and degradative enzymes (PPO), and depending on which mechanisms are triggered to a higher degree, an increase or decrease in PC content can be observed. For instance, anthocyanidins and chlorogenic acid content, found in berries, decrease at higher temperatures in a time-dependent mode, due to the activation of PPO and other metabolic responses such as respiration. The effect of temperature on the PC content is higher in the peel, where the highest

cold/heat stress is observed, while in the pulp their content remains practically unchanged.

12.4.2 Effect of Modified Atmosphere

Modified or controlled atmosphere (MAP) storage is a technology normally used in combination with low temperature to preserve the quality of fruits and vegetables during long-term storage. It consists of lowering the content of oxygen and/or carbon dioxide, by replacing them with nitrogen, carbon dioxide (for oxygen removal), or another inert gas, to control the growth of aerobic microorganisms and oxidative metabolic reactions. A high carbon dioxide atmosphere is known to produce several physiological changes during postharvest storage (decreased respiration rate and energy production), which may induce damage in cell membranes and enzymatic browning. It has been reported that carbon dioxide can regulate the activity of PPO and PAL enzymes, and consequently PC synthesis, oxidation, and tissue browning in a dose-dependent pattern. Carbon dioxide may also activate antioxidant enzymes (POD and CAT), that can accelerate the synthesis of PCs. Interestingly, the activation of these enzymes will depend on the maturity degree of the product. For example, it has been shown that in late-harvested apples, PAL activity increases during storage, while for early-harvested apples, this enzyme remained practically unchanged. This result may suggest that the metabolic stage of the produce plays an important role in the activation of the defense mechanisms.

Studies have also shown that when samples with low initial PC content are kept under passive MAP storage (when the produce is stored in a low permeability container, O_2 is reduced, and CO_2 is increased due to respiration), their final PC content increases to a much larger degree, compared to samples with higher initial PC content, in which the PC content remained practically unchanged. This is because PAL is activated in the low PC samples, resulting in an increase in PC biosynthesis, especially phenolic acids such as caftaric and chicoric acids. MAPs containing high O_2 concentrations may facilitate the oxidation and degradation of PCs; however, in combination with ascorbic and citric acid, it allowed for a better preservation of PCs, probably because ascorbic acid can reduce oxidized quinones back into PCs, while citric acid can inhibit the activity of PPO by reducing pH and chelating copper. It is also suggested that PAL activity is enhanced at high O_2 concentration.

12.4.3 Effect of Light Irradiation

Irradiation with ionizing (gamma rays) and nonionizing radiation has been extensively used as a postharvest technology to protect products against microbiological deterioration, and in this way, extend their shelf-life. It has been described that low doses of these irradiation sources into fruits and vegetables may increase the content of PCs by a phenomenon known as hormesis (low doses show a beneficial effect, while high doses show a

damaging effect). This accumulation of PCs is the result of plant defense against the photobiological stress, which induces the activation of the plant defense system, which involves both antioxidant enzymes (SOD, CAT, POD, APx) as well as enzymes involved in the synthesis of PCs (PAL).

UV light is one of the main sources of nonionizing radiation used for this purpose. UV energy can be divided according to its energy level from lower to higher energy in UV-A (315−400 nm), UV-B (280−315 nm), and UV-C (100−280 nm). The impact of UV light treatment on phenolic content and antioxidant capacity of fruits and vegetables depends on both the energy level (UV-C and UV-B show higher effect than UV-A), and the exposition time (at short irradiation times there is a dose−response effect in the PC content, but longer irradiation periods induced PC decline). UV-C and UV-B induced the synthesis of flavonoids and stilbenes in leaves, due to the activation of PAL, CHS, and stilbene synthase. This increase in flavonoid content has been linked to their property to reduce photobiological stress. Visible light can also affect the PC content of fruits and vegetables, for example, kale sprouts produced under blue light (470 nm) exhibited higher PC, anthocyanin, and antioxidant capacity than those grown in white (440−660 nm) or red (660 nm) light.

Gamma rays (10^{-12} m) are a form of high-energy radiation that induces atom ionization and that has been used at low doses to preserve fruit and vegetables against microbiological spoilage. Similar to UV radiation, gamma rays are known to trigger PC synthesis. Samples treated with small doses of gamma rays (0.25−1.5 kGy) show a dose-dependent increase in the content of total PC, due to the activation of PAL and also to the degradation of polymeric condensed tannins to oligomeric proanthocyanidins and monomeric flavan-3-ols.

12.4.4 Effect of Phytohormones and Natural Compounds

Another postharvest method used to modify the PC concentration is the use of phytohormones, as well as other natural compounds. As with other previously described postharvest treatments, these compounds were initially used to increase the shelf-life of fruits and vegetables; however, they increased the PC content of treated products due to the activation of antioxidant enzymes (SOD, CAT, APX) and metabolic enzymes (PAL, LOX). For example, in lettuce, preharvest and postharvest treatment with salicylic acid, Harpin protein, and methyl jasmonate elicited an increase in PCs, especially caffeic acid derivatives, triggered by diverse intracellular signals, such as an increase in ROS concentration, activation of membrane G-proteins, or cytosol acidification, all of which can activate phenylpropanoid biosynthesis. Similar responses have been observed by ethylene treatment.

Finally, a combination of several postharvest treatments showed a larger effect than the same treatments alone, for example, UV-C treatment combined with the use of natural compounds, combination of cold storage with UV

treatment, cold storage with modified atmosphere, and modified atmosphere with natural compounds, among others.

12.5 CONCLUDING REMARKS

PCs found in fruits and vegetables can be classified as monomeric or polymeric, flavonoids or nonflavonoids. Among nonflavonoids, the monomers hydroxycinnamic and hydroxybenzoic acids are the most relevant. Among monomeric flavonoids, flavones, flavonols, flavan-3-ols, and anthocyanidins are the main compounds found in fruits and vegetables. Polymeric PCs derived from phenolic acids (gallotanins and elagitanins) and flavan-3-ols (proanthocyanidins) are also reported in fruits and vegetables. PAL, CHS, and ANS are the main enzymes responsible for the synthesis of phenolic acids, flavonoids, and anthocyanidins, respectively. Postharvest treatments with temperature, modified atmosphere, ionizing and nonionizing radiation, and natural compounds and elicitors can induce an increase in the PC content in fruits and vegetables, mainly due to the activation of antioxidant (SOD, CAT, APx) and metabolic enzymes (PAL, LOX); however, they can also, under some conditions, increase the activity of enzymes that degrade PCs. The effect of the different treatments on the PC content is dependent on several factors, including treatment dose and time, type of fruit or vegetable, type of PC, and other characteristics of the produce including their metabolic state and initial content of PC. More research is needed to better understand the effect of these stressors in the different PC metabolic pathways, as well as to analyze the effect of the PC increase due to these treatments on the sensorial quality and shelf-life of the postharvest fruits and vegetables.

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CHAPTER 13
Lipids

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13.1 BRIEF DESCRIPTION OF LIPID CLASSIFICATION AND METABOLISM

13.1.1 Definitions and Nomenclature

The term lipid is extremely broad, and can be used to describe thousands of molecules with different chemical structures and functions. The most defining characteristic that is shared by all lipids, is that they are not soluble in water. Learning about lipids begins by considering the simplest and most abundant ones, which are fatty acids and triacylglycerols (TAGs). This can be further simplified, because a handful of them are the most relevant. The study of lipids requires a basic knowledge of chemistry; to aid beginners in understanding this chapter without requiring external sources, the chemical terms mentioned will be described using simple terminology.

Fatty acids are the simplest lipids, and are chemically defined as: a linear, nonbranched, nonpolar hydrocarbon chain that can be saturated or unsaturated, with a single carboxyl group at one end. In this definition, the term linear indicates that they are a chain with a defined beginning and end, that is, they do not contain any rings. Nonbranched means that there are only two ends present on the molecule. Nonpolar indicates that they do not dissolve in water, in contrast to polar molecules which do. A hydrocarbon chain indicates that they only contain hydrogen and carbon; the typical length is 16–22, but shorter and longer molecules can also be found. Saturated fatty acids (SFAs) contain no double bonds (also known as unsaturations), while unsaturated fatty acids contain at least one double bond; triple bonds are not found on fatty acids. Unsaturated fatty acids can be further classified as monounsaturated (MUFAs, monounsaturated fatty acids) if they contain a single double bond, or as polyunsaturated (PUFAs, polyunsaturated fatty acids), if they contain two or more double bonds. A carboxyl group (abbreviated as COOH or

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as COO^- in its ionized form) is chemically an acid group, and its presence is responsible for the term acid in fatty acid. Note that the carboxyl group contains one double bond between the carbon and one of the oxygens (C = O), this double bond is always present, but it does not count towards the number of unsaturations of the fatty acid, only carbon−carbon bonds (C = C) do. These simple rules and definitions have some exceptions, but they are rarely encountered, if at all.

Fatty acids have several names, ranging from trivial to highly systematic, in accordance with the intended audience. For example, the trivial name of a common fatty acid is oleic acid, and although this may be simple to remember and pronounce, it does not include any structural information about it. The same fatty acid can also be named *cis*-9-octadecenoid acid, a name that fully describes the chemical structure of the molecule, but requires fluency in chemical terms to thoroughly understand.

An alternative, and less complex, naming scheme requires only numbers to indicate the basic properties of a fatty acid: the m:n $\Delta^{x,y,z}$ system. Using this nomenclature, oleic acid or *cis*-9-octadecenoid acid is known as 18:1 Δ^9. This indicates the number of carbons in the chain (18 carbons), the number of double bonds (one in this case, but can also be zero, two, or more), and their position in the chain (Δ^9, in the ninth carbon relative to the carboxyl group). The delta (Δ) can be omitted for further simplicity, leaving only 18:1 as the "name" of the compound, and is never written when the fatty acid has zero double bonds. The position of the double bond can also be indicated relative to the last carbon. The carbon from the COOH group is the first, and the carbon on the opposite end is the omega (ω) or *n* carbon, regardless of the length of the chain. Thus, if a fatty acid is known as ω-3 (or *n*-3), this means that the first double bond is located on the third carbon relative to the noncarboxyl, or ω, end. The m:n $\Delta^{x,y,z}$, omega, and trivial nomenclature can be used to complement each other and to avoid chemical jargon.

Fig. 13.1 shows the structure of fatty acids and the various ways to name them.

Referring to fatty acids with numbers, instead of other names, is also useful because chain length and saturations (or lack thereof), are the main characteristics that define the properties of a fatty acid. For example, saturation indicates in what physical state we find the fatty acids: at room temperature, SFAs are solid, while unsaturated fatty acids are liquid. Regarding chain length: the longer the chain, the higher the temperature required to melt them into liquids, while shorter chains require less temperature to melt. Lipids that are solid at room temperature are known as fats, while lipids that are liquid at room temperature are known as oils. Fats consumed by humans usually come from animals (such as lard) and oils from plants (such as corn oil), which is why solid lipids are sometimes known as "animal lipids," and oils are known as "vegetable lipids." These phrases are common, but incorrect, because lipids from plants and animals both contain varying amounts of saturated and unsaturated fatty acids, and neither one is made up of 100% saturated or 100% unsaturated fatty acids.

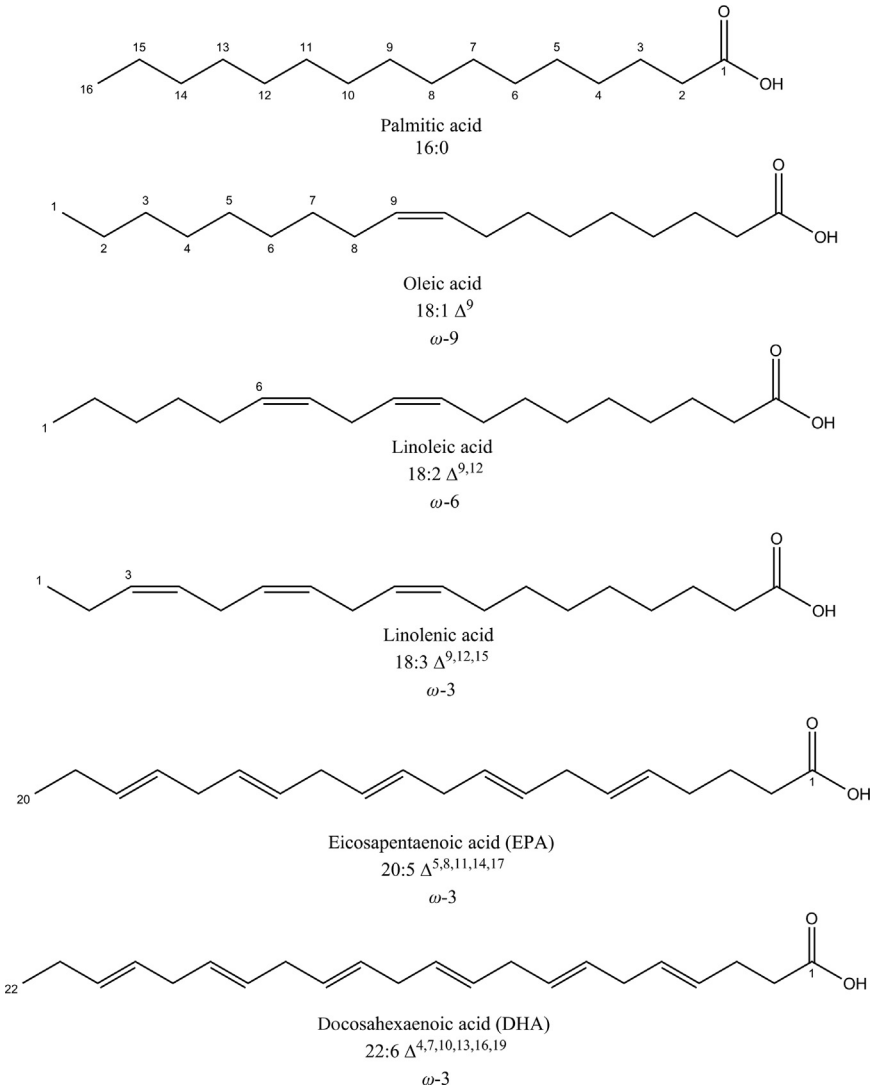

FIGURE 13.1

Chemical structures of fatty acids.

Fatty acids are linear (do not contain rings), nonbranched (have only two ends), nonpolar (do not dissolve in water) hydrocarbon chains (contain only hydrogen and carbon) that can be saturated (no double bonds in the chain) or unsaturated (at least one double bond in the chain) with a single carboxyl group (COOH). The carbon–oxygen double bond (C = O) of the carboxyl group is not considered when counting the unsaturations of a fatty acid, only carbon–carbon bonds (C = C). The trivial names can be substituted for numbers, indicating carbon numbers and double bonds present (16:0, 18:1, etc.). The presence of the first double bond can also be stated by specifying its position relative to the carbon opposite of the carboxyl group, the omega (ω) or *n* carbon.

Fatty acids are also classified as essential or nonessential to humans. An essential fatty acid is one that humans need for normal physiologic processes, but that is impossible for our bodies to make from any other molecule, or the amount that can be made is insufficient, and must be therefore acquired from the diet. A nonessential fatty acid is one that, if required, can be made from other molecules in sufficient amounts if not consumed from the diet. Essential fatty acids for humans are linoleic acid (18:2, ω-6) and α-linolenic acid (18:3, ω-3). Examples of nonessential fatty acids are palmitic acid (16:0) and stearic acid (18:0).

When fatty acids are not attached to any other molecule they are known as free fatty acids (FFAs). Some can be found as FFAs, but the majority are found chemically attached to a molecule of glycerol, in a three-to-one ratio. These molecules are known as TAGs or as triglycerides in older texts. The name exactly specifies their composition: three (tri-) fatty acids chains (-acyl-) and one glycerol (-glycerol). Related molecules are also frequently encountered, such as monoacylglycerols (MAGs) and diacyglycerols (DAGs), which have one or two fatty acids attached to glycerol. The chemical bond that links a fatty acid to glycerol is known as an ester bond (RCOOR′), or it can be said that fatty acids are esterified to glycerol.

TAGs are not a single molecule, but rather, a class of molecules. A particular TAG can have three identical 16:0 fatty acids attached to glycerol, another one can have two 16:0 and one 18:0, and yet another one can have one 16:0, one 18:0, and one 18:1, etc. When discussing lipids (in this chapter and elsewhere), it is usually referring to the specific fatty acids, but with the understanding that most of them are attached to glycerol as TAGs, and not free. Phospholipids, the main components of cell membranes, are closely related to TAGs, they have two fatty acids attached to glycerol, and a phosphate group in place of the third fatty acid.

Fig. 13.2 shows the molecular structure of glycerol, TAGs and ester bonds.

13.1.2 General Mechanism of Fatty Acid and Triacylglycerol Synthesis in Plants

The chemical reactions that lead to fatty acid and TAG synthesis are complex, and are also interconnected to other reactions that synthesize other types of lipids and related molecules. The process is described in this section and is illustrated in Fig. 13.3.

In plants, fatty acid synthesis takes place within plastids, a double-membraned organelle. The carbon atoms that are used for this purpose come from the simple sugars produced during photosynthesis, mainly glucose, which is then oxidized to pyruvate during glycolysis. Pyruvate is then converted to the two-carbon molecule acetyl, which is attached to a molecule of coenzyme A (CoA) to form acetyl-CoA; the enzyme pyruvate dehydrogenase catalyzes this reaction. A fraction of acetyl-CoA is transformed into malonyl-CoA by the enzyme acetyl-CoA carboxylase (ACC), which adds a third carbon to the acetyl group. The CoA

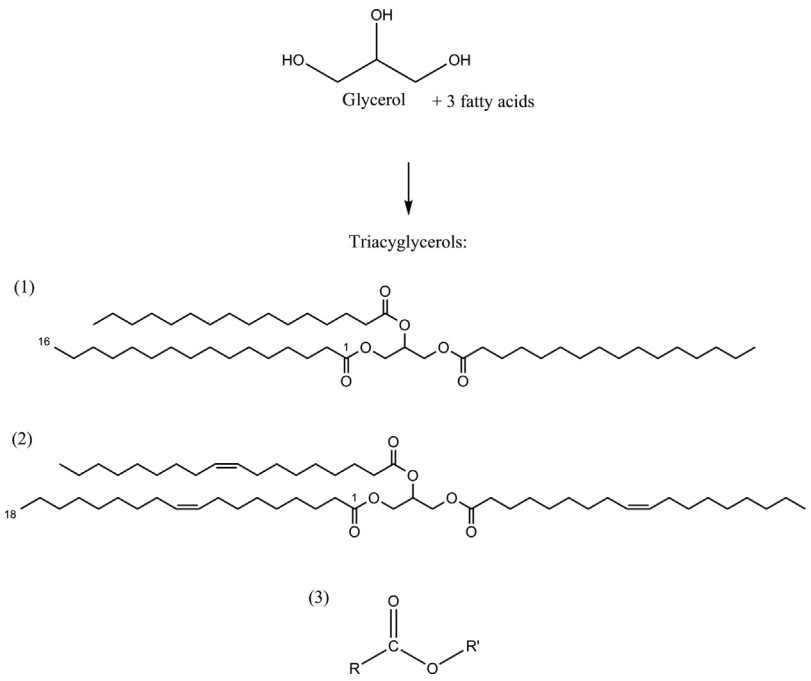

FIGURE 13.2

Chemical structures of TAGs.

Glycerol has three carbons where three fatty acids can bind to in order to produce a TAG or triglyceride molecule. Different combinations of fatty acids bound to glycerol will yield a particular TAG, in this example, the first (1) TAG has three 16:0 fatty acids, while the second one (2) has three 18:1 fatty acids. Fatty acids bind to glycerol through an ester bond RCOOR' (3).

molecules do not directly participate in the reactions, and can be thought of as transporters of both acetyl and malonyl. In order to proceed along the pathway, another transporter will take the place of CoA: the acyl carrier protein (ACP). The enzymes acyl-carrier-protein S-acetyltransferase and acyl-carrier-protein S-malonyltransferase attach the acetyl and malonyl groups to molecules of ACP. The products of these reactions are acetyl-ACP and malonyl-ACP.

Once acetyl-ACP and malonyl-ACP are both present, the first reaction attaches two of the carbons from the malonyl to the two carbons of the acetyl, producing a four-carbon precursor (the third carbon of malonyl is discarded as CO_2). The four-carbon precursor is subsequently rearranged by the enzyme fatty acid synthase (FAS), until a 4:0 fatty acid is formed, but that is still attached to ACP. These are the basic steps of fatty acid synthesis, which are then repeated once more, beginning with the incorporation of an additional molecule of malonyl-ACP. Therefore, the 4:0 fatty acid becomes 6:0, another repetition yields an 8:0 fatty acid, and the process concludes when a 16:0 or 18:0 fatty acid is formed. Because every step adds two carbons, the vast majority of fatty acids found in nature have even-numbered chain lengths.

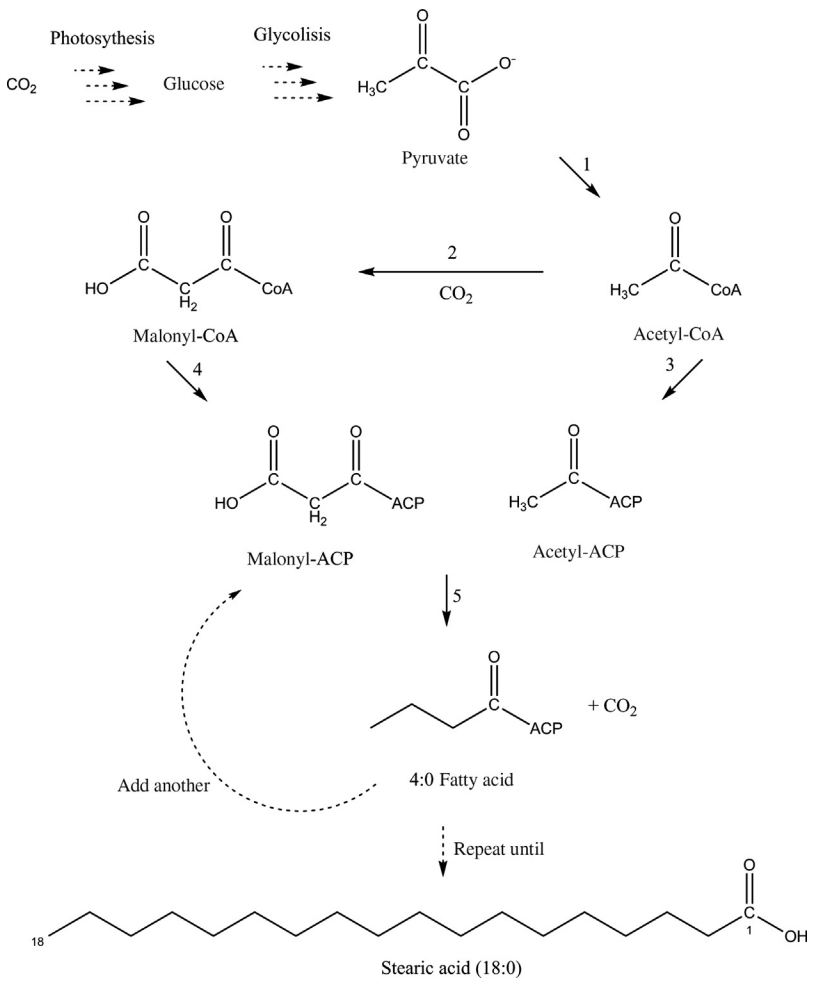

FIGURE 13.3

Fatty acid synthesis.

See Section 13.1.2 for a step-by-step description. Enzymes are represented by numbers: 1, pyruvate dehydrogenase; 2, ACC; 3, acyl-carrier-protein S-acetyltransferase; 4, acyl-carrier-protein S-malonyltransferase; 5, FAS.

The newly synthesized fatty acids can be directed to various other metabolic pathways, as illustrated in Fig. 13.4. One possibility is the introduction of a double bond to the 18:0 molecule to produce 18:1. This reaction is catalyzed by stearoyl-ACP Δ^9-desaturase. A second double bond can be introduced by a Δ^{12} fatty acid desaturase to produce 18:2, and a third double bond can be introduced by a Δ^{15} fatty acid desaturase to produce 18:3. 18:2 and 18:3 are of particular interest to humans, because, as previously mentioned, they are essential ω-6 and ω-3 fatty acids, respectively. The desaturases are contained within the plastid, which means that fatty acid synthesis and desaturation take

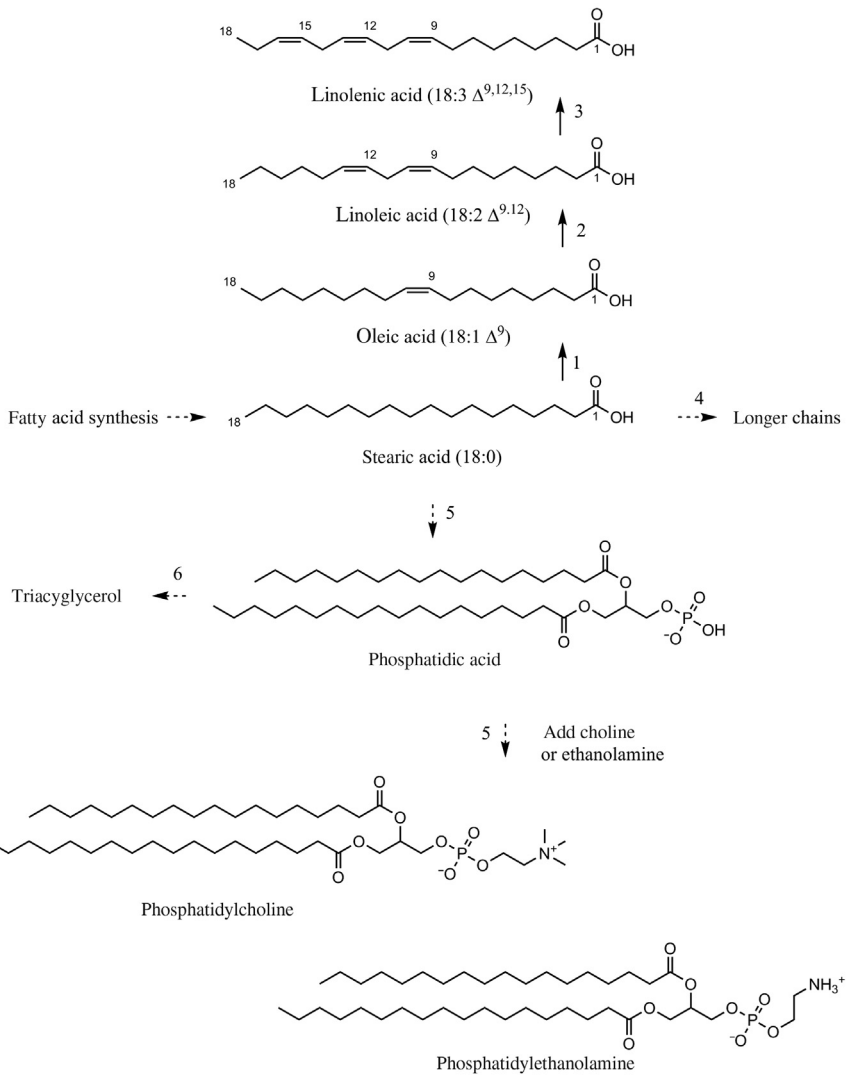

FIGURE 13.4

Reactions involving fatty acids.

See Section 13.1.2 for a step-by-step description. Enzymes and other pathways are represented by numbers: 1, stearoyl-ACP Δ^9-desaturase; 2, Δ^{12} fatty acid desaturase; 3, Δ^{15} fatty acid desaturase; 4, microsomal FAE; 5, transferases; 6, PAP and DGAT.

place in the same organelle. The chains can be further elongated to a length of 20 or more carbons, by the enzyme microsomal fatty acid elongase (FAE), which uses similar reactions to the main pathway, until the final length is achieved. Humans and other animals synthesize fatty acids in a similar manner to plants, but a key difference is the ability of plants to synthesize 18:2 and 18:3 fatty acids, which is not possible in humans because we lack Δ^{12} and Δ^{15}

desaturases, thus making it necessary for us to consume them from plants that do produce them.

Fatty acids can also be used to synthesize phospholipids or TAGs. In either case, it is first necessary to hydrolyze (separate) them from the ACP, which is done by the enzyme acyl-ACP thioesterase, which attaches a CoA in place of ACP. The acyl-ACP is then sent to the endoplasmic reticulum, where successive reactions attach two fatty acids to glycerol 3-phosphate by different transferases, yielding phosphatidic acid, the simplest phospholipid. If phospholipids are being produced, ethanolamine or choline are attached to the phosphate group, yielding phosphatidylethanolamine or phosphatidylcholine, respectively. If TAGs are being produced instead, the phosphate group is hydrolyzed by the enzyme phosphatidic acid phosphatase (PAP) to yield a DAG, to which the third and final fatty acid is attached by diglyceride-acyl transferase (DGAT), thereby producing a TAG molecule. The plant can use phospholipids for various purposes, but most are incorporated into the membranes of all cells. TAGs are mainly used as energy storage in the embryo, endosperm, or both.

Fatty acids and TAGs are highly energetic, requiring substantial energy investment by the plant. Humans who consume them can use that metabolic energy or store it in adipose tissue. The balance of storage and use of fatty acids as an energy source is a highly complex process, but in the absence of disease, diet is a key factor. Because the quality and quantity of consumed lipids are critical to human health, it is of great interest to clearly identify and quantify the types of lipids in fruits and vegetables, and how they will affect our health when consumed in the diet.

13.1.3 Other Lipids of Interest

From a nutritional perspective, plant foods do not contain cholesterol, but vegetable cells do synthesize minute amounts of cholesterol, which are negligible as compared to animal cells. Phytosterols are produced by plants in higher concentrations, and can be considered the vegetable equivalents of cholesterol, of which β-sitosterol, campesterol, and stigmasterol are the most prominent. Their synthesis also begins with acetyl-CoA, but requires additional and more intricate steps to reach a final product through the mevalonate or methylerythritol phosphate pathways, which take place in the cytoplasm or the plastid, respectively. Vitamins A, D, E, and K are also classified as lipids. Fig. 13.5 shows the molecular structure of cholesterol and phytosterols.

13.2 FRUITS AND VEGETABLES WITH LOW (<10%) TO MODERATE (10%–40%) LIPID CONTENT AND ASSOCIATED HEALTH EFFECTS

The definitions of fruits and vegetables used throughout the text are according to their culinary use and not their botanical meaning, for example, tomatoes

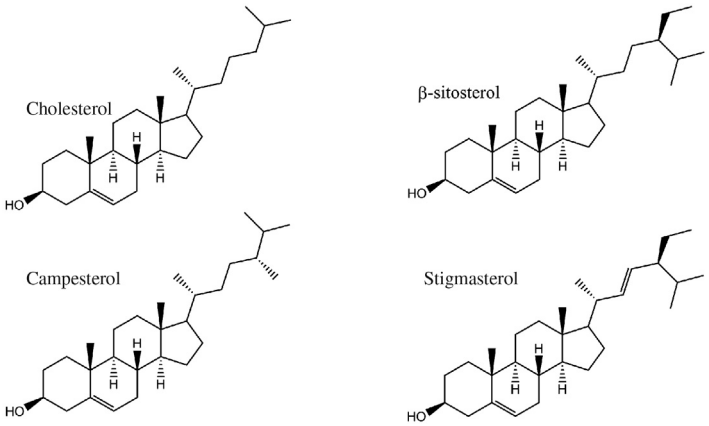

FIGURE 13.5
Molecular structure of cholesterol and the main phytosterols.

are botanically fruits, but are vegetables in the culinary sense. Nuts and legumes are also grouped within vegetables. Fruits and vegetables can be classified according to their lipid and water contents, which typically show an inverse correlation, in other words, low-lipid content indicates a high water content, and vice versa. Since this classification can be arbitrary, the intended meanings in this chapter are $<10\%$ for low, $10\%-40\%$ for moderate, and $>40\%$ for high-lipid content.

Selected examples of fruits and vegetables with low to moderate lipid content include apple, avocado, blackberry, blueberry, broccoli, carrot, celery, chia seeds, coconut, lettuce, mango, olive, pineapple, raspberry, spinach, strawberry, tomato, watermelon, and others; their macronutrient composition is detailed in Table 13.1.

Low-lipid items contain mostly structural lipids, such as those of the cell membrane, and have the minimum amount of lipids from any edible substance of vegetable origin. The majority of the mass is water (up to 95%), with sugars, fiber, and protein completing the macronutrient profile. Because water is the main component, the metabolic cost for the plant to generate these structures is low; consequently, low-lipid items have less than 100 kcal/100 g. The high water content also favors colonization and proliferation of microorganisms, like bacteria and mold, which limits their shelf-life when stored at room temperature. They are produced by the plant to attract animals to eat the fruit and disperse the seeds (apples or watermelon), others are the leaves or stalks of the plant and take part in photosynthesis (lettuce or celery), and others are part of the plant's root system (carrots).

Fruits and vegetables with moderate lipid content have storage lipids (TAGs) concentrated in the pulp or in the seeds. Their synthesis is therefore more energy-intensive for the plant, as compared to low-lipid foods. Lipids are

Table 13.1 Macronutrient Profile of Selected Fruits and Vegetables With Low (<10%) and Moderate (10%–40%) Lipid Content, Ranked in Increasing Lipid Percentage

	Lipids	Water	Sugars	Fiber	Protein	Energy
			g/100 g			kcal/100 g
Pineapple	0.1	86.0	9.9	1.4	0.5	50
Apple (no skin)	0.1	86.7	10.1	1.3	0.3	48
Watermelon	0.2	91.5	6.2	0.4	0.6	46
Lettuce	0.2	95.0	0.8	1.3	1.4	15
Celery	0.2	95.4	1.3	1.6	0.7	16
Tomato	0.2	94.5	2.6	1.2	0.9	18
Carrots	0.2	88.3	4.7	2.8	0.9	41
Strawberry	0.3	91.0	4.9	2.0	0.7	32
Blueberry	0.3	84.2	10.0	2.4	0.7	57
Broccoli	0.4	89.3	1.7	2.6	2.8	34
Mango	0.4	83.5	13.7	1.6	0.8	60
Spinach	0.4	91.4	0.4	2.2	2.9	23
Blackberry	0.5	88.2	4.9	5.3	1.4	43
Raspberry	0.7	85.8	4.4	6.5	1.2	52
Olive	10.7	80.0	0.0	3.2	0.8	115
Avocado	14.7	73.2	0.7	6.7	2.0	160
Chia seed	30.7	5.8	7.7	34.4	16.5	486
Coconut	33.5	47.0	6.2	9.0	3.3	354

Source: From United States Department of Agriculture, 2016. USDA Food Composition Databases. <https://ndb.nal.usda.gov/ndb/> (accessed 02.12.16.).

stored within oil bodies, structures that consist of a monolayer of phospholipids that surrounds a TAG core. They have increased caloric content (150–500 kcal/100 g) as compared to low-lipid items.

Table 13.2 lists the full fatty acid profile of fruits and vegetables with low to moderate lipid content. The main fatty acids present are 16:0, 18:0, 18:1, 18:2, and 18:3, but as the lipid content is higher than 10%, other fatty acids can also be found. Coconuts are the best example of this, because they contain a very high concentration of 12:0 that surpasses the concentration of all other fatty acids.

13.2.1 Health Effects

As previously mentioned, the intake of vegetable-derived lipids in the diet has important consequences for human health. In the case of low-lipid fruits and vegetables, these effects are mainly attributed to nonlipid molecules. Regardless, their consumption is highly recommended as part of a healthy diet, which has been thoroughly demonstrated by various studies. For example, consuming high amounts of these items can exert cardioprotective effects, that is, they can promote and maintain an optimal function of the heart and circulatory system in general (Mozaffarian et al., 2011).

Table 13.2 Fatty Acid Profile and Content of Phytosterols of Selected Fruits and Vegetables With Low (<10%) and Moderate (10%–40%) Lipid Content, Same Order as Table 13.1

	16:0	18:0	18:1	18:2	18:3	PS
			g/100 g			mg/100 g
Pineapple	0.005	0.003	0.012	0.023	0.017	6
Apple (no skin)	0.017	0.002	0.005	0.031	0.007	–
Watermelon	0.008	0.006	0.037	0.050	–	2
Lettuce	0.018	0.002	0.005	0.024	0.058	38
Celery	0.037	0.004	0.031	0.079	–	6
Tomato	0.020	0.008	0.030	0.080	–	7
Carrots	0.035	0.002	0.012	0.115	0.002	–
Strawberry	0.012	0.003	0.042	0.090	0.065	12
Blueberry	0.017	0.005	0.047	0.088	0.058	–
Broccoli	0.029	0.006	0.010	0.017	0.021	–
Mango	0.072	0.004	0.075	0.019	0.051	–
Spinach	0.049	0.004	0.005	0.026	0.138	9
Blackberry	0.012	0.003	0.044	0.186	0.094	–
Raspberry	0.016	0.004	0.059	0.249	0.126	–
Olive[a]	1.179	0.236	0.086	0.847	0.064	–
Avocado[b]	2.075	0.049	9.066	1.674	0.125	83
Chia seed[c]	2.170	0.912	2.203	5.835	17.830	–
Coconut[d]	2.839	1.734	1.425	0.366	–	47

PS, phytosterols. In addition to the common fatty acids listed, other fatty acids are present:
[a]*16:1.*
[b]*17:1, 20:1, 20:3.*
[c]*14:0, 15:0, 17:0, 20:0, 22:0, 14:1, 16:1, 20:1.*
[d]*6:0, 8:0, 10:0, 12:0 (the major fatty acid found in coconuts is 12:0, at 14.858 g/100 g, which is superior to all common fatty acids).*
Source: From United States Department of Agriculture, 2016. USDA Food Composition Databases. <https://ndb.nal.usda.gov/ndb/> (accessed 02.12.16.).

Consumption of more than five servings of raw vegetables per week can contribute to good asthma control. Fruits and vegetables with these effects include tomatoes, carrots, leafy greens, citrus fruits, fruit juices, vegetable juices, and others (Iikura et al., 2013). A healthy diet that includes high fruit and vegetable intake can also mitigate the risk of tumor and cancer development. This is likely through a combination of effects on the organism, such as reduced obesity, inflammation, and others, which are altogether anticarcinogenic (Ruiz and Hernandez, 2014). Fruit and vegetable consumption has also been correlated with a decrease in all-cause mortality (Oyebode et al., 2014).

13.3 FRUITS AND VEGETABLES WITH HIGH (>40%) LIPID CONTENT AND ASSOCIATED HEALTH EFFECTS

Selected fruits and vegetables with high-lipid content are mostly nuts and seeds, such as almonds, cashews, flaxseeds, hazelnuts, macadamia nuts,

peanuts, pecan nuts, pine nuts, pistachios, and walnuts. When lipids are the main components, the water content can be as low as 1%, sugar content is also low (<10%), and protein is high (up to 25%). Table 13.3 lists the full macronutrient profile of high-lipid items.

Table 13.4 lists the full fatty acid profiles of selected fruits and vegetables with high-lipid content. The fatty acid profile can vary widely, but MUFAs or PUFAs are the major components. When comparing the fatty acid profiles of low- and moderate-lipid items (Table 13.2) with the profile of high-lipid items (Table 13.4), it is evident that they are markedly different, for example, the concentration of phytosterols is significantly increased, which has important effects on human health. Also, the lengths of the fatty acid chains range from 6 up to 24 carbons in most items, whereas those of low-lipid content have only molecules of 16 or 18 carbons in length.

Low water content hinders the proliferation of microorganisms, thus favoring a significantly longer shelf-life of years when properly stored. Nevertheless, some microorganisms are still able to contaminate them and are potential health hazards, such as the molds *Aspergillus flavus*, *Aspergillus paraciticus*, and *Aspergillus nomius*, which can produce aflatoxin, a highly carcinogenic compound.

13.3.1 Lipid Oxidation

Lipids in these foods can spoil by oxidization in the presence of atmospheric oxygen, light, or heat. The double bonds present in MUFAs and PUFAs are chemically weaker than the single bonds of SFAs, and are therefore more susceptible to oxidation. Foods with oxidized lipids are known as rancid, and are

Table 13.3 Macronutrient Profile of Selected Fruits and Vegetables With High Lipid Content, Ranked in Increasing Lipid Percentage

	Lipids	Water	Sugars	Fiber	Protein	Energy
			g/100 g			kcal/100 g
Flaxseed	42.2	7.0	1.6	27.3	18.3	534
Cashew	43.9	5.2	5.9	3.3	18.2	553
Pistachio	45.3	4.4	7.7	10.6	20.2	560
Peanut	49.2	6.5	4.7	8.5	25.8	567
Almond	49.9	4.4	4.4	12.5	21.2	579
Hazelnuts	60.8	5.3	4.3	9.7	15.0	628
Walnuts	65.2	4.1	2.6	6.7	15.2	654
Pine nuts	68.4	2.3	3.6	3.7	13.7	673
Pecan nuts	72.0	3.5	4.0	9.6	9.2	691
Macadamia nuts	75.8	1.4	4.6	8.6	7.9	718

Source: From United States Department of Agriculture, 2016. USDA Food Composition Databases. <https://ndb.nal.usda.gov/ndb/> (accessed 02.12.16.).

Table 13.4 Fatty Acid Profile and Content of Phytosterols of Selected Fruits and Vegetables With High Lipid Content, Same Order as Table 13.3

	16:0	18:0	18:1	18:2	18:3	PS
			g/100 g			mg/100 g
Flaxseed[a]	2.165	1.330	7.359	5.903	22.813	146
Cashew[b]	3.916	3.223	23.523	7.782	0.062	122
Pistachio[c]	5.265	0.478	22.674	14.091	0.289	214
Peanut[d]	5.154	1.100	23.756	15.555	0.003	–
Almond[e]	3.083	0.704	31.294	12.324	0.003	139
Hazelnuts[f]	3.097	1.265	45.405	7.833	0.087	109
Walnuts[g]	4.404	1.659	8.799	38.093	9.080	121
Pine nuts[h]	3.212	1.390	17.947	33.15	0.164	152
Pecan nuts[i]	4.366	1.745	40.594	20.628	0.986	126
Macadamia nuts[j]	6.036	2.329	43.755	1.296	0.206	116

In addition to the common fatty acids listed, other fatty acids are present:
[a] *14:0, 15:0, 17:0, 20:0, 22:0, 24:0, 16:1, 20:1, 22:1, 24:1, 20:2.*
[b] *8:0, 10:0, 12:0, 14:0, 17:0, 20:0, 22:0, 24:0, 16:1, 20:1.*
[c] *6:0, 10:0, 14:0, 20:0, 22:0, 16:1, 20:1.*
[d] *14:0, 20:1.*
[e] *14:0, 17:0, 20:0, 22:0, 16:1, 17:1, 20:1, 20:2.*
[f] *20:0, 16:1, 20:1.*
[g] *20:0, 20:1.*
[h] *20:0, 22:0, 16:1, 20:1, 20:2, 20:3.*
[i] *20:0, 20:1.*
[j] *12:0, 14:0, 17:0, 20:0, 22:0, 24:0, 16:1, 22:1, 24:1.*
Source: From United States Department of Agriculture, 2016. USDA Food Composition Databases. <https://ndb.nal.usda.gov/ndb/> (accessed 02.12.16.).

easily perceived by humans, because the end-products of rancidity have strong unappealing sensorial characteristics, such as smell and taste.

The process of lipid oxidation is known in great detail and occurs in three phases: initiation, propagation, and termination. The process of lipid oxidation described in this section is depicted in Fig. 13.6. A hydrogen atom of a fatty acid is removed by light, heat, or other conditions in the initiation phase, typically adjacent to a double bond, and an alkyl radical is formed ($R^{\bullet}$). The alkyl radical reacts with oxygen in the propagation phase to produce a peroxyl radical ($ROO^{\bullet}$). The peroxyl radical is more reactive, and removes a hydrogen atom of another nearby fatty acid, in a reaction that generates two products: (1) the initially oxidized fatty acid becomes a lipid hydroperoxide (ROOH) with the hydrogen atom it removed and (2) a new alkyl radical from the second fatty acid whose hydrogen was removed, which restarts the process. The hydroperoxides are easily cleaved by the same conditions that initiated the oxidation (light, heat, etc.), which then produce alkoxy radicals ($RO^{\bullet}$). The newly formed alkoxy radical can remove a hydrogen atom of yet another fatty acid, restarting the process once more. Alkoxy radicals can reach the termination phase by inducing chain breakage, which generates compounds like malondialdehyde, 4-hydroxynonenal, pentane, or others. These are volatile

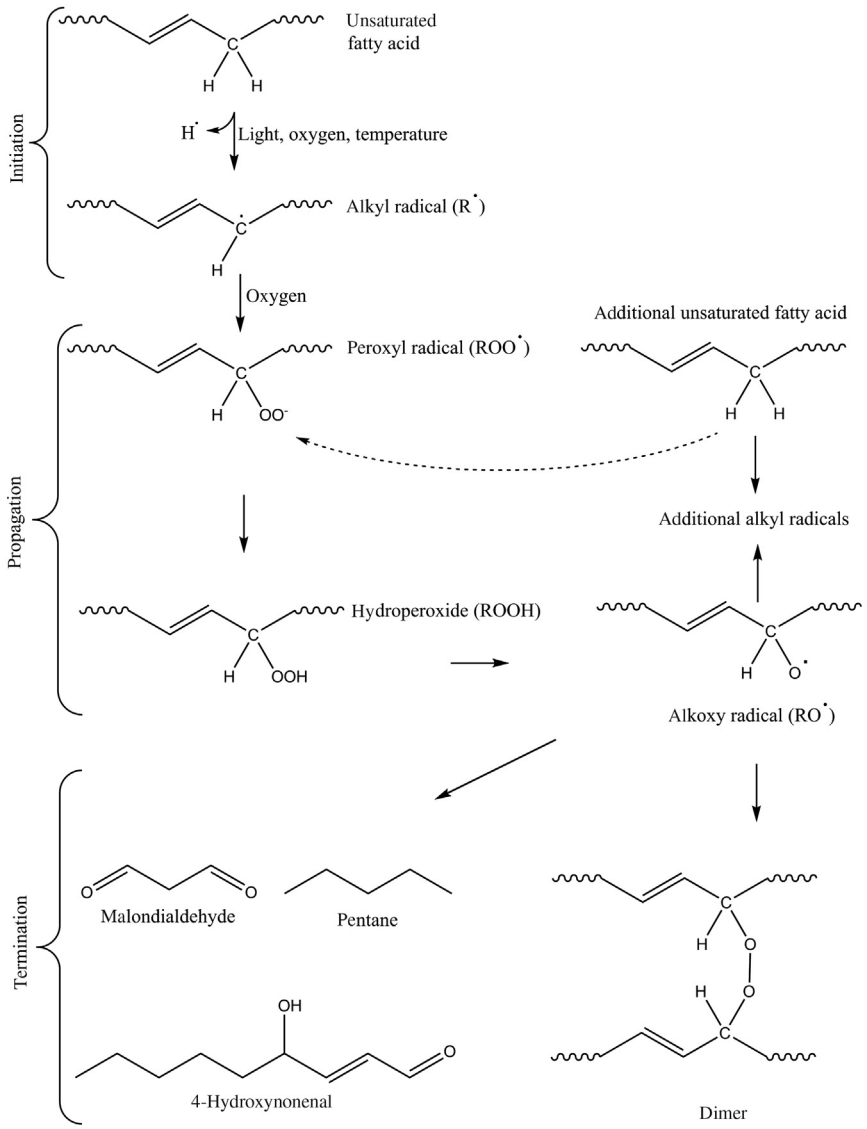

FIGURE 13.6

General mechanism of lipid oxidation.

See Section 13.3.1 for a step-by-step description. The reactions are focused on a double bond of the fatty acid, while the rest of the molecule is omitted, as indicated by the use of wavy bonds.

compounds of low molecular weight that are easily detected by our senses, and the food is now perceived as rancid. Two radicals can also react with each other and produce a single nonradical dimer, trimer, or oligomer. Antioxidants naturally present in these foods, such as vitamin E, stop or slow down these reactions, thereby contributing to an extended half-life.

Antioxidants can also be artificially added to various foods, in order to prevent lipid oxidation and increase their shelf-life. Storing foods under dry, cool, and dark conditions also prevents oxidation.

13.3.2 Health Effects

The health effects of consuming high-lipid items can be directly attributed to the lipids and to minor compounds dissolved in them. These molecules have a profound impact on the central nervous system and on the cardiovascular system. The brain has a high-lipid content of approximately 60%, and is therefore sensitive to dietary lipids throughout all stages of life. The cardiovascular system has direct contact with the lipoproteins that transport lipids to and from the organs via the blood, and it is also sensitive to dietary lipids.

Adequate brain development requires sufficient consumption of docosahexaenoic acid (DHA, 22:6 $\Delta^{4,7,10,13,16,19}$) and eicosapentaenoic acid (EPA, 20:5 $\Delta^{5,8,11,14,17}$) during pregnancy, because their deficiency cannot be compensated for postnatally, and is manifested in visual and behavioral impairments. Because of this, infant formulas include both compounds in order to prevent deficiencies (Zou et al., 2016).

The effects of fatty acid intake extend into adulthood. Dietary habits have been broadly categorized as Western diets and Mediterranean diets. The Western diet favors high consumption of saturated fats and refined sugars, along with reduced fruit and vegetable intake. Regularly consuming a Western diet has been shown to produce functional and anatomical variations of the central nervous system, such as depression and a reduced hippocampus (Jacka et al., 2015). In contrast, the Mediterranean diet includes high intakes of lipids, but mostly from vegetable sources like olives (and olive oil), avocado, nuts, and seeds. Studies involving individuals who consume the Mediterranean diet have shown a decreased risk for cognitive decline in the form of Alzheimer's disease and dementia (Lourida et al., 2013). Adherence to a Mediterranean diet is also associated with higher brain volumes in elderly adults, in contrast with those who do not consume a Mediterranean diet (Gu et al., 2015). The benefits also extend to a reduced risk for depression in all age groups (Psaltopoulou et al., 2013). Sufficient consumption of DHA and EPA seems to favor neurotransmitter-mediated brain signaling and adequate intracellular signaling (Haast and Kiliaan, 2015).

The effects on the cardiovascular system are related to long-term effects on cholesterol, TAG, and lipoprotein concentration. As previously mentioned, plant foods contain negligible or null cholesterol concentration, but they do contain phytosterols, both of which compete to be absorbed in the digestive system due to their similar structure (Fig. 13.5). A phytosterol-rich diet can decrease total serum cholesterol in a period of weeks or months, and can aid in maintaining these low values throughout life if regularly consumed (Abumweis et al., 2014). Because fatty acids are absorbed in their entirety, their effects are relevant when incorporated into lipoproteins and in body

organs. First, the MUFAs and PUFAs from high-lipid items tend to decrease low-density lipoprotein (LDL, commonly referred to as bad cholesterol) concentration and TAG concentration (Del Gobbo et al., 2015). Second, tissues will store less lipids, which results in decreased body weight and waist circumference (Damasceno et al., 2013). Although these effects are generalized among various nuts and high-lipid vegetables, particular items contain certain fatty acids not found elsewhere. For example, pine nuts contain pinolenic acid (18:3 $\Delta^{5,9,12}$), which can reduce inflammation, appetite, body weight, serum lipids, and exert other benefits (Xie et al., 2016). The overall outcome of the previously mentioned effects is a decrease in atherosclerosis and cardiovascular disease (Urpi-Sarda et al., 2012).

The fatty acid profile of foods of vegetable origin has a positive impact on the nervous and cardiovascular systems, but the benefits have been documented on the individual as a whole. It should also be noted that lipids of vegetable origin are generally considered healthier than those of animal origin, but because lipids are highly energetic, an excess of either is still detrimental. A moderate intake of vegetable lipids and low intake of animal and processed lipids can have significant benefits on human health throughout life (Chen et al., 2016).

13.4 VEGETABLE OILS

Vegetable oils are the most concentrated form of lipids, and can be extracted from the pulp (e.g., avocado pulp) or the seeds of an edible plant. Selected examples include canola, corn, cottonseed, coconut, flaxseed, grape seed, olive, sesame, and sunflower oils.

Table 13.5 lists the fatty acid profile of vegetable oils. The fatty acid profile of oils is characteristically rich in MUFAs and/or PUFAs; coconut and cottonseed oil are notable exceptions, as they contain substantial percentages of saturated lipids.

Vegetable oils are used for several purposes, such as human or animal consumption, as industrial energy sources, in cosmetic products, pharmaceuticals, etc., and there are also different methods available to obtain them. One of the simplest methods is pressing, which can be cold or hot pressing. Cold pressing is done at low temperatures ($<40^{\circ}C$), uses no solvents, and requires only mechanical presses to extract the oil. Among the advantages of cold pressing is reduced oxidation, it is an environmentally friendly process, and it preserves the original aroma, taste, and nutritional components. Some disadvantages include lower oil yield, inconsistent sensory characteristics from different sources or harvest seasons, and higher average prices. Hot pressing can increase oil yield and decrease prices, but changes or losses in sensorial parameters or nutritional components can take place.

Solvent extraction is done by adding organic solvents to the oil source and separating them from the remaining matter. Solvent extraction is highly efficient, up to 99% or more of the oil can be recovered, but the solvents used

Table 13.5 Fatty Acid Profile of Selected Vegetable Oils

	16:0	18:0	18:1	18:2	18:3	PS
			g/100 g			mg/100 g
Canola[a]	4.298	2.087	61.744	19.005	9.137	657
Cottonseed[b]	22.700	2.300	17.000	51.500	0.200	324
Coconut[c]	8.636	2.516	6.253	1.683	1.676	86
Flaxseed[d]	5.109	3.367	18.316	14.327	53.368	334
Grape seed[e]	6.700	2.700	15.800	69.600	0.100	180
Olive[f]	11.290	1.953	71.269	9.762	0.761	221
Safflower	4.282	1.915	14.355	74.623	–	444
Sesame[g]	8.900	4.800	39.300	41.300	0.300	865
Sunflower	5.900	4.500	19.500	65.700	–	100

In addition to the common fatty acids listed, other fatty acids are present:
[a]*20:0, 22:0, 16:1, 20:1.*
[b]*16:1, 20:4.*
[c]*4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 15:0, 17:0, 20:0, 22:0, 24:0, 16:1, 20:1 (the major fatty acids found in coconut oil are 12:0 and 14:0, at 41.840 and 16.653 g/100 g, respectively).*
[d]*10:0, 12:0, 14:0, 15:0, 17:0, 20:0, 22:0, 24:0, 14:1, 16:1, 22:1, 24:1, 20:3, 20:3, 22:4.*
[e]*14:0, 16:1*
[f]*17:0, 20:0, 22:0, 16:1, 17:1, 20:1.*
[g]*16:1, 20:1.*
Source: From United States Department of Agriculture, 2016. USDA Food Composition Databases. <https://ndb.nal.usda.gov/ndb/> (accessed 02.12.16.).

(typically hexane) are highly toxic and must be thoroughly eliminated before the oil can be used. The remaining oil in vegetable matter that was initially cold pressed can be subsequently reextracted with solvents to minimize waste.

If no additional process is done to the oil, it is known as unrefined or virgin. Refined oils undergo further treatments to obtain a product of uniform characteristics. The process removes different components such as phospholipids, proteins, volatiles, waxes, etc., and the resulting oil is odorless, clear, stable, and with a fixed pH. Potential negative consequences from refining include *cis–trans* isomerization, which is a conformational change of the unsaturated fatty acids into *trans*-fatty acids. *Trans*-fatty acids have the same chemical composition as the *cis*-fatty acids produced by the plant, but their spatial orientation is different. Consumption of *trans*-fatty acids formed during industrial processing is considered detrimental to human health, particularly to the cardiovascular system (Dawczynski and Lorkowski, 2016), but fortunately, the problem has been recognized and addressed by the industry, and modern procedures minimize the generation of *trans*-fatty acids during oil refining (Aldai et al., 2013).

13.4.1 Health Effects

Because oils are >99% TAGs, their health effects are largely attributed to the fatty acids, but other dissolved molecules (such as vitamins or phytosterols)

are also bioactive and can contribute to the overall effects of the oil. A comparative study analyzed the effects of grape seed, corn, and coconut oils on healthy rats (Wall-Medrano et al., 2017), and found that coconut oil increased total cholesterol and TAGs in the serum and liver, and decreased serum HDL cholesterol. The collective effects of this study indicated that grape seed oil promoted changes related to the reverse cholesterol transport (cholesterol excretion) of the rats, but coconut oil had an opposite effect. Part of this effect is related to the vastly different fatty acid profiles of the oils (Table 13.5), which is rich in SFAs in the case of coconut oil, and rich in PUFAs in the case of grape seed oil; but grape seed oil also contains various antioxidants (such as polyphenols) that can synergize with the fatty acids.

Similar evidence has been obtained when analyzing the pattern of lipid consumption in humans. For example, if saturated fats or oils are replaced with oils rich in oleic acid, total and LDL cholesterol can significantly decrease; and if oils rich in *trans*-fatty acids are replaced with oils rich in oleic acid, total cholesterol, LDL cholesterol, TAGs, and other parameters can significantly decrease (Huth et al., 2015). This shows that the metabolic and physiological effects of vegetable oils can be different, and the evidence supporting this fact can be obtained through animal models and through epidemiological analysis in humans.

Another important factor to consider is the content of ω-6 (18:2, linoleic acid) and ω-3 (18:3, linoleic acid) fatty acids, as well as their ratio. The metabolism of fatty acids in humans is complex, but in general, the products of ω-6 metabolism are inflammatory, and the products of ω-3 metabolism are antiinflammatory. High consumption of oils rich in ω-6 (corn, cottonseed, safflower, sunflower) can promote an inflammatory state, favor the generation of adipose tissue, increase TAG concentration, decrease fatty acid oxidation, increase thrombus formation, increase waist circumference, alter hormone secretion, etc. In contrast, oils rich in ω-3 (canola, chia, flaxseed) can have the opposite effects (Simopoulos and Dinicolantonio, 2016). Mankind evolved consuming diets that had a low ratio of ω-6 to ω-3, but modern dietary patterns have dramatically increased this ratio, and the surplus of ω-6 cannot be converted to ω-3. This altered ratio promotes weight gain and generalized changes that induce cardiovascular disease and related conditions that are highly prevalent in modern societies (Simopoulos, 2016).

13.4.2 Vegetable Oils as Potential Fuel Sources

One final note on vegetable oils is that their energy can be used as a renewable source of fuel, known as biodiesel. Biodiesel can be made from almost any vegetable oil, but nonedible oils or waste cooking oils are preferred to avoid food versus fuel competition. Serious consideration and effort into biodiesel has been made since the 1980s, but progress has been slow. Some challenges remain that have to be fully solved in order for biodiesel to become a viable energy source in the transport industry, such as economic viability, thorough knowledge

of its impact on food production and food prices, adequate land use, etc. The ultimate goal of biodiesel is to decrease dependence on fossil fuels and to decrease emissions of greenhouse gases, but until the current setbacks are overcome, vegetable oils are mostly used for human consumption (Aghbashlo and Demirbas, 2016).

13.5 CONCLUSIONS

Lipids are highly diverse molecules that are insoluble in water. The simplest lipids, fatty acids, are also the most abundant, and are found as TAGs. TAGs are a concentrated form of storage energy, which require significant energy investment from the plant. Phospholipids are chemically similar to TAGs, but are mainly used as structural lipids in the cell membrane. Humans consume vegetable lipids in foods or as vegetable oils, and their composition has important effects on our health. In particular, we cannot produce linoleic acid (18:2 ω-6) or linolenic acid (18:3 ω-3), which makes them essential fatty acids that must be consumed from vegetable sources, in order to maintain our health. Vegetable lipids can also be an adequate source of other health-promoting micronutrients (such as phytosterols) that can contribute to cognitive, cardiovascular, and overall wellbeing. Finally, vegetable lipids can also be used as fuels (biodiesel), but at the time, this is not a major application. The study of lipids from edible plants must be done without forgetting that they will be ultimately consumed by humans who seek basic nutrition from them, as well as maintaining and improving their health.

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CHAPTER 14
Texture

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HIGHLIGHTS

- Postharvest changes involve three stages: maturation, ripening, and senescence.
- Softening is caused by enzymatic ripening occurring before and after harvest.
- Texture can be evaluated by instrumental (destructive and nondestructive protocols) and sensory methods.
- Appearance and texture are the most important attributes for consumer acceptability of fruits and vegetables.

14.1 INTRODUCTION AND COMPONENTS OF THE TEXTURE IN FRUITS AND VEGETABLES

The texture of a fruit or vegetable is an essential, complex property determining consumer acceptance and informing consumers about the structure and quality of the product. An inappropriate texture will lead to an absolute

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rejection by consumers; for instance, a too soft and woolly peach will inform consumers of a too advanced ripening stage or inappropriate postharvest storage. Thus, it is essential to understand the physiology and biochemistry of fruits and vegetables during postharvest to ensure proper texture attributes for each product. *There is not a "general" optimal texture*, specific texture attributes are required for each fruit/vegetable; for instance, a high level of juiciness is appropriate for oranges, but it is totally inappropriate for almonds.

It is well known that the cell wall elasticity and force, the turgor, the cell bonding and density, among other factors, will determine the texture of a fruit/vegetable, therefore, it is essential to understand how the biochemical changes, happening during postharvest, affect them. This aspect will ensure that high-quality fruits/vegetables reach the final consumer.

As a summary, during postharvest different biochemical reactions will affect the physiology of fruits and vegetables, being finally reflected in the texture attributes, their quality, and consumer acceptance.

14.1.1 Definition of Texture

Finding a proper definition for "fruit texture" is not an easy task because this complex property is composed of many different attributes, which are mainly affected by the cell wall composition, tissue type, water content, water stress, etc. Texture can be defined as the deformation or fragmentation of a fruit/vegetable when a force is applied, and can be perceived by both the sense of touch (hand and mouth), and instrumentally by destructive and/or nondestructive methods. Another definition of texture can be "the sensory manifestation of the structure or inner structure of a fruit/vegetable, in terms of type of cells, reaction to stress, and amount and type of moisture/oil" (Meilgaard et al., 2006).

In the 21st century consumers are demanding and want to eat/drink products of the highest possible quality, and are willing to pay high prices for these commodities; thus, it is very important to control, optimize, and ensure the desired texture for fruits/vegetables. Consumers are willing to pay a reasonably high price for a crunchy organic carrot, but their willingness to pay will be reduced if the carrot is withered and poorly washed. Then, for optimal acceptability of fruits and vegetable is vital to assure a high-quality and proper product texture.

The evaluation of fruit/vegetable texture can be conducted using objective methods, including imitative methods, such as a texture-meter, or by subjective methods, when the subjective opinion of consumers is sought. Even when instrumental objective methods are used for the texture evaluation, there are destructive and nondestructive protocols, as will be discussed later in this chapter.

14.1.2 Texture and Its Components

Texture is a complex property and includes many different attributes. These attributes can be classified into three groups: geometrical, mechanical, and compositional (Meilgaard et al., 2006; Vázquez-Araújo et al., 2012).

The first group is represented by the *geometrical attributes* and is related to the size, shape, and organization of the cells or particles of the fruit and vegetables. This group includes, among others, the following attributes:

- *Smoothness* is the absence of any type of particles (no particles). Example: Greek yogurt.
- *Grittiness* is the presence of small but hard particles. Examples: polenta or poorly washed carrots.
- *Graininess* is the presence of small particles. Example: grain bread.
- *Chalkiness* is the presence of fine particles. Example: some strawberry yogurts.
- *Fibrousness* is the presence of long and stringy particles; they are better perceived after five to eight chews (long particles). Examples: celery and asparagus.
- *Lumpiness*: presence of large and even pieces or protrusions. Example: some baby foods.
- *Mealiness* is the perception of fine and smooth particles equal distributed in the product. Example: wooly peaches, improperly stored.

The second group of *mechanical attributes* informs about the response of a fruit or vegetable to different types of forces or stress (cutting, compression, etc.). This group can be divided in two subgroups "primary" and "secondary". The main "primary" mechanical attributes are listed and defined as follows:

- *Hardness* is the force needed to reach a certain deformation or the force necessary to compress completely the food with the molar teeth.
- *Cohesiveness* is the degree of sample deformation before breaking or the degree to which a product is compressed between the teeth before breaking.
- *Denseness* is the compactness of the cross-section of a fruit or vegetable.
- *Springiness* is the rate of a deformed product to return to the initial condition after the deformation force is removed or the degree to return a product to the original condition after teeth compression.
- *Adhesiveness* is the force required to remove a sample from a surface or the effort to remove a sample from the palate using the tongue.
- *Crunchiness* is the force needed to break a product (instead of deforming) using the molars. Usually the crunchiness is related to fresh foods, such as fruits and vegetables, while for snacks or chips the term used is *crispiness*.

Meanwhile, the "secondary" mechanical attributes which depend, at least, on two primary attributes, are listed below:

- *Fracturability* is the force necessary to rupture a product after the first bite using molars. To have a product with high fracturability, a combination of high hardness and high friability is required, this is a hard product that breaks into many pieces.
- *Chewiness* is the energy required to chew a product until is ready to be swallowed or it can be defined as the number of chews used to prepare the product to be swallowed. Chewiness is a combination of hardness, cohesiveness, and springiness.

Finally, the third group of *compositional attributes*, also known as moisture/oil parameters, informs about the presence, release, and absorption of water and/or oil. This group includes, among other, the following attributes:

- *Juiciness* is the amount of liquid released from the fruit/vegetable after, for example, two bites with the molars. Example: banana (low juiciness) and watermelon (high juiciness).
- *Succulence* is the release of high amounts of water from the fruit/vegetable when being chewed. Example: the cladodes of *Opuntia ficus-indica*.
- *Oiliness* is the release of oils or essential oils from the fruit/vegetable during its manipulation. Example: orange peel.

14.2 STRUCTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL CHARACTERISTICS OF LIVING CELLS IN FRUITS AND VEGETABLES

14.2.1 Types of Cells

A fleshy fruit is composed mostly of parenchymatous tissue specialized in water and organic compound accumulation, leading to its characteristic juiciness. The *parenchyma cells* vary in shape and elongation rate. They are characteristically living cells surrounded by a general thin wall controlling cell shape and the relation between turgidity and volume.

Parenchyma cells are usually depicted as the typical unspecialized plant cell but they are the best storage for organic products. As they are composed of photosynthates, they are the machinery for plant metabolism. In the mesophilic tissue of leafy vegetables, the photosynthesis is intensive and the parenchyma cell contains numerous chloroplasts (called chlorenchyma); the extensive development of intercellular spaces denotes the active gaseous exchange that also represents this type of vegetable.

In fleshy fruit, *ripening* is an event involving dramatic changes in parenchyma cells and represents the most intensively investigated process of postharvest fruit physiology. Among changes accompanying ripening, *softening* is

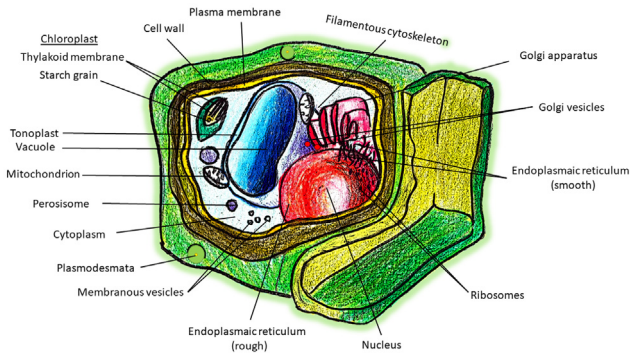

FIGURE 14.1
Cell structure. *Drawn by Mr. José Antonio Granero Vicente.*

considered the second phenomenon distinctive of the progressive hormonal-driven processes (Barry and Giovannoni, 2007).

The modification of parenchymatous wall structure and dynamics during ripening contributes to cell swelling for seed dispersion. *Ethylene* plays the most significant role as an inductor of metabolic changes at the level of solubilization and depolymerization of cell wall components, that is, neutral sugars size, composition, and rearrangements (Barry and Giovannoni, 2007; Fig. 14.1).

Cell wall classification. The cell wall is usually classified in terms of function as primary or secondary walls. The primary wall is a heterogeneous structure composed by pectins, hemicelluloses, and cellulose, as well nonpolysaccharide compounds such as proteins, phenolic acids, minerals, and lipids. The fibrillary network of primary wall is assembled by the hemicellulose–cellulose matrix linked together, whereas pectin is the highly hydrated matrix embedding the fibrillary network. The dense layer of pectins arranged along the edge of fibrillar structures for wall coating, neighboring cell binding, and apoplastic porosity is microscopically observed as a well-defined lamellae region. In the secondary cell wall, the pectin is substituted by lignin and is deposited between the primary cell wall and plasma membrane when the cell stops the expansion, rendering support, strength, and protection (Carpita and McCann, 2015; Jarvis et al., 2003).

14.2.2 Cell Wall: Strength and Elasticity

Under microscopic observation, parenchyma cell walls are viewed as a thin layer surrounding plant cells. The conjunction of the primary wall with protoplast is capable of producing a mechanically strong but elastic network, determining the unique structural characteristics of cell volume and cell shape regulation.

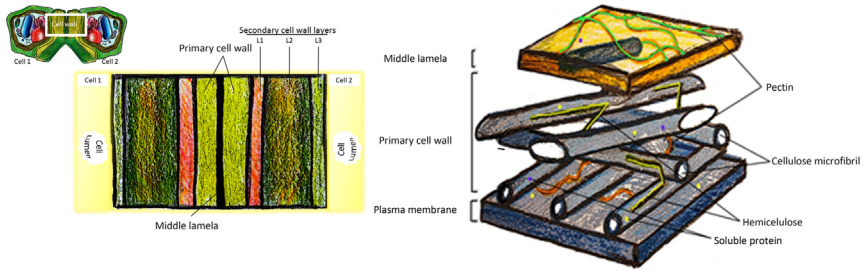

FIGURE 14.2
Cell wall structure. *Drawn by Mr. José Antonio Granero Vicente.*

The cell wall strength is influenced by intrinsic factors, such as the tight association of polymers, chain conformation, presence of proteins with "glue" properties, interactions with phenolic compounds, and the presence of ionic-bonded minerals. An estimation of mechanical properties of cell walls gives an understanding of structure; measurements of *hardness*, *firmness*, and *elasticity* are common quality-related attributes in postharvest studies used to relate mechanical properties to fruit texture. On the other hand, fruit tissues have different patterns of elastic behavior, and this property changes over the ripening process. There is a negative relationship between the *elasticity* (able to resume its normal shape spontaneously after an effort, compression, or bite) and the *plasticity* (able to undergo permanent deformation under an effort, compression, or bite). *Immature "hard" fruit possesses*, in general, *a highly elastic but slightly plastic behavior*; while *ripe "soft" fruit is only slightly elastic and very plastic*. Understanding the plant cell walls through the use of valuable tools and methods will provide critical information about the physical and biochemical properties (Fisher and Bennett, 1991; Fig. 14.2).

14.2.3 Cellular Arrangement, Density, Bonding, and Turgor

The cellular organization and distinctive characteristics are a function of plant tissues and the specialization level. In this chapter, the description will be devoted to the parenchyma cells as the representative cell type of fleshy tissues.

The *parenchyma cell* is frequently arranged in a spherical or cylindrical shape and is subjected to constant tension toward the corners of cellular joining, known as tricellular junctions (usually conformed by three cells). Molecules specialized in cell-to-cell adhesion are extremely important for matrix bridge regulation. Amorphous polysaccharides are arranged in layers to form the cements of neighboring plant cells. Cell adhesion is associated with the lamella media that in a simple definition is the region of the extracellular matrix where cellulose microfibrils are absent. The main macromolecules in the lamella media are pectins and proteins.

Other forms of parenchyma cells might be found, but in general they are not much longer than wide, arranging close to an isodiametric form. Factors such as cell density or tissue developmental stage influence the occurrence of changes in shape (Esau, 1977).

During parenchyma cell growth an irreversible change occurs in volume that might implicate the modification of cell size in more than one dimension or the elongation in one dimension. Under this condition, the protoplast drives turgor pressure over cell walls driven by osmotic forces. In the sense of hydrostatic pressure, the *turgor* is the force exerted by intracellular liquids over the cellular membrane. Under weak cell wall assembly, the intake of large amounts of water might cause cracking, whereas a phenomenon known as *plasmolysis* can occur under an excessive loss of water or the simple cell exposition to hypertonic solutions (causing the protoplast to shrink to a volume smaller than the enclosed space) (Hopkins and Hüner, 2009). Therefore, *cell walls are responsible for turgor, pressure maintenance, indicating that a close relationship between turgor and the mechanical properties of the cell wall clearly exists*. This feature is of significant importance in texture parameters of juicy fruits and vegetables.

14.2.4 Pectic Substances, Hemicellulose, Cellulose, Proteins, and Lignin

The components of the cell wall have been the subject of constant study, in part because of the implications of the postharvest quality of fruits and vegetables. Several models have been proposed to describe the spatial arrangement of cell wall components, as well as their interactions; however, the main limitation is the scarcity of techniques for the study in situ for the appropriate definition of structure and interrelationship instead of the study of chemically extracted and isolated components. The major components of cell walls are macromolecules and they vary among species, cell type, developmental stage, and cell position. The primary wall of parenchyma cells is composed of polysaccharides, pectin, hemicellulose, and cellulose. Other important macromolecules cementing or connecting polysaccharides are proteins and lignin (Carpita and McCann, 2015).

Pectin. The pectin group consists of diverse polysaccharides consisting of acidic and neutral sugars. The main component of pectin is galacturonic acid, which is present as free acid or methyl esterified, depending on its function and developmental stage. The pectic matrix is usually classified into two regions based on the arrangement of polysaccharides: the smooth and the hairy region. The smooth region contains mainly a pectin type known as homoglacturonan, which is composed of $(1\rightarrow4)$ α-galacturonic acid and might polymerize approximately until 200 units. Galacturonic acid is commonly esterified with methanol at carbon 6 and substituted at carbon 2. The hairy region is more flexible than the smooth region and is composed of the rhamnogalacturonan types I and II. Rhamnogalacturonan

type I contains repeated disaccharide (1→2) α-D-rhamnose-(1→4)α-D-galacturonic acid at position C-4 of rhamnose, that can be branched with other complex and less abundant polysaccharides known as arabinans, galactans, arabinogalactans type I, and arabinogalactans type II. Rhamnogalacturonan type II contains the highest diversity of sugars in its structure, including apiose, aceric acid, 2-*O*-methyl fucose, 2-*O*-methyl xylose, 3-deoxy-D-manno-2-octulosonic acid, and 3-deoxy-D-lyxo-2-heptulosaric acid; their complexity might be the cause of their important interconnection functions (Mohnen, 2008).

Pectin changes during growth and development, playing functions from structural to biological purposes. The precursor of pectin, protopectin, is a high-molecular-weight methylated polymer of galacturonic acid found in immature fruit; it is insoluble in water and cannot form gels. As the fruit ripens, natural enzymatic modification of protopectin leads to the production of pectinic acids, a group of high-molecular-weight polymers and a varied degree of methyl-esterification, frequently known as *pectins*. Pectins are soluble in water and can form gels in suitable conditions. The pectic acid predominates in overripe fruits, as a consequence of the action of polysaccharidases. Some hydroxyl groups might be acetylated or substituted with phenolic acids. The polymerization degree of pectin is diverse, and the molecular weight is also variable as a function of its origin and method of extraction. Pectic acids do not contain methyl groups in their molecules, they can be found in overripe fruits, and are formed by the action of the enzymes that cause depolymerization and demethylation of the pectinic acids, producing demethylated short chains unable to form gels, and its salts are called pectates. Pectin can represent as much as one third (33%) of the dry matter and it is mainly located at the middle lamella region. Continuous research is ongoing into pectins and some limitations related to isolation methods have been overcome with modern tools, such as subcellular localization by immunomicroscopy tools and polysaccharide microarrays. These tools are also used for the study of other polymers and proteins associated with the cell wall (Mohnen, 2008).

Hemicelluloses are spatially arranged as linear, flat polymers with a backbone of (1→4) β-type bonds and contain relatively short side chains. They connect to cellulose microfibrils by hydrogen bonds and can join adjacent microfibrils of cellulose but also cross the fibrils. Xyloglucan is the predominant type of hemicellulose that crosses cellulose fibrils, but xylans, glucomannans, and glucurono-arabinoxylans are other types of hemicelluloses with the same function but with a lesser extent since the formation of hydrogen bonds with these polysaccharides is weaker than those formed by the xyloglucans. The pectins that fulfill the cellulose–hemicellulose matrix are highly hydrated and are intersected with adjacent spaces through ester bonds and ionic bonds. It is also possible that the cellulose–hemicellulose matrix is bound to the pectins by covalent bonds between xyloglucans and the side branches of the pectins (Carpita and McCann, 2015).

Cellulose represents the most abundant polysaccharide in plant tissues and is constituted as a homopolymer of glucose. It is recognized in all fields of science, as one of the structures with the highest resistance to digestion by chemical or enzymatic hydrolysis. Its strong structure is due to the linear chain elongation that takes the (1→4) β-D-glucose bonds raising degrees of polymerization to between 2000 and 6000 in primary cell walls and more than 10,000 in secondary walls. Because each glucose residue rotates 180 degrees with respect to its neighbors, it is considered that the basic unit of cellulose is the cellobiose. The microfibrils join each other forming mainly crystalline regions that are responsible for the high-tension force attributed to cellulosic materials; however, these arrays may present disruptions, losing their crystallinity, and eventually forming what are known as semicrystalline or amorphous regions that turn into hydrolysis-susceptible polymer, hydrolyzable by enzymes with β-1,4-glucanase activity. From the biological aspect, it is important that the length of the cellulose chains, the degree of aggregation, and the degree of crystallinity are the same. The crystallinity of the cellulose depends on the alignment of the hydroxyl groups of the glucose in a symmetric arrangement, the presence of water molecules, and the interaction with residues of hemicellulose polysaccharides. The type and content of cellulose fibrils is different in each tissue and organ of the same plant or plant tissue (Carpita and McCann, 2015).

Proteins are deposited in the cell wall to carry out catalytic or structural functions. From the postharvest interest, the group of enzymatic proteins acting as machinery for polysaccharide disassembly is of interest. Depolymerization of matrix glycans mediated by polysaccharides is thought to be a major contributor to cell wall loosening and fruit softening. The endo-glycanases which include, among others, polygalacturonase, (1→4) β-glucanase, and β-galactanase, in addition to pectin methylesterase, are the most studied proteins although other nonenzymatic proteins like expansions might be important in impacting major structural changes to the wall during ripening. Polygalacturonase might be named the king of the softening associated enzymes. It catalyzes the hydrolysis of (1→4) α-bonds between galacturonic acid residues of pectins as exo- or endo-cleavage. The endo-PG acts on de-esterified galacturonic acid residues in random order and is considered to be the catalytic form with the greatest influence on the texture modification. The exo-PG catalytic form acts on the de-esterified galacturonic residue of the nonreducing end. This requirement of de-esterification explains the cooperative role of pectin methylesterase and polygalacturonase.

The four major classes of structural proteins are:

- Hydroxyproline-rich glycoproteins;
- Proline-rich proteins;
- Glycine-rich proteins;
- Arabinogalactan proteins.

These proteins are glycosylated, hydroxyproline-substituted and contain repetitive sequences (except glycine-rich proteins). The majority of cell wall proteins are crosslinked into the wall and probably in addition to structural functions they may also participate in morphogenesis. Despite arabinogalactan proteins being described as hydroxyproline-rich glycoproteins, their complexity and key functions at the cell surface of plants gets special attention as another class of cell wall-associated proteins. Arabinogalactan protein structure is characterized by a large arabinogalactan-type II glycan decorating the protein backbone; they have important functions during growth and development, either as a tethered macromodular protein anchored to the plasma membrane by a ceramide-class glycosylphosphatidylinositol group or as soluble molecules (Carpita and McCann, 2015; Fisher and Bennett, 1991).

Lignin is the compound that distinguishes the secondary wall of plants. It is the second most abundant natural compound after cellulose, occurring as a high-molecular-weight aromatic polymer resulting from the oxidative combinatorial condensation of 4-hydroxyphenylpropanoids. These polymers are variable in hydroxyl and/or methoxy substitution in the positions para- and meta- from aromatic rings, respectively. In plant tissues they fulfill three important functions, growth, facilitator of the movement of water by vascular tissue, and protection from the attack of pathogens and predators. Lignin is synthesized in response to biotic and abiotic stress as a mechanism of apoptosis (programmed cell death). On the other hand, it prevents degradation of polysaccharides from the cell wall. Lignin polymers are composed primarily of three phenylpropanoid subunits: guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H); which are derived from precursors (alcohols or monolignols) and phenylpropanoids (*p*-coumaric acid, coniferylic acid, and synapylic acid). The precursors of these subunits are transferred to the cell wall where they are oxidized enzymatically (presumably by peroxidases and laccases) and then bonded by ether and carbon−carbon bonds for the random conformation of an amorphous polymer of high complexity, heterogeneity, and resistance to digestion by digestive enzymes, such as lignin (Carpita and McCann, 2015; Hopkins and Hüner, 2009).

14.2.5 Monosaccharides and Polysaccharides

The cell wall of fruit and vegetables is assembled by carbohydrate moieties that offer versatile functions. As described before, polysaccharide polymers form the cell wall and monosaccharides conform with their individual building blocks.

Monosaccharides are conformed by carbon, oxygen, and hydrogen atoms in the form of polyhydroxyl aldehydes (aldoses). They exist in the form of ketoses and aldoses; the cell wall sugars are predominantly aldoses of five to six carbons. The arrange of monosaccharides in polymeric compounds is in heterocyclic rings like furanoses (five-membered ring formed by four carbons

and one oxygen) or pyranoses (six-membered ring formed by five carbons and one oxygen). The seven neutral sugars that are most abundant are glucose, galactose, mannose, xylose, arabinose, rhamnose, and fucose; whereas the acidic sugars are galacturonic acid and glucuronic acid. Other conformational units are apiose, mannuronic acid, methylated xylose, methylated galacturonic acid, and 3-deoxy-D-manno-2-octulosonic acid, among others. Most monosaccharides are derived from glucose through the participation of enzymes with epimerase and oxidase in decarboxylation or dehydration catalytic actions (Carpita and McCann, 2015).

Polysaccharides are polymers of covalently linked sugars with a long and variable chain length. The cell wall framework contains *homopolysaccharides* and *heteropolysaccharides* dependent on whether there is repetitive sugar conformation or not. During the synthesis of polysaccharides via synthases, the anomeric carbon of one sugar molecule is linked to the hydroxyl group of the contiguous sugar by a glycosidic bond. The available hydroxyl positions for glycosidic linkages in a pyranose like glucose, are at carbons 2, 3, 4, or 6. Position 5 is not available because it forms part of the ring structure. This relatively diverse position is what makes multiple possibilities of sugar linkages and conformations. In addition, the anomeric carbon can take the orientation of α or β. For example, the homogalacturonan region from pectins is constituted of $(1\rightarrow4)$ α-D-galacturonic acid, whereas cellulose is of $(1\rightarrow4)$ β-D-glucose. To facilitate the domain of the nomenclature of wall polysaccharides, it is recommended to review the chemistry of organic molecules. The Complex Carbohydrate Structure Database (CCSD) and database management system (CarbBank) were created for carbohydrate science. The platform encompasses most findings about carbohydrates, glycoproteins, and glycolipids and was designed to support the information about plant and bacterial glycocompounds.

Hydrolases acting in the performance of exo- or endo-catalyst, depending on the nonreducing end or random site of cleavage, respectively, fundamentally drive the disassembly of polymers. Other enzymes associated with the matrix disassembly are from the groups of lyases and transferases. All these enzymes have also been used for studies into polymer linkage and composition.

14.3 CHANGES OVER TIME OF THE MAIN COMPONENTS OF TEXTURE DURING POSTHARVEST

The most relevant changes in the fruit/vegetable texture take place during its growth and development on the tree or while in the soil; however, the changes continue during postharvest, with the main changes in texture (fruit softening) being a result of changes in the chemistry of the primary cell wall components and the middle lamella (pectin, cellulose, and hemicellulose). As previously described, during fruit postharvest the pectins of the primary cell wall are solubilized and sequentially disassembled by increasing the rate of

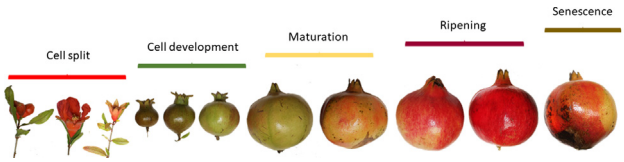

FIGURE 14.3
Texture changes during pomegranate evolution. *Photo courtesy Mr. José Antonio Granero Vicente.*

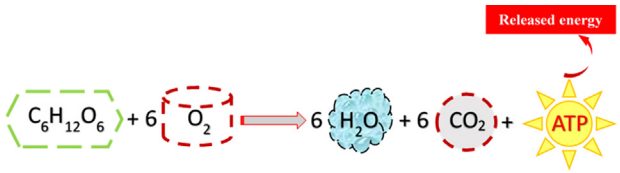

FIGURE 14.4
Respiration process.

depolymerization and loss of neutral sugars (e.g., arabinose and/or galactose) (Valero and Serrano, 2010).

In general, the ripening of fruits/vegetables is accelerated in postharvest if inappropriate storage conditions are used, leading to softening of the fruit/vegetable texture and rejection by the final consumers. *Excessive fruit softening is one of the main factors limiting shelf-life, storage, marketability, and consumer acceptance*; this statement is especially true in fruits/vegetables with accelerated softening patterns, such as stone fruits. However, there are several postharvest treatments (e.g., heat treatment, calcium application) that can delay or slow down the softening process, and these will be later discussed in this chapter (Fig. 14.3).

14.3.1 Respiration

Respiration is a chemical process in which fruit/vegetable carbohydrates (e.g., glucose) are broken, in the presence of oxygen (O_2), into their constituent molecules (carbon dioxide (CO_2), and water (H_2O)), to produce the energy necessary to perform cellular processes that keeping the cells alive (Fig. 14.4).

This metabolic activity (respiration) can continue after fruit/vegetable harvesting. This is, during postharvest, the fruits and vegetables are still alive, and to ensure long shelf-life and high quality, it is necessary to control the respiration rate through proper storage temperature. *If temperature is maintained at low levels, the respiration rate is decreased, the senescence is retarded, and the storage life is extended.* Thus, the time the product is exposed to high temperatures is critical for a low respiration rate and also for high-quality products. Beside time and temperature, other factors such as oxygen and carbon dioxide concentrations may be controlled to maintain or reduce the respiration process. Respiration results in

product spoilage, including nutritional and aromatic damage, as well as texture and weight losses (Ahmad and Siddiqui, 2015).

14.3.2 Ethylene

Ethylene ($CH_2 = CH_2$, C_2H_4) is a colorless gas generated naturally by plants, acting as a plant growth controller. This plant hormone is effective at very low concentrations, in the range of parts-per-million (ppm) to parts-per-billion (ppb).

Fruit ripening is a process started by ethylene during the plant growth cycle and produces changes in plant organs, including textural decay. These changes are ideal when it comes to ripening but if ethylene is applied for a long time, the ripening process advances into senescence. In this situation the flesh gets overly soft, and so damage and premature degradation appear. It is well known that, depending on the product, the texture can be more or less affected by ethylene; for example, if cucumber and peppers are exposed to ethylene the crunchiness attribute of texture diminishes or disappears. Also, tomatoes and peaches would obtain a grainy texture and a mealy texture, respectively. Moreover, if watermelon is exposed to ethylene (5 mL/L) at 18°C for 3 days, the flesh becomes soft and maceration appears. And finally, the firmness of kiwi is also affected by ethylene and become softer at 30 ppb concentration (Saltveit, 1998).

14.3.3 Water Loss

The transpiration or water loss is a process by which fruits and vegetables lose their moisture during postharvest handling. The water loss process consists of the transportation of the moisture through the skin, the evaporation of the moisture from the product surface, and convective transportation of the humidity mass. Water is the main constituent of fruits and vegetables, and is responsible for the appearance and texture of fresh products. Thus, *it is very important to assure adequate relative humidity during storage conditions to avoid dehydration and texture changes*. Consumers correlate firmness and crunchiness with fruit/vegetable freshness; therefore, if water loss happens, the intensity of these two attributes will decrease, resulting in shriveled and low-quality stale products. To avoid dehydration and an inappropriate produce texture it is suggested to maintain 90%–95% relative humidity and temperatures from 0°C to 15°C (depending on the product) for both storage and transportation (Ahmad and Siddiqui, 2015).

14.3.4 Ripening

The ripening of fruits and vegetables starts at harvest, when they are picked from the tree or extracted from the soil, and immediately lots of processes start taking place. In fact, there are products, such as avocado or tomatoes, that are harvested unripe, and they continue their ripening process during storage and transport; however, there are other products, such as grapes or berries, which must stay on the plant until fully ripe. This process, which is fundamental for

a product to be edible, produces many changes in fruit appearance including flesh softening and textural changes. The *softening* phenomenon happens due to ripening enzymes that produce changes in the cell wall polysaccharide. The changes produced in cell walls alter the structure and resistance of the walls, resulting in fruit softening.

14.3.5 Senescence

The senescence of fruits/vegetables is the final phase of their growing and development cycle, and several irreversible processes take place, leading to cellular breakdown and fruit tissue death. The senescence of fruits and vegetables leads to a loss of texture and flavor caused by the natural deterioration of the tissue. In this process, cell wall breakdown may result from limited synthesis of some constituents and a coordinated activity of various enzymes, but not due to a specific one. In this way, many enzymes have been reported to be vital for cell wall degradation as well as polygalacturonases, glycosidases, β-galactosidase, xyloglucan, endotransglycosylase, and cellulases.

Senescence is a regular plant tissue aging process accelerated by ethylene or other compounds activating intense respiration. Beside other quality attributes, texture is also affected by the senescence mechanism, due to the disruption of the integrity of the cell wall. For apples and tomatoes the disintegration of intercellular adhesion between cells contributes to mealiness of the product which means a loss of textural quality (Aked, 2002).

14.3.6 Dormancy

Dormancy is a period in which plant regular growth is stopped. This is important because it permits plants to survive for a long time in climates where growth is not possible, especially in the winter season. The crops as tubers, bulbs, or roots have a regular dormancy cycle that can be significantly extended, if adequate storage conditions are assured. Under moisture conditions, there are plants such as potatoes or onions, which develop roots or shoots during dormancy; if so, they are not accepted in the market because of loss of internal quality. The internal quality of the vegetable is decayed during the breaking for dormancy due to the conversion of stocked starch into sugars, which are transported to the shoots or roots. Thus, the quality and texture of the product change diminishes the hardness and increases the roughness or other textural defects, which makes them unsuitable for selling.

14.3.7 Physiological Disorders

The physiological disorders are also key factors for the texture quality of the fruit or vegetable. *Chilling injury* happens when fruits or vegetables are maintained below freezing or when very low oxygen and/or high carbon dioxide concentrations exist; both situations affect the texture due to the breakdown of the tissue. Also, chilling disorder produces external and internal discoloration,

pitting, soaked areas, and a rapid occurrence of surface decay and molds. In addition, *calcium (Ca) deficiency* can produce bitter pitting of apples. Heat damage results from excessive exposure to direct sunlight and also produces surface scalding, desiccation, and significant softening.

14.4 POSTHARVEST TREATMENTS THAT CAN INFLUENCE TEXTURE

14.4.1 Heat Treatment

Heat treatment is a pesticide-free method to care for texture quality during storage. This method is conducted using hot air, hot water dips, or vapor heat, and is an alternative to cut down the need to use chemical products to assure the quality of fruits or vegetables. The application of heat treatment significantly reduces fruit softening, by maintaining firmness and extending shelf-life. Thus, heat treatment applied at temperatures between 30−40°C in products such as plums, pears, avocados, and tomatoes reduces their softness more than if applied at 20°C (Klein and Lurie, 1992).

14.4.2 Calcium

Calcium solutions are applied as postharvest treatments and are also used as foliar spray to increase the firmness of the fruits and its shelf-life. Calcium is a fruit cell wall stabilizer and if used in adequate amounts helps in holding fruit firmness and diminishing the internal breakdown, bitter pitting, and water core. This mineral (Ca) is very important for the growth cycle of the plant and for the maintenance of cell functions. Increasing the Ca content is important to maintain fruit quality, and to diminish softening and respiration. To assure an improvement of the texture quality, an adequate concentration of Ca must be applied. If the Ca content is higher than the proper threshold, damage can appear on the surface of the product.

14.4.3 Others

Other treatments used to maintain the freshness of the fruits/vegetables are:

1. *Edible coatings*, which preserve the color and also the texture. These are applied by using layers of external coating on the surface of the fruits, which create a barrier to avoid moisture movement leading to water loss, and slowing down respiration, enzymatic oxidation, and senescence;
2. *Irradiation*, meaning exposing the fruit or vegetable to radiant energy γ-rays and e-beam (high-energy electrons), breaks through the product and destroys the molecular bonds. Using this method, at an appropriate level, a significant delay in ripening can be achieved, leading to a longer shelf-life. On the other hand, if too high levels of irradiation are applied to the product, texture defects and accelerated senescence can be the result (Mahajan et al., 2014).

It is important to highlight that the intensity of the changes described in Section 14.3 and the effectiveness of the treatments described in Section 14.4 are dependent on the fruit/vegetable type and even the cultivar. For example, if stone fruits such as apricot, peach, or nectarine are stored for 1 week at room temperature, their firmness will decrease considerably more than that of tomatoes or lemons (Valero and Serrano, 2010).

14.5 TEXTURE MEASUREMENTS

The texture measurements can be performed by both instrumental and sensory methods. Usually instrumental methodologies are adopted instead of the sensory ones because of the difficulty in properly training a panel to conduct objective sensory measurements of the fruit/vegetable texture. However, the use of both methods and a comparison of the obtained results will drastically increase the amount and quality of the data produced, especially when the consumer opinion is also studied. For instance, the firmness of fruits/vegetables is a key factor for consumer acceptability and whether the products can be stored for a longer period of time. If after the harvest of some fruit (e.g., pears), their firmness is high and the storage conditions are adequate, the product can be stored for a long time.

The texture attributes of fruits/vegetables can be measured using different methods which are described in the Sections 14.5.1 and 14.5.2, respectively.

14.5.1 Instrumental Methods

14.5.1.1 DESTRUCTIVE INSTRUMENTAL METHODS

The destructive instrumental methods include, among others, puncture, Magness–Taylor, compression, cutting, and TPA tests. These tests must be conducted using a texture-meter, such as the Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, United Kingdom). The most important destructive methods are briefly described below:

1. The puncture test is a method which measures the firmness of the *peel* of fruits and vegetables. A stainless-steel probe (needle of 2 mm of diameter) is used and applied in the center of the product, and the maximum force in the force *versus* time curve is taken as representative of the product firmness, and is expressed in N (Fig. 14.5A);
2. The Magness–Taylor test is an indicator of the pulp firmness (N) and is measured with a cylindrical stainless-steel probe of 8 mm diameter. For this test, it is necessary to remove the peel using a sharp knife to avoid measuring the peel firmness (Fig. 14.5B);
3. The deformation test consists of compressing the fruit with, for instance, a 75 mm diameter cylindrical probe at an established speed up to a given percentage of the sample height. The necessary force used to compress the percentage of the sample height represents the result of the test and is expressed in N;

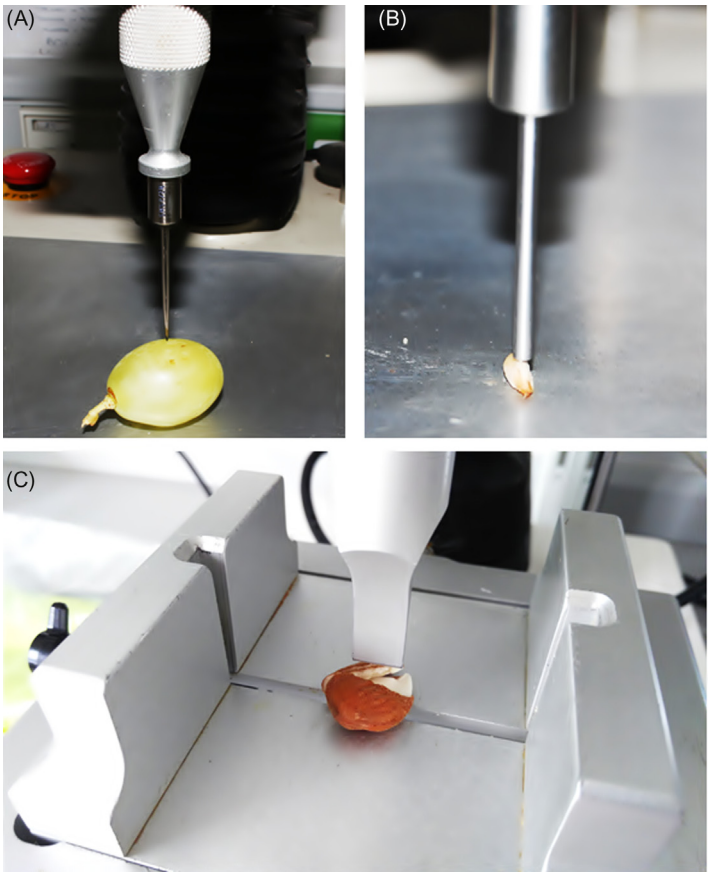

FIGURE 14.5
Texture measurements: (A) puncture, (B) Magness–Taylor, (C) cutting force.

4. The cutting test is about simulating the action of teeth by using a knife blade to cut through a fruit/vegetable and measuring the maximum force (N) needed to cut it. The descriptors measured using this method are firmness, hardness, and fibrousness (Fig. 14.5C).
5. Texture profile analysis (TPA) is the last destructive method and one of the most important in texture analysis and consists of two full compression cycles (with about 10 s between cycles) to give an instrumental texture profile of food (Bourne, 1978). Samples are compressed to approximately 25% of their original height with a 75 mm diameter cylindrical probe (the probe must always be wider than the food). The textural attributes of hardness, adhesiveness, cohesiveness, springiness, and chewiness were calculated from the texture profile curve as seen in Fig. 14.6 (Bourne et al., 1978). The above-mentioned attributes obtained using the TPA method are briefly described below:

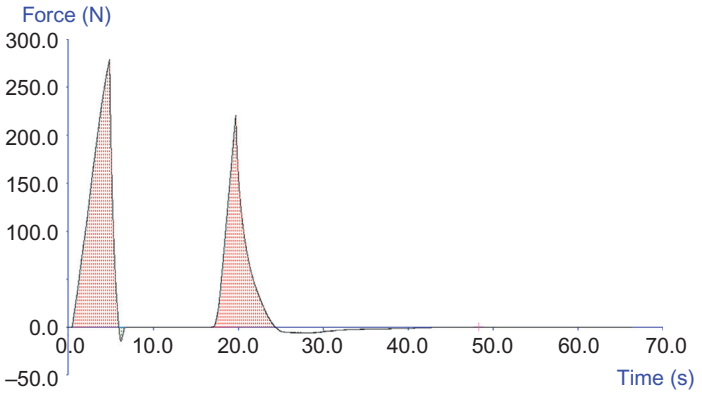

FIGURE 14.6
Model of texture profile analysis.

a. *Hardness* (N) is defined as the "maximum force required to compress a sample," and is calculated as the peak force of the first compression of the product.
b. *Adhesiveness* (N s) is defined as the "work to separate a product stuck to the compression plate"; it is assumed to measure the adhesiveness of a sample to the palate and teeth, and is calculated as the negative area between the first and second compression cycles.
c. *Cohesiveness* (%) is a measure of how well the structure of a product withstands a second deformation relative to how it behaved under the first deformation (A_2/A_1, A_1 being proportional to the total energy required for the first compression and A_2 being proportional to the total energy required for the second compression).
d. *Springiness* (%) is a measure of how well a product physically springs back after the first compression, and is calculated as the ratio between the distance of the second compression peak/distance of the first compression peak.
e. *Chewiness* (N) is assumed to be a measure of the work to masticate the sample for swallowing (springiness × hardness × cohesiveness, with springiness and cohesiveness being expressed as fractional numbers between 0 and 1).

14.5.1.2 NONDESTRUCTIVE METHODS

Nondestructive methods are able to measure fruit or vegetable internal variables without destroying the product. The firmness can be measured by many approaches, such as evaluation of the variables obtained from force–deformation curves, examination of collision forces, Ricochet's method, nuclear magnetic resonance method, evaluation of acoustic responses to collision and vibrations, and assessment of the optical properties (García-Ramos et al., 2005).

One of the devices created to measure the *force—deformation* is the durometer. A *Durometer* is a piece of equipment which measures deformation and is based on a small cylinder movement when pressed on the fruit. The sample is measured in two points of the fruit equatorial diameter and the average between those two measurements is used. The nondestructive equipment has the advantage that the fruit can be used for further analyses because it does not destroy the sample and also because it is small and so is easier to use directly in an orchard.

The examination of *collision forces* refers to the impact of a hard surface (sphere) on a fruit. This impact force and duration are analogous with the product firmness. This method uses sensors to measure the reaction of fruit to impacts and consists of hitting the product with a surface which contains the sensor, it also can be measured by placing the fruit above a load cell and applying a weight through it, and lastly by setting the product on a plate and a load cell bellow it.

The *ricochet technique* refers to the theory of impact which explains that the firmness quantification can be done in accordance with the fruit ricochet after impact against a surface. This type of apparatus was developed to be used also in the separation of poor-quality soft fruits/vegetables (olives or clementines) from high-quality ones. The machine is equipped with a conveyor belt which transports the product to a horizontal cylinder covered with lining material that spins in the opposite direction to the belt. The fruits/vegetables are separated because the soft products' ricochet is less than the high-quality ones and they drop near to the impact point.

The evaluation of fruits/vegetables by *acoustic vibration* depends on the elasticity, shape, and mass. There are different equipments, which are appropriate for fruit/vegetable firmness determination, calculated using the mean of the first two nearby resonant frequencies obtained by sensors. This apparatus is formed from three hammers with an electromechanical impulse and three piezoelectric sensors. The product mass is measured with a force transductor and in this way the signal is balanced.

The texture also can be measured in a nondestructive way by *nuclear magnetic resonance* (NMR), which measures the internal characteristics of a product based on the magnetic properties of the atomic nuclei which form the material. The NMR method does not measure the firmness directly, but the features associated with internal defects or ripening.

The optical sensors, such as *visual (VIS)* and *near-infra-red (NIR)*, can be used to evaluate the firmness of fruits and vegetables. These sensors are characterized by a light origin and a receiver needed for the optical signal register. The optical signal emits different wavelengths. Reflectance and transmittance are two principal optical techniques depending on the light route inside the fruit or vegetable. The first technique is characterized by the incident light which percolates the external matter and egresses close to

the entrance point beside the sensor. In addition, in the transmittance technique the incident light crosses the matter at 90° to entrance point and hits the sensor in the reverse side.

14.5.2 Sensory Analysis Methods

From a sensory point of view, the texture of fruits and vegetables is mainly perceived by the sense of touch, including hands and mouth, during the chewing process. Our skin is endowed with nerves and the tactile stimuli are recognized in their endings. Using the sense of touch, consumers are able to obtain information about the temperature, quality, shape, etc., of the products. Touching the fruits and vegetable with the hands is the first step in deciding if the texture of fruits and vegetables is of high quality by measuring the freshness. Beside this, hand-feel, mouth-feel, and chewing sounds are also essential for textural quality determination. Mechanical properties, such as hardness, crunchiness, softness, adhesiveness, etc., are measured by the corporal sense in the muscle of hands, lips, tongue, and jaw. The geometrical and moisture properties, such as grainy, gritty, oily, dry, wet, etc., are determined by the tactile feel in the skin of the hands, lips, or tongue. All these mouth and chewing movements are responsible for a lot of information about the texture quality of fruits and vegetables.

The sensory methods used in the determination of fruit/vegetable texture can be classified into three main groups: discriminative, descriptive, and affective tests.

Discrimination tests are used to evaluate a specific characteristic of the product (e.g., hardness). This is an analytical test and includes difference tests, such as triangle, duo-trio, ranking, and paired comparison. The triangle test consists of the preparation of three samples from which two are the same. The duo-trio test also involves three-sample preparations, in which two are the same, but in this case one of the same samples is the control and the panelist must choose from the two remaining samples which is the same as the control one. For the ranking test, the panelist must rank in order different samples depending on the presence or absence of a certain attribute (e.g., hardness). The paired comparison test consists of analyzing an attribute (i.e., hardness) for two different samples and the panelist has to decide which of the samples is *harder*.

Descriptive sensory analysis is an analytical method used to document the sensory characteristics of food (full description of the product) and allows comparison between products. In this approach, product lexicons are prepared and include the preparation of reference materials to allow objective measurement of the intensity of key sensory attributes. For descriptive analysis a trained panel made of 7–10 panelists is used and trained to describe a product from the sensory point of view using terms and definitions easy to understand by the panelists. The panel must be trained to evaluate the intensity of each descriptor and to establish their own list of attributes from the studied product category. For this, it is important to use reference products to be understood

by all members of the panel and it is advisable to use two or three reference products per attribute. These tests are conducted in tasting rooms, equipped with a room for sample preparation and the tasting place itself. The tasting place must be isolated from noise and odor, have air conditioning, and individual boots to allow individual work with proper light (natural and fluorescent).

Affective tests deal with the consumers' responses on whether they like or dislike the texture, or any other sensory attribute, of the product using a nine-point hedonic scale. Where 0 means extremely dislike, 5 neither like nor dislike, and 9 means extremely like. Usually it is conducted by approximately 100 consumers, who are regular consumers of the product under study (for instance, they consume fruits and vegetable at least once per week). These studies are mainly conducted at central locations and/or households, trying to reproduce normal buying or consumption conditions.

14.6 CONCLUSIONS

Appearance and texture are the most important attributes when it comes to consumer acceptability of fruits and vegetables. Thus it is essential to understand the degradative processes happening during postharvest, to take action, and reduce them to offer consumers with high-quality products and extended shelf-life; this will lead to higher consumer acceptance. Knowing the biochemical mechanisms behind the loss of texture during postharvesting will allow us to establish proper measurement to control them and reduce, for instance, tissue softening. In this way, it is well known that softening and loss of cohesiveness are linked to a decrease in the degree of intermolecular bonding among cell wall polymers, and higher solubility of pectin and/or other cell wall components. Both instrumental and sensory analysis techniques are important to determine the texture of fruits/vegetables and it will be very useful to correlate them with purchase intention and consumer satisfaction. Even though sensory analysis is sometimes avoided because of the difficulty to properly train a panel to be able to produce objective data, linked to consumer acceptance, it is important that instrumental values are compared with sensory data to be able to select an instrumental technique that reproduces and represents consumer opinions and emotions towards fruits/vegetables texture.

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CHAPTER 15
Protein

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15.1 INTRODUCTION

Proteins are important macromolecules that participate in every aspect of plant growth and development. They are involved in many processes such as chemical reaction catalysis, membrane transport facilitation, intracellular structure maintenance, and also in energy-generating reactions involving electron transport. Initially, nitrogen is assimilated and organically incorporated into glutamine, glutamate, asparagine, and aspartate, which constitutes important nitrogen carriers inside the plant. These primary amino acids are the main compounds of the total free amino acid pool in many plants, which are utilized for the synthesis of other amino acids. In order for protein synthesis to occur, the supply of amino acids must be sufficient to make up the proteins required. In plants, the presence of protein is low due to the large carbohydrate content of most plant structures. However, in some legume plants, the protein content is high. In addition to addressing the structure and prevalence of all proteins, a fascinating potential of proteomics is represented by a rapidly developing large-scale approach to study changes in the level of protein expression. Using proteomic analysis, the differences in protein levels and protein modifications can be measured.

15.2 NITROGEN AS A PRIMARY PRECURSOR OF PROTEIN IN PLANT

Nitrogen is an essential nutrient for plant growth, development, and reproduction. It is the fourth most abundant element in living organisms. The nitrogen in soil that might eventually be used by plants has two sources: nitrogen-containing minerals and the vast storehouse of nitrogen in the atmosphere.

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The nitrogen in soil minerals is released as the mineral decomposes. This process is generally quite slow and contributes only slightly to nitrogen nutrition in most soils. Atmospheric nitrogen is a major source of nitrogen in soils. In the atmosphere, it exists in the very inert N_2 form and must be converted before it becomes useful in the soil.

Plants from different species and environments have diverse strategies for acquiring nitrogen. Some heterotrophic bacteria living in the soil can fix significant levels of nitrogen without direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of *Azotobacter*, *Bacillus*, *Clostridium*, and *Klebsiella*. These bacteria have chemolithotrophic capabilities and can thus utilize inorganic compounds as a source of energy. Because of the insufficiency of suitable energy sources for these organisms, their contribution to global nitrogen fixation rates is generally considered negligible. Species of *Azospirillum* are able to form a close association with several members of the Poaceae family, mostly in cereal crops, such as rice, wheat, corn, oats, and barley. *Azospirillum* can convert atmospheric nitrogen into ammonium under microaerobic conditions at low nitrogen levels, through the action of the nitrogenase complex.

Another type of bacteria called *Rhizobium* can fix appreciable amounts of nitrogen within the rhizosphere of the host plants compared to other species. The *Rhizobium* bacteria colonizes the host plant's root system and cause the roots to form nodules to house the bacteria. The plant provides sugars from photosynthesis that are utilized by the *Rhizobium* for the energy it needs for nitrogen fixation. The exchange allows the plant to increase photosynthetic capacity, which in turn yields nitrogen-rich seed. By far, the relationship between legumes and rhizobacteria is considered to be the most favorable in developing sustainable agriculture. Important legumes used in agricultural systems include alfalfa, beans, clover, cowpeas, lupines, peanut, and soybean.

Plants commonly acquire nitrogen as NH_4^+, which can be assimilated immediately into amino acids, or as NO_3^-, which must be reduced to NH_4^+ for further metabolism. With the help of some bacteria, atmospheric N_2 is reduced to NH_4^+. The assimilation is carried out by the nitrogenase complex. Two components of enzymes are dinitrogenase and dinitrogen reductase. Dinitrogenase accumulates electrons and catalyzes the reduction of substrates, including N_2. Dinitrogenase reductase is a unique and specific electron donor to dinitrogenase. The nitrogenase complex carries out the reduction of molecular dinitrogen to ammonium.

Ammonium ions that are not immobilized or taken up quickly by higher plants are usually rapidly converted to NO_3^- ions by a process called nitrification. This is a two-step process, during which bacteria called *Nitrosomonas* convert NH_4^+ to nitrite (NO_2), and then other bacteria, *Nitrobacter*, convert the NO_2^- to NO_3^-. This process requires well-aerated soil and occurs rapidly enough that one usually finds NO_3^- rather than NH_4^+ in soils during the growing season.

Some plants, such as tea plants, have a nitrogen content, particularly in young shoots, that is closely related to the tea quality. Higher uptake of NH_4^+ was found to significantly increase their total nitrogen and free amino acids. In several higher plants the nitrogen uptake through NO_3^- was suggested to involve two different uptake systems called the high-affinity transport system (HATS) and low-affinity transport system (LATS) that show different affinity towards external N (Yang et al., 2014). HATS displays Michaelis–Menten kinetics, with a Km typically between 10 and 100 μM. LATS usually displays nonsaturating uptake kinetics. NO_3^- uptake is also associated with the proton gradient established by depolarization of the plasma membrane.

In the soil solution, nitrate is carried towards the root by bulk flow and is absorbed into the epidermal and cortical symplasm. Nitrate may be processed by both root and leaf cells. It is used by higher plants in various processes, including absorption, vacuole storage, xylem transport, reduction, and incorporation into organic forms. Within the root symplasm, NO_3^- has four fates: (1) reduction to NO_2^- by the cytoplasmic enzyme nitrate reductase; (2) efflux back across the plasma membrane to the apoplasm; (3) influx and storage in the vacuole; or (4) transport to the xylem for long-distance translocation to the leaves. Following long-distance translocation, NO_3^- must leave the xylem and enter the leaf apoplasm to reach leaf mesophyll cells, where NO_3^- is again absorbed and either reduced to NO_2^- or stored in the vacuole.

NO_3^- reduction to NH_4^+ is mediated by two key enzymes, nitrate reductase and nitrite reductase. Nitrate reductase catalyzes the reduction of two electrons for the conversion of nitrate into nitrite. Nitrite reductase turns nitrite into ammonium and the ammonium is then converted to glutamine, which is essential for amino acid synthesis via the glutamine synthetase (GS) and glutamate synthase (GOGAT) systems. The absorption of plant species to a particular form of nitrogen varies from species to species and their preference for NH_4^+ and NO_3^- is usually related to their physiological adaptation to the ecosystem. Nitrate assimilation requires synthesis of organic acids, especially α-oxoglutarate which acts as the acceptor for ammonium in the GOGAT pathway, and malate which acts as a counteranion and substitutes for nitrate to prevent alkalization. Inorganic nitrogen is assimilated and organically incorporated into glutamine, glutamate, asparagine, and aspartate, which constitute important nitrogen carriers inside the plant. These primary amino acids are the main compounds of the total free amino acid pool in many plants, which are utilized for the synthesis of other amides, like ureides, other amino acids, and amines.

Plants contain a wide array of endogenic proteases, which play crucial functions in each metabolic pathway. It has been shown that a few plant species such as *Arabidopsis thaliana* and *Hakea actities* can take up intact amino acids. The occurrence of amino acids in soils is primarily in the form of proteins. To access this source of nitrogen, the action of proteases is needed. Proteases secreted by the roots help in digesting proteins to low-molecular-mass products and these belong to the cysteine protease family. Their activity is highest at pH 10 and they function mainly as endopeptidases.

15.3 BIOSYNTHESIS OF AMINO ACIDS

The assimilation of inorganic nitrogen into an organic form contributes to the positive effects on plant productivity, biomass, and crop yield. Inorganic nitrogen usually first reduces to ammonia prior to its incorporation into an organic form. Ammonia is assimilated into organic form as glutamine and glutamate, which serve as the nitrogen donors in the biosynthesis of essentially all amino acids, nucleic acids, and other nitrogen-containing compounds such as chlorophyll. Several isoenzymes have been identified to play major roles in ammonium assimilations which include GS, glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH). These enzymes involve the primary assimilation of ammonium into Glu/Gln and Asp/Asn N-transport amino acids.

GS catalyzes the ATP-dependent assimilation of ammonium into glutamine, using glutamate as substrate. Purification of GS using ion-exchange chromatography shows that they are localized in the cytosol (GS1) and chloroplast (GS2), respectively. GS work with GOGAT in catalyzing the reductive transfer of an amide group from glutamine to α-ketoglutarate, forming two molecules of glutamate. Two major classes of GOGAT have been identified as ferredoxin-dependent GOGAT that is usually found in higher plant systems and NAD(P)H-GOGAT that is generally found in plants and bacteria and localized in the plastid, respectively. The glutamine synthase–glutamate synthase (GS/GOGAT) pathway is thought to be the main path of ammonium assimilation.

GDH is an enzyme involved reversibly in amination of α-ketoglutarate and deamination of glutamate to yield α-ketoglutarate and ammonium. Two classes of GDH have been found to localize in both mitochondria (NADH-dependent GDH) and chlorophyll (NAD(P)H-dependent GDH), respectively. Evidence suggests that GDH primarily plays a role in the glutamate catabolic process, however it also may assimilate inorganic nitrogen into glutamate when ammonium is high. After the assimilation of ammonium into glutamate and glutamine is completed, nitrogen can be distributed to many other compounds by the action of aspartate aminotransferase (AAT). Synthesis of aspartate regenerates the carbon skeleton required for the next generation of nitrogen assimilation by transferring an amino group from glutamate to oxaloacetate. Aspartate is active in the transport of carbon and nitrogen within and between cells as occurred in some C3 and C4 plants and is needed for synthesis of other compounds.

Amino acids such as lysine, threonine, methionine, and isoleucine are derived from branched pathway from aspartate as demonstrated in *Arabidopsis thaliana*. As the committing enzyme leading to the formation of lysine, methionine, threonine, isoleucine, and other downstream metabolites, aspartate kinase is subject to extensive allosteric regulation. L-aspartate-4-semialdehyde is known to become an intermediate towards the formation of aspartate-derived amino acids. Dihydrodipicolinate synthase enzyme (DHDPS) then catalyzes the first reaction leading to lysine biosynthesis. Meanwhile homoserine dehydrogenase catalyzes the formation of homoserine from aspartate-4-semialdehyde as the

first committing step in the pathway leading to the biosynthesis of threonine and methionine. Through the catabolism process of threonine, deaminase enzyme converts threonine to 2-oxobutanoate. This 2-oxobutanoate is then converted into isoleucine in the presence of acetolactate synthase.

Amidation of aspartate by glutamine or ammonium yields asparagine, an inert amino acid used to store nitrogen and transport it into several parts of plants. The route of asparagine biosynthesis is thought to be through asparagine synthetase (AS). AS catalyzes the transfer of the amido group from glutamine to aspartate, generating glutamate and asparagine. AS preferentially uses glutamine as a nitrogen donor but it can catalyze the assimilation of inorganic nitrogen when ammonium is abundant.

Tryptophan, phenylalanine, and tyrosine are aromatic amino acids that serve as precursors of a wide variety of plant natural products that play crucial roles in plant growth, development, reproduction, defense, and environmental responses. For example, tryptophan is a precursor of alkaloids and indole glucosinolates as well as the plant hormone auxin, whereas tyrosine is a precursor of isoquinoline alkaloids, pigment betalains, and quinones. Phenylalanine is a common precursor of numerous phenolic compounds, which include flavonoids, condensed tannins, lignans, lignin, and phenylpropanoid/benzenoid volatiles. All these aromatic amino acids are produced from the final product of the shikimate pathway called chorismate. The seven enzymatic reactions of the shikimate pathway connect the central carbon metabolism and the aromatic amino acids network by converting phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P) intermediates in glycolysis and the pentose–phosphate pathways, respectively, to chorismate, the universal precursor for aromatic amino acids. The tryptophan pathway converts chorismate to tryptophan via five enzymatic reactions in the plastids. While in Phe and Tyr, chorismate is converted by CM to prephenate, whose subsequent conversion to phenylalanine and tyrosine is through two alternative pathways, called the arogenate pathway and the phenylpyruvate or 4-hydroxyphenylpyruvate pathway. Through the arogenate pathway, prephenate is first transaminated to L-arogenate followed by dehydration/decarboxylation to phenylalanine or dehydrogenation/decarboxylation to tyrosine. Using the phenylpyruvate or 4-hydroxyphenylpyruvate pathway, prephenate is first subjected to dehydration/decarboxylation by prephenate dehydratase (PDT) or to dehydrogenation/decarboxylation by prephenate dehydrogenase (PDH), followed by transamination of the corresponding products, phenylpyruvate and 4-hydroxyphenylpyruvate, to phenylalanine or tyrosine, respectively.

Proline is a unique amino acid that is increasingly being associated with many important functions in biology. Proline accumulation represents one of the major strategies used by plants as a response to various abiotic and biotic stress conditions. Biosynthesis of proline occurs via two routes: the glutamate and ornithine pathways. In plants, a single bifunctional enzyme, namely Δ^1-pyrroline-5-carboxylate synthetase (P5CS), catalyzes both reactions. Glutamate

γ-semialdehyde undergoes a spontaneous cyclization to δ1-pyrroline-5-carboxylate (P5C). In the terminal step, thisat is catalyzed by pyrroline-5-carboxylate (P5CR), P5C is reduced by the cofactor NAD(P)H to yield proline and the oxidized cofactor $NAD(P)^+$. The enzymes ornithine amino transferase (OAT) and P5CR are required for the biosynthesis of proline from ornithine.

15.4 BIOSYNTHESIS OF PROTEIN

The central dogma of molecular biology describes the two-step process, transcription and translation, by which the information in genes flows into proteins. In cells, DNA is present in the double-stranded form. Each cellular chromosome contains two strands of DNA, each strand containing nucleotides linked by phosphodiester bonds. Each strand is made up of a five-carbon sugar (2′-deoxyribose), phosphoric acid, and four nitrogen-containing bases. Two bases are purines, which have a double ring structure; the other two are pyrimidines, which contain a single ring. The purine bases are adenine (A) and guanine (G), while the pyrimidine bases are thymine (T) and cytosine (C). The strands themselves associate with one another through hydrogen bonds that form the nucleotides from one strand and nucleotides of the other. Specific base pairing through hydrogen bonds occurs between G and C, and A with T. The genetic information for all cellular processes is stored in DNA in the sequence of bases along the polynucleotides chain. In plant cells, the vast majority of genes are located in the nucleus instead of the mitochondria and plastids.

The replication mechanism first introduced by Watson and Crick was that the strand of the original parent duplex separates, and each individual strand serves as a template for the synthesis of a new strand, a process called semiconservative replication. DNA replications begin at the specific site called the primer. The primer is a site at which the DNA polymerase can attach the first nucleotide. DNA polymerase adds a new strand of DNA by extending the 3' end of an existing nucleotide chain, adding new nucleotides matched to the template strand one at a time through phosphodiester bonds. As DNA synthesis continues, the original DNA strands continue to unwind, forming a replication fork. It is created by helicases, an ATP-dependent enzyme which breaks the hydrogen bonds that hold the two DNA strands together. These two strands serve as the template for the leading and lagging strands.

The leading strand is the strand of nascent DNA which is being synthesized in the same direction as the growing replication fork. The lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork. Because of its orientation, replication of the lagging strand is more complicated as compared to that of the leading strand. On the leading strand, DNA synthesis can occur continuously because there is always a free 3'-OH at the replication fork to which new nucleotides can be added. But on the opposite strand, the lagging strand, DNA

synthesis must occur discontinuously. A small RNA primer must be synthesized by primase to provide free 3'-OH. A DNA polymerase extends the primed segments, forming Okazaki fragments. The RNA primers are then removed and replaced with DNA, and the fragments of DNA are joined together by DNA ligase.

The transcription of genetic information from DNA to RNA is carried out through the action of the enzyme RNA polymerase, which catalyzes the formation of phosphodiester bonds between ribonucleotides. The particular sites on the DNA where RNA polymerase are called promoters. Gene promoters are DNA sequences located upstream of gene-coding regions and contain multiple *cis*-acting elements, which are specific binding sites for proteins involved in the initiation and regulation of transcription. As RNA polymerase moves down the DNA chain, it causes a temporary opening of the double helix and transcription of one of the DNA strands. As the newly synthesized RNA dissociates from the DNA, the opened DNA closes into the original double helix. Transcription stops at specific regions called transcription terminators.

Translation is generally divided into three stages: initiation, elongation, and termination. The first step of the initiation stage is the binding of a specific initiator methionyl tRNA and mRNA to the small ribosomal subunit. The initiation factor eIF-3 first binds to a 40S ribosomal subunit. The complex of eIF-3-40S is then stabilized by another protein called eIF-4C. In a separate reaction, another protein complex, Met-tRNAf, is activated by the binding of GTP and another initiation factor, eIF2. This step is followed by binding of mRNA. The attachment of the complex of eIF-3-40S and Met-tRNAf-eIF2 is then ready to bind mRNA with the help of eIF-4 and ATP. After the initiation complex has formed, translation proceeds by elongation of the polypeptide chain.

The ribosome has three sites for tRNA binding, designated the E (exit), P (peptidyl), and A (aminoacyl), sites. The E site is the site when tRNA is actually released from the ribosome. The P site, the peptide site, is the site where the growing peptide is held by a tRNA. Menawhile, the A site, the acceptor site, is the site where the new AA-tRNA first attaches. The initiating N-formylmethionyl tRNA is positioned in the P site. The second aminoacyl tRNA (e.g., alanyl tRNA) is then brought to the A site by the elongation factor of EF-Tu (complexed with GTP). Following GTP hydrolysis, EF-Tu (complexed with GDP) leaves the ribosome, with alanyl tRNA inserted into the A site. A peptide bond is then formed, resulting in the transfer of methionine to the aminoacyl tRNA at the A site. The ribosome then moves three nucleotides (one codon) at each step along the mRNA. This movement translocates the peptidyl (Met-Ala) tRNA to the P site and the uncharged tRNA to the E site, leaving an empty A site ready for the addition of the next amino acid. Translocation is mediated by EF-G, coupled to GTP hydrolysis. Elongation of the polypeptide chain continues until they reach a stop codon (UAA, UAG, or

UGA), which serves as the stopping point for protein synthesis. Release factor will recognize the signal of codon and serve to cleave the attached polypeptide from terminal tRNA. Finally, the ribosome dissociates, and the subunits are free to form a new initiation complex.

Protein biosynthesis by ribosomes first yields an unfinished polypeptide. In many cases, multiple polypeptide chains must assemble into three-dimensional conformations through proper folding to become a functional protein. The proper folding of proteins within cells is mediated by a special protein called a chaperon. Chaperons appear to function by binding and stabilizing unfolded or partially folded polypeptides to prevent incorrect folding and allowing the polypeptide chain to fold into its correct conformation. The binding of chaperon can occur during translation in which the nascent polypeptide chains are being translated on ribosomes, thus preventing incorrect folding or aggregation of the amino-terminal portion of the polypeptide before synthesis of the chain is finished.

15.5 PROTEIN DEPOSITION IN PLANT ORGANS

Plants store proteins in the embryo and vegetative cells to provide nutrient resources such as carbon, nitrogen, and sulfur for subsequent growth and development. The storage of proteins is very critical to the lifecycle of the plant because they are utilized following germination to support early growth of the seedling (from seed) or shoots (from tuber), which allows it to survive before it begins photosynthesis activity. In addition to storing nitrogen, sulfur, and carbon for future seedling growth, certain storage proteins also have secondary role(s) including pest and disease resistance. In vegetative cells, stored proteins provide the building block for seed and fruit setting during the reproduction stage and for rapid expansion in the vegetative structure. Among horticultural products, the seed is perhaps the best-studied example of protein deposition and storage.

Legume and cereal seeds are important agricultural commodities around the world. The demand for food has caused rice, wheat, soybean, and maize to become among the most valuable crops as the global population has increased. Through the domestication process, humans have been selecting and improving seed quality in order to have greater prospects of producing a healthy crop with improved yields. Cereal and leguminous seeds have a large part in human food consumption. During early development of seed, up to enlargement of the embryo, protein accumulates more, until desiccation of seed begins which prepares them for dormancy. Storage proteins are synthesized on the rough endoplasmic reticulum and transported to the Golgi apparatus. Then they are sent to their final destination based on their special tag (for example, amino acid sequences). Proteins are sorted into secretory vesicles that bud off from the trans face, and they eventually fuse with the plasma membrane to deliver their contents.

Storage proteins deposited in organelles may contain one or both types of protein storage organelles, ER-derived protein bodies (PB) or Golgi-derived protein storage vacuoles (PSV). The PB is distinct from PSV in terms of their origin, contents, and biological characteristics. Analysis of seed storage proteins based on their solubility, categorized as: water-soluble (albumins), dilute saline-soluble (globulins), alcohol-soluble (prolamins), and dilute acid- or base-soluble (glutelins). In monocots, the endosperm becomes the main tissue, while most dicot endosperm has only a temporary role and cotyledons carry out the reserve function. Dormancy ends in seed germination when seed storage proteins are utilized for seedling development.

Proteins are also deposited in the bulb, bark, or parenchyma tissue of the plant. Tuber parenchyma cells gare enerally rich in starch, however, they do contain protein, which varies in amounts up to 10% in dry weight. Significant variation exists in tuber protein as they are derived from different botanical origins. Potato, for example, is derived from stem while sweet potato and cassava from root, and taro and yam from corm. About 40% of the total protein was determined as patatin in potato, both G1 and G2 globulin in taro corm (Colocasia), respectively. The major storage protein of sweet potato is known as sporamin, which accounts for 80% of the total protein in the swollen root. Yams contain about 1%−3% protein on a dry weight basis and dioscorin protein is reported as the major group of protein in their fleshy corm.

15.6 PROTEIN CONTENT AS AFFECTED BY PRE- AND POSTHARVEST FACTORS

Protein typically occurs in a very small amount in horticultural produce except in legumes. Protein constituents are required at different stages to uphold certain activities for growth and maturation. Different types of genes are expressed in relation to the requirement of the plant. Changes in maturation are highly coordinated and involve many biochemical steps. In some plants such as tomato, there are some clues that may provide an understanding about the genes involved in the most important physiological changes, such as the accumulation of soluble sugars, cell wall disassembly, and synthesis of pigments during fruit ripening. Nevertheless, protein occurrences are affected by preharvest and postharvest factors. Climatic conditions, especially temperature, soil type, and cultural practices, can affect the composition and quality attributes of harvested parts. Inaccuracy in determining maturity at harvest, harvesting procedure, and handling of horticultural produce can greatly increase the injury rate and cause low quality. The degree of losses will be increased if they are stored outside the range that is optimum for each produce during postharvest storage.

Diverse fruit species and genotypes mature at different rates, leading to variations in their nutrient composition, which includes protein. Plant breeders have been successful in selecting soybean cultivars with much higher protein composition. A high-protein (BRS 258) soybean cultivar was found to have

42.29 g/100 g of dry weight of total protein and has higher mineral and bioactive peptide contents, such as lunasin. However, soybean cultivar BRS 133 contains 37.36 g/100 g of dry weight of total protein, accompanied byh higher isoflavones and saponins. Research done by Matsuda and Kubota (2010) has discovered the total soluble protein content of green and red fruit among six tomato cultivars to be different. Approximately seven- to eightfold variation was observed among cultivars for both green and red fruit. Meanwhile different types of proteins were discovered at different stages of ripening in papaya (*Musa acuminate* cv Dwarf Cavendish). A protein with 23 kDa is prominent in immature fruit, however, it decreases with ripening, while several proteins such as a 42-kDa protein, polygalacturonase-related protein, is found to increase from the peclimacteric to the postclimacteric periods. In kiwifruits (Abbot, Allison, and Hayward), total protein content increases significantly during fruit development and then maintains a steady-state level until harvesting time. When kiwifruits are stored at ambient temperature, total protein concentration is increased in all three cultivars significantly. The increase in the total protein content in horticultural produce during maturation might be to help the plant system cope with all the biochemical processes accompanying ripening.

Horticultural products require application of fertilizers in order to sustain crop yields. Nitrogen, phosphorus, and potassium are nutrients which are taken up by plants in relatively large amounts. The application of nitrogen usually causes an incredible increase in the size of certain plant organs and total protein, for example, tuber, however, it brings about a decrease in certain amino acids and proteins such as albumin and globulin, as happens in cereal grains. The application of nitrogen fertilizer at the flowering stage of rice has been shown to increase its protein content. The increase in protein content in milled rice is reflected mainly in the storage proteins, glutelin and prolamin, located in protein bodies. Higher protein content in milled rice will provide consumers with higher protein intake and better eating quality.

Horticultural produce is perishable and subject to continuous damage from the ripening stage to the senescence stage through postharvest storage. They are exposed to different environmental factors that potentially cause unfavorable effects to living tissues. As they cannot avoid exposure to those effects, they have to deal with it through a series of molecular responses. The complex plant response to the unfavorable effects is through the cell signaling pathway and cellular responses, such as the production of stress proteins, upregulation of antioxidants, and accumulation of compatible solutes.

Under low-temperature conditions, a total of 108 differentially accumulated protein spots in citrus were found. The results showed that the proteins are involved in the metabolism and stress response related to sugar and polysaccharide metabolism, secondary metabolism, protein destination and storage, and response to stimulus. Fruits that are prone to chilling injury, as occurred in peach when stored at 0°C, indicated several membrane stability-related

proteins were enhanced, while proteins related to phenolic compound metabolization were repressed. Other commonly identified proteins by low-temperature manipulation included heat shock protein which was well known to play a key role in plant stress responses. Tomato has the capability to accumulate heat shock proteins (HSPs) at low-temperature storage (4°C), which may be responsible for the enhanced chilling injury resistance. Instead of HSPs, peach fruit under cold storage upholds the capacity for ROS scavenging via induction of the expression of genes related to key elements from the antioxidant system, such as GR, CAT, and SOD.

Employing hot treatment storage is a promising postharvest method for reducing disease incidence and severity. Analysis of the proteomic analysis of peach after hot treatment has shown that a large number of identified protein spots have been proposed to play a role in plant metabolism, such as the defense and stress response, cytoskeleton organization, primary metabolism, transcription and translation regulation, and protein storage and catabolism. Interestingly, one-third of the identified proteins correspond to the family of HSP protein and exhibited molecular masses less than 20 kDa.

15.7 FRUITS AND VEGETABLES RICH IN PROTEIN

Table 15.1 outlines the content of proteins of selected fruits as reported by the United States Department of Agriculture (USDA). Among the listed fruits, guava demonstrated the highest protein content, with 2.55 mg/100 g of the edible portion, followed by dates, deglet noor types, with 2.45 mg/100 g of the edible portion. Avocados account for 2.00 mg proteins/100 g of the edible portion, which ranks as the fruit containing the third highest content of protein. Meanwhile other fruit such as apricots, bananas, carambola, red sour cherries, durians, jackfruits, kiwifruits, longan, mulberries, nectarines, pomegranates, and sour soup ranged between 1.00−1.99 mg of proteins/100 g of edible portion. Very low protein content is found in apple, with 0.2 mg of proteins/100 g of edible portion.

The total production of primary vegetable accounted for 1223 million tonnes in 2016 (FAOstat). Table 15.2 summarizes the content of protein of selected raw vegetables as reported by the USDA. Among the listed vegetables, soybeans contain the highest total protein content with 12.95 mg/100 g of the edible portion. This is followed by drumstick leaf and lentil sprouts with 9.40 and 8.96 mg/100 g edible portions, respectively. Some beans such as navy beans, winged beans, and also garlic contain a high content of protein, in the range of 6.0−6.9 mg/100 g of the edible portion. Low protein contents are found in several vegetables (below 1.0 mg/100 g of edible portion) such as celery, chayote, cucumber, eggplant, jalapeno peppers, sweet peppers, radishes, squash, tomatillos, and turnips.

In summary, the protein content in vegetables is demonstrated to be higher than in fruits.

Table 15.1 Protein Content in Selected Fruits (Amount mg/100 g, Edible Portion)

Fruits	g/100 g
Apples, raw, fuji, with skin	0.20
Apricots, raw	1.40
Avocados, raw, all commercial varieties	2.00
Bananas, raw	1.09
Blackberries, raw	0.39
Blueberries, raw	0.74
Carambola, (starfruit), raw	1.04
Cherries, sour, red, raw	1.00
Cherries, sweet, raw	1.06
Cranberries, raw	0.46
Dates, deglet noor	2.45
Durian, raw or frozen	1.47
Elderberries, raw	0.66
Figs, raw	0.75
Gooseberries, raw	0.88
Grapes, red or green (European type, such as Thompson seedless), raw	0.72
Guavas, common, raw	2.55
Jackfruit, raw	1.72
Kiwifruit, green, raw	1.14
Limes, raw	0.70
Litchis, raw	0.83
Longans, raw	1.31
Mangos, raw	0.82
Melons, cantaloupe, raw	0.84
Melons, honeydew, raw	0.54
Mulberries, raw	1.44
Nectarines, raw	1.06
Oranges, raw, all commercial varieties	0.94
Papayas, raw	0.47
Peaches, yellow, raw	0.91
Pears, Asian, raw	0.50
Pears, raw	0.36
Pineapple, raw, all varieties	0.89
Plums, raw	0.70
Pomegranates, raw	1.67
Pummelo, raw	0.76
Raspberries, raw	1.20
Roselle, raw	0.96
Sapodilla, raw	0.44
Soursop, raw	1.00
Strawberries, raw	0.67
Tangerines, (mandarin oranges), raw	0.81
Watermelon, raw	0.61

Source: USDA National Nutrient Database for Standard Reference. From US Department of Agriculture, Agricultural Research Service. Release 28. Home page. https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/.

Table 15.2 Protein Content in Selected Vegetables (Amount mg/100 g, Edible Portion)

List of Vegetables	Range of Protein Content (mg/100 g, Edible Portion)
Celery (raw), chayote (fruit, raw), cucumber (with peel, raw), eggplant (raw), peppers, jalapeno (raw), peppers, sweet (green, raw), radishes (raw), squash (all varieties, raw), tomatillos (raw), turnips (raw), waxgourd, (Chinese preserving melon, raw), yambean (jicama, raw)	0.0–0.9
Beans (snap, green, raw), burdock root (raw), cabbage (raw), carrots (raw), cassava (raw), cauliflower (raw), chard (swiss, raw), chicory greens (raw), endive (raw), fennel (bulb, raw), ginger root (raw), gourd (dishcloth (towelgourd), raw), kohlrabi (raw), leeks, (bulb and lower leaf-portion, raw), lemon grass (citronella, raw), lettuce (raw), mountain yam, (hawaii, raw), mushrooms (chanterelle, raw), mushrooms (maitake, raw), nopales (raw), okra (raw), onions (raw), parsnips (raw), pepper, banana (raw), peppers hot chili, (red, raw), pumpkin (raw), radicchio (raw), rutabagas (raw), sesbania flower (raw), taro (raw), tomatoes (raw), waterchestnuts, (Chinese, matai, raw), yam (raw)	1.0–1.9
Amaranth leaves (raw), asparagus (raw), bamboo shoots (raw), beet greens (raw), broccoli (raw), corn (sweet, white, raw), cornsalad (raw), cress (garden, raw), dandelion green (raw), dock (raw), lotus root (raw), mushrooms (enoki, raw), mushrooms (portabella, raw), mushrooms (shiitake, raw), mustard greens (raw), mustard spinach, (tendergreen, raw), peas, edible-podded (raw), peppers, hot chili (green, raw), potatoes (flesh and skin, raw), purslane (raw), shallots (raw), spinach (raw), squash (zucchini, baby, raw), swamp cabbage, (skunk cabbage, raw), sweet potato leaves (raw), watercress (raw), yardlong bean (raw)	2.0–2.9
Alfalfa seeds (sprouted), artichokes (raw), Brussels sprouts (raw), chives (raw), chrysanthemum (leaves, raw), collards (raw), corn (sweet, yellow, raw), cowpeas (young pods with seeds, raw), mung beans (mature seeds, sprouted, raw), mushrooms (morel, raw), mushrooms (oyster raw), mushrooms (white, raw), parsley (fresh), pumpkin (leaves, raw)	3.0–3.9
Beans (kidney, mature seeds, sprouted, raw), eppaw (raw), fireweed (leaves, raw), jute potherb (raw), kale (raw), taro leaves (raw), wasabi (root raw)	4.0–4.9
Arrowhead (raw), bitter gourd (leafy tips, raw), grape leaves (raw), peas (green, raw)	5.0–5.9
Beans (navy, mature seeds, sprouted, raw), garlic (raw), winged beans (immature seeds, raw)	6.0–6.9
Lentils (sprouted, raw)	8.96
Drumstick leaves (raw)	9.40
Soybeans (green, raw)	12.95

Source: USDA National Nutrient Database for Standard Reference. From US Department of Agriculture, Agricultural Research Service. Release 28. Home page. https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/.

15.8 HEALTH ASPECTS OF PLANT-BASED PROTEIN

Protein is an important nutrients and it exists in varying degrees in plants. It is found to be rich in legumes and pulses, and low in fruits and vegetables, and is essential for the human body. Plant proteins are somewhat compromised by their limitation of one or more amino acids. Plant proteins have a reduced content of essential amino acids such as methionine, lysine, and tryptophan in comparison to animal proteins but provide greater amounts of the nonessential amino acids arginine, glycine, alanine, and serine. As compared to plant-based proteins, animal-based proteins are used more readily and rapidly than plant proteins as they are much more similar to human proteins. Some of the profile differences between animal and plant proteins have been previously distinguished, which in turn gives a unique physiologic effect, especially in the tissue response.

Legumes, such as soybean, are one of the most consumed staple food worldwide. It is one of the richest sources of protein, which is approximately 40% of dry weight. Soybeans are an important protein source in Asian countries. They are utilized in various forms such as tofu (soybean curd), miso (fermented soybean paste), tempeh (fermented soya beans), and other soybased food products. Proteins in soybean are present as storage proteins in the subcellular particles of cotyledons called "protein bodies." The accumulation of protein is high during seed maturation, however it declines as the seed germinates. Proteins in soybean mainly consist of four fractions of globulins, which are classified based on their sedimentation properties: 2S, 7S, 11S, and 15S fractions. About 70%–80% of storage protein is constituted by two protein fractions: glycinin and β-conglycinin. Soybeans also contain biologically active or metabolic proteins, such as enzymes, trypsin inhibitors, hemagglutinins, and cysteine proteases. Soybeans provide a good balance in amino acid composition since all the essential amino acids are contained. In addition, it offers physiologically beneficial components which are shown to lower cholesterol, and reduce the risk of hyperlipidemia and cardiovascular diseases.

Proteins originating from soy protein have been shown to exhibit antioxidant activity. The predominant antioxidant activity of soy protein is through inhibition of lipid peroxidation. The mechanisms of inhibition are related to their biological system through antioxidant enzymes and iron-binding protein and by a nonspecific mechanism. This antioxidant mechanism contributes to the internal antioxidant capacity of the protein and can also be added to food as an additive. The addition of antioxidant protein in many food applications, including the development of foods fortified with antioxidants, will produce healthy foods.

Cereals are the dominant source of carbohydrates in the human diet, providing the major source of energy and contributing significantly to protein intake. The range of proteins in cereals ranges from 5%–15% on a dry weight basis, as found in rice, maize, and barley. Most cereal grains contain a higher

concentration of cysteine in comparison to pulses. Depending on the requirement, cereals such as barley with low protein content will go for malting, while barley with higher protein content is required for animal feeding.

The awareness of people of the good health benefits have been reflected in different personal food choices and preferences. Higher plant-based diets may have advantages as numerous epidemiological studies have shown their beneficial effects in the prevention of cancer, coronary heart disease, and/or many chronic diseases. Since fruits, vegetables, and legumes vary widely in protein and other components, it is expected to have divergent physiological effects. Even though the composition of protein in fruits and vegetables is low, they are commonly mixed with different molecules such as carbohydrates, lipids, antioxidants, and other dietary compounds in a food matrix. Different compositions in the food matrix may provide a better nutritional package which contributes to the beneficial effects of fruits and vegetables.

A great number of proteins from plant sources have been identified and characterized as causing allergic reactions with a variety of symptoms. They can be classified into prolamins superfamily, cupin superfamily, pathogenesis-related proteins (PR-proteins), enzyme and protease inhibitors, and other proteins. This classification is done based on their sequence homology, which is related to conserved three-dimensional structures and possibly function. According to the characteristics of the clinical manifestation, the percentage of allergic individuals reacting to a protein of a given allergenic source, major ($>50\%$) and minor ($<50\%$) allergens can be distinguished. The seed storage proteins, for example, are considered as major allergens of the prolamin superfamily, possibly acting as potent class I food allergens, while profilins and PR-proteins are regarded as class II food allergens.

15.9 METHODS TO DETERMINE TOTAL PROTEIN CONTENT IN FRUITS AND VEGETABLES

Food proteins are very complex and composed of several elements including hydrogen, carbon, nitrogen, oxygen, and sulfur. The building blocks of proteins consist of 20 α-amino acids and they are linked by a peptide bond. Among the elements, nitrogen is the most common element present in proteins. To analyze the protein content in foods, especially in fruits and vegetables, numerous methods have been developed. The basic principles of these methods include the determinations of nitrogen, peptide bonds, aromatic amino acids, dye-binding capacity, ultraviolet absorptivity of proteins, and light-scattering properties. Those analyses are very important to be used for specific purposes, such as nutrition labeling in food products, to investigate functional properties of proteins, and to determine the activity of enzyme. Table 15.3 shows some of the common analyses have been used in determining the protein content in fruits and vegetables.

Table 15.3 Common Method Used to Determine Content of Protein in Fruits and Vegetables

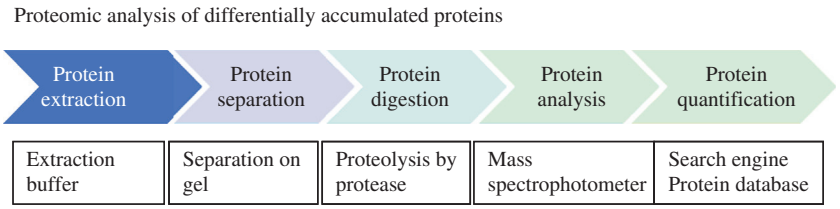
Methods	Principle	Advantages	Disadvantages
Kjeldahl method	Food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. Measures only organic nitrogen plus ammonia	• An official method for crude protein content • Applicable to all types of foods • Inexpensive	• Measures total organic nitrogen, not just protein nitrogen • Time consuming • Poorer precision
Dumas (nitrogen combustion) method	Determination of the total nitrogen content in a usually organic matrix. The sample is combusted at high temperature in an oxygen atmosphere. Via subsequent oxidation and reduction tubes, nitrogen is quantitatively converted to N_2. The total nitrogen released is carried by pure helium and quantified by gas chromatography using a thermal conductivity detector. Measures total nitrogen, including the inorganic fraction (i.e., including nitrate and nitrite)	• Requires no hazardous chemicals • Can be accomplished in a short time • Automated instruments	• Expensive equipment is required
Infrared spectroscopy	Absorption of radiation (near- or mid-infrared regions) by molecules of protein. Different functional groups, backbones, and bonding of protein structure in a food absorb different frequencies of radiation	• Application range from small soluble proteins to large membrane proteins	• Preparation of a stable film can be difficult • The incident light is split into its spectral components • Sensitivity of band intensities on the structure of the metal surface. Structural modifications due to protein interaction with the metal surface

(*Continued*)

Table 15.3 (Continued)

Methods	Principle	Advantages	Disadvantages
Biuret method	Combination of cupric ions with peptide bonds (substances containing at least two peptide bonds under alkaline conditions and measured at 540 nm)	• Simplest method for analysis of proteins	• Not very sensitive as compared to the Lowry method • Interfered with high concentration of ammonium salts
Lowry method	Combination of the biuret reaction with the reduction of the Folin–Ciocalteau phenol reagent (phosphomolybdic phosphotungstic acid) by tyrosine and tryptophan residues in the proteins and measured at 750 nm	• More sensitive than 280 nm UV absorption method • Less affected by turbidity of the sample	• Color is not strictly proportional to protein concentration • Interfered with varying degrees of sucrose, lipids, phosphate buffers, monosaccharides, hexoamines, ammonium sulfate, and sulfhydryl compounds
Bradford dye-binding method	When the dye (Coomassie Brilliant Blue G-250) binds to protein, it changes from reddish to bluish, and the absorption is measured at 595 nm	• Rapid reaction • Reproducible • More sensitive than the Lowry method	• Interfered with by both nonionic and ionic detergents, such as Triton X-100 and sodium dodecyl sulfate • Protein–dye complex can bind to quartz cuvettes • Color varies with different types of proteins
Bicinchoninic acid method	Protein reaction with cupric ions under alkaline conditions to form cuprous ions, which react with bicinchoninic acid (BCA) to form purple color, measured at 562 nm	• More sensitive than Lowry method (sensitivity, procedure, and stability of reagent)	• Any compound capable of reducing Cu^{+2} to Cu^{+} will lead to color formation • Interference of reducing sugars and ammonium sulfate also interfere
Ultraviolet 280 nm absorption method	Absorption in the region at ultraviolet (UV) 280 nm, primarily due to tryptophan and tyrosine residues in the proteins	• Rapid and relatively sensitive • No interference from ammonium sulfate and other buffer salts • Nondestructive samples	• Interference of nucleic acid and other aromatic amino acid can influence the sensitivity

15.10 PROTEOMIC ANALYSIS OF DIFFERENTIALLY ACCUMULATED PROTEINS



Proteomics represents a rapidly developing approach to investigating complex biological processes such as fruit maturation and their postharvest life. This technique provides a full understanding of fruit maturation which includes genetic regulatory elements and biochemical networks and establishes functional correlations between genotypes and phenotypes. Protein profiling in different states of a cell or tissue of fruit can be compared to identify proteins that are affected by different stages of life or conditions of interest. It has recently been used to describe protein changes during ripening in many fruits at different points from development stages towards senescence such as in peach, papaya, banana, tomato, apple, kiwi, and apricot.

The method of sample preparation depends on the purpose of the research and is key to the success of the experiment. For good reproducibility of results, sample preparation is a critical step. The extraction of high-quality protein from recalcitrant, low protein content fruit tissue is always a challenge. Factors such as the solubilities, sizes, charges, and isoelectric points (pI) of the proteins of interest are considerations for sample preparation. Plant cells contain many components that may interfere with the process, for example, pigments, starch, polyphenols, and polysaccharides that can cause inactivation of proteins. The choice of buffer for extraction is also very important to maximize the protein extracted and to get reproducible separation of the protein from the gel.

After the protein is completely extracted, the protein is applied to the gel. Several choices of gels for separation are available such as PAGE, 1D-SDS PAGE, 2D-SDS PAGE, and Western blot. There are many points of comparisons among the techniques, which include separation efficiency and targeting of proteins. Staining such as Coomassie, fluorescent, silver, and negative stains can be used to visualize the protein bands. In certain techniques, for example, 2D-SDS PAGE, imaging software can be applied to measure the protein quantitatively. Using a special scanner, the gel spot can be aligned and the intensity of protein can be determined.

After protein separation on the gel, the entire gel lane is excised and divided into slices prior to proteolytic digestion. A specific protease sequence such as trypsin is utilized to cleave protein into smaller fragments, or peptides.

Separate and sequential digestion can be carried out with alternative proteases to improve targeting protein, following which the peptide fractions are subjected to the second separation in a mass spectrometer. Mass spectrometers (MSs) consist of an ion source, the mass analyzer, and an ion detection system. Analysis of proteins by MS occurs in three major steps: (1) protein ionization and generation of gas-phase ions, (2) separation of ions according to their mass to charge ratio (m/z), and (3) detection of ions. Two main ionization sources, which include matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI), are commonly used for protein identification and characterization. In MALDI the sample of interest is crystallized with the matrix on a metal surface and a laser ion source causes excitation of matrix along with the analyte ions, which are then released into the gas phase. Meanwhile, in ESI, the analyte is ionized from a solution and transferred into the gas phase by generating a fine spray which results in multiple charging of the analyte and generation of multiple consecutive ions. Four major mass analyzers, which are time-of-flight, ion trap, quadrupole, and Fourier transform ion cyclotron, are usually incorporated with the ionization source to separate the ions. Using tandem mass spectrometry (MS/MS), ions are further fragmented to provide information on the protein of interest on account of their specific and characteristic fragmentation patterns. These are searched against sequence databases containing known protein amino acid sequences using database search software. This software will give a statistical score for how well the experimental data match the database sequence. For a positive protein identification, the score has to be above the 95% confidence level. Proteins are then functionally classified according to their primary biological function and relevant data as reported in the literature.

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CHAPTER 16
Enzymes

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16.1 INTRODUCTION

The ripening of fruits is a highly synchronized, genetically programmed, and irrevocable phenomenon encompassing a chain of physiological, biochemical, and organoleptic alterations. These changes, in turn, transform an unripe fruit into an edible ripe fruit with an attractive blend of color, taste, aroma, and texture. These associated changes may happen either at the preharvest stage when fruits are still tethered to the plants or at the postharvest stage. The fruits can be called "climacteric" or "nonclimacteric" on the basis of their ripening mechanism. Climacteric fruits such as mango, banana, avocado, apple, and pear exhibit a steep rise in respiration and ethylene production with the advancement of ripening, whereas, nonclimacteric fruits such as strawberry, citrus, grape, and pineapple do not show such an increase in respiration and often have no requirement for ethylene to complete ripening. However, these distinctions are not always foolproof, as closely related melon and capsicum may behave as climacteric as well as nonclimacteric fruits. On the other hand, nonclimacteric fruits may exhibit enhanced ripening characters in response to exogenous application of ethylene. All fruits undergo a number of modifications during their ripening. The major changes include fruit softening, sweetening, decreased bitterness, and color alterations in ripe fruit, as well as some other desirable quality attributes like aroma and flavor. These ripening changes take place both in climacteric as well as nonclimacteric fruits; however, the level of and response to ethylene may vary from fruit to fruit. Fruit softening is a major factor that decides fruit quality and is thought to be progressed through the cell wall-degrading enzymes. In addition, other metabolic

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changes occurring during fruit ripening also involve a myriad of enzymatic activities and transporters.

Unripe fruits are usually green, sour, odorless, hard, and mealy. As the ripening process progresses, the fruit becomes more appealing with the alterations in skin color due to chlorophyll breakdown and in some cases due to the foundation of new pigments (carotenoids, terpenoids, phenolics, etc.). The acids are broken down, the mealy starches are transformed into sugars, hard pectin is softened, and macromolecules are transmuted into micromolecules which then can emit an aroma. All these changes lead to a soft, juicy, sweet, fragrant, colorful fruit ready for consumption. This makeover of fruit is accomplished by a group of enzymes that are made on cue. The enzymes' coordinated efforts succeed after a ripening signal—a burst of ethylene. Ethylene is a plant hormone produced during fruit ripening which mobilizes the genes to synthesize the enzymes which are responsible for fruit ripening. However, disintegration of the cell wall is highly intricate in ripening fruits due to the wide range of fruit types, encompassing the dismantling of multiple polysaccharide networks by diverse families of wall-modifying enzymes. Although several members of each such family are expressed in the same fruit tissue, it is unclear whether the same reflects functional redundancy. This emphasizes that a clear understanding of cell wall structure, its changes, and the enzymes involved in cell wall degradation is a prerequisite to control ripening in order to initiate and accentuate it. This chapter describes the processes and enzymes involved in fruit ripening, their characteristics, classification, and their role in bringing various compositional changes like fruit softening, cell wall degradation, color, flavor, and aroma changes leading to fruit ripening. Fruits are living commodities, even after harvest, and unlike any other living organism; they carry out a diverse physiological and biochemical transformation for their normal growth and development. These transformations may include seed germination, growth, development, differentiation, fertilization, fruit ripening, softening, color development, etc. In all these processes, enzymes play a pivotal role, without exception. In the absence of enzymes, many of these processes would not occur even over a period of months and years. In fact, at the heart of all these processes lie chemical reactions that are catalyzed by enzymes. The catalytic activity of enzymes depends upon the integrity of their native conformation. The various enzymes involved in fruit ripening come from various enzyme classes. Therefore, a brief overview of enzyme classification and certain general properties of enzymes has been appended before beginning the discussion on ripening and its associated changes.

16.2 ENZYME CLASSIFICATION

The enzymes are grouped into six major classes based on their reactions (see Table 16.1). Enzymes involved in various reactions in this chapter can be identified by their enzyme commission number, the first letter of which corresponds to enzyme class, that is, oxidoreductase, transferase, or any other of the six enzyme classes.

Table 16.1 Classification of Enzyme Based on Their Reactions

Sr. no.	Enzyme Class	Type of Reaction Catalyzed	Examples
1	Oxidoreductases	Oxidation–reduction reactions involving addition and removal of hydrogen, electrons, or oxygen	Lipoxygenase, malate dehydrogenase, alcohol dehydrogenase, ACC (1-aminocyclopropane-1-carboxylic acid) oxidase
2	Transferases	Transfer of functional group from one molecule to another, except hydrogen	DOXP synthase, starch synthase, aminotransferases
3	Hydrolases	Cleavage of molecules involving water as substrate	Chlorophyllase, α-amylase, lipases, esterases (pectin methyl esterase)
4	Lyases	Cleavage of substrate without involving water; double bonds created	Pectate lyase, hydroperoxide lyase, ACC (1-aminocyclopropane-1-carboxylic acid) synthase
5	Isomerases	Isomerization reactions within molecule	Isomerization (carotenoid isomerase, hexose phosphate isomearase) Epimerases (glucose to mannose or galactose) Mutases: phosphoglucomutase, chorismate mutase
6	Ligases	Bond formation coupled with ATP hydrolysis; also called synthetases	Hydroxycinnamoyl-CoA ligase; acetyl-CoA carboxylase

16.3 FRUIT RIPENING

Ripening is the ultimate stage of fruit development that results in enhanced ethylene production along with other associated biochemical alterations. The specific biochemical changes occurring during fruit ripening vary from species to species vis-à-vis variety to variety. Various physiological and biochemical changes generally associated with fruit ripening are as follows:

- Hormonal change (phytohormone ethylene);
- Increase in respiration rate;
- Flesh softening and textural changes;
- Accumulation and exhaustion of organic acids;
- Synthesis of volatile compounds;
- Change in chlorophyll content (chlorophyll degradation);
- Synthesis of other secondary metabolites (terpenoids);
- Synthesis of colored pigments (carotenoid, anthocyanin, and xanthophyll).

16.3.1 Role of Ethylene and Associated Enzymes During Fruit Ripening

The induction and development of ripening are tightly controlled steps in the overall developmental cycle of fruit. Climacteric fruits are harvested when they

are still mature green. Once the fruits are picked, hormones in the fruit convert certain amino acids (methionine) into ethylene gas. Ethylene is a simple colorless gaseous compound containing two carbon and four hydrogen atoms and one double bond (C_2H_4). It is a plant hormone that mainly triggers fruit ripening through a coordinated route which results in multifarious processes. These processes include enhanced respiration and changes in color, texture, and flavor of fruits. In climacteric fruits, ethylene not only induces ripening but also regulates and participates in its progression. Biochemically, the ethylene biosynthesis pathway starts with methionine, a sulfur-containing amino acid (Fig. 16.1). The continuous supply of methionine is ensured by methionine recycling by the Yang cycle. It is synthesized in methionine salvage pathway through the following three steps:

1. Conversion of L-methionine to S-adenosyl-L-methionine (SAM) through the action of enzyme L-methionine–S-adenosyl transferase (SAM synthetase; 2.5.1.6).
2. Formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM through the action of 1-aminocyclopropane-1-carboxylic acid synthase (ACS; 4.4.1.14).
3. Conversion of ACC to ethylene, which is catalyzed by 1-aminocyclopropane-1-carboxylic acid oxidase (ACO; 1.14.17.4).

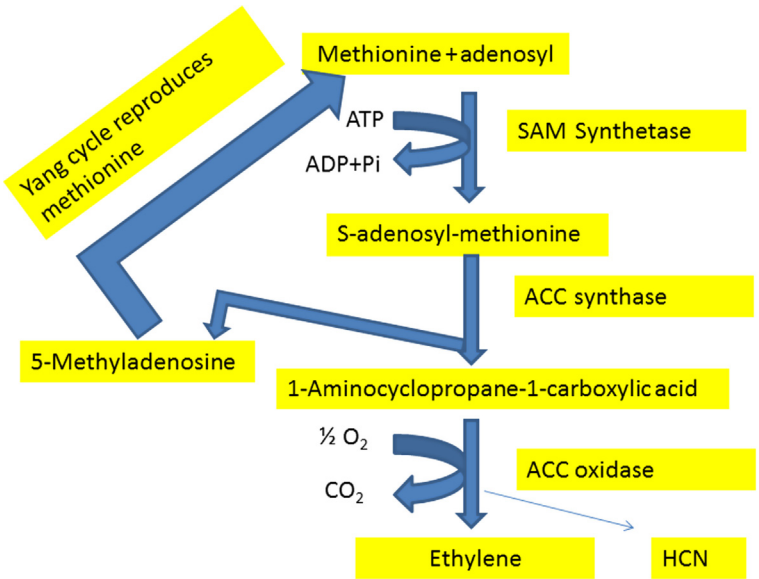

FIGURE 16.1

Ethylene biosynthetic pathway. *Taiz, L., Zeiger, E., 2010. Plant Physiology, second ed. Sinauer Associates, Sunderland, MA, pp. 347–376.*

S-adenosyl-methionine (SAM) is the precursor for ethylene biosynthesis as a major methyl donor. Nearly 80% of cellular methionine is channeled to SAM by the enzyme SAM synthetase. This is an energy-intensive process and accomplished at the expense of ATP. Pyridoxal 5-phosphate acts as an essential cofactor for ACS activity and remains prebound in the active site of unliganded enzyme. The rate-limiting step of ethylene synthesis is the conversion of SAM to ACC by ACC synthase. Finally, ACC is oxidized by ACC oxidase to form ethylene, CO_2, and cyanide. ACC oxidase requires absolutely ascorbic acid, ferrous ion, and dioxygen for its activity, while bicarbonate is required for its activation. The resulting cyanide is detoxified to β-cyanoalanine by β-cyanoalanine synthase (β-CAS, 4.4.1.9). This helps in preventing cyanide-mediated toxicity while ethylene synthesis is at its peak.

The pathway is well established and conserved in higher plants. Both enzymes (ACC synthase/ACS and ACC oxidase/ACO) are limited in unripe fruit but greatly induced during the ripening. The formation of ACC also leads to production of 5-methylthioadenosine, which is recycled via the methionine cycle to produce a new molecule of methionine via the Yang cycle. The genes encoding ACS and ACO are transcriptionally regulated through two distinct ethylene biosynthetic systems. System 1 belongs to low ethylene production and is present throughout the development of fruits, while system 2 is specific to climacteric fruits, and speaks of an autostimulated massive ethylene production. Therefore, climacteric fruits can be differentiated from nonclimacteric ones by the presence of autocatalytic ethylene production.

16.4 CHANGES IN FRUIT RESPIRATION DURING RIPENING

The visible changes in fruit leading to ripening are accompanied by a rapid increase in respiration followed by a decline as fruit undergoes senescence. This steep increase in respiration at the beginning of fruit ripening is called climacteric rise. The climacteric rise in respiration fulfills high energy requirement during the initial stages of fruit ripening. The major storage products of fruits, namely sucrose and starch, are fully oxidized to CO_2 and H_2O with the release of energy (ATP). Major compositional changes can be categorized into the following three stages:

1. Starch degradation (starch phosphorylase, α1,6-glucosidase, α- and β-amylase);
2. Tricaboxylic acid (TCA) cycle and glyoxylate cycle;
3. Sucrose synthesis and degradation (UDP-glucose pyrophosporylase, sucrose phosphate synthase, sucrose phosphate phosphatase).

16.4.1 Starch Degradation (Starch Phosphorylase, α1,6-Glucosidase, α- and β-Amylase)

The products of photosynthetic assimilation (formed in chloroplasts) are either stored as starch in chloroplasts or may be transported via specific

translocators to the cytosol where they are converted into sucrose by a different set of enzymes. Also, some photosynthetic products are translocated from leaf chloroplasts in the form of sucrose to other nonphotosynthetic tissues of plants, where it is reconverted to starch (excess one) and stored in chloroplasts in case of plants like spinach. Starch is composed of amylose (linear; α1-4 glycosidic bonds) and amylopectin (with branching; α1-4 and α1-6 glycosidic bonds). During ripening, starch is broken down to simple sugars so that it can enter respiration. It remains deposited in plastids as water-insoluble granules. Various hydrolytic enzymes listed below carry out its degradation inside plastids where a water molecule is used to break glycosidic bonds between glucose monomers of starch.

1. α-Amylase (3.2.1.1): A hydrolytic endoenzyme which acts on α1-4 glycosidic bonds of amylose and amylopectin randomly. However, the enzyme cannot initiate its action on α1-6 glycosidic bonds or α1-4 glycosidic bonds, which are in the close vicinity of α1-6 glycosidic bonds. Random cleavage gives rise to the majority of maltose units though a few glucose and dextrins are also produced.
2. β-Amylase (3.2.1.2): A hydrolytic enzyme which catalyzes selective cleavage of α1-4 glycosidic bonds of amylose and amylopectin at every second (β) bond and thus produces only maltose. However, similar to α-amylase, it cannot initiate its action on α1-6 glycosidic bonds or α1-4 glycosidic bonds resting in the close neighborhood of α1-6 glycosidic bonds. The enzyme works only from the nonreducing end to degrade short chains and cannot work in the core chain.
3. Starch phosphorylase (2.4.1.1): Starch in plants can be mobilized by a phosphorolytic reaction catalyzed by starch phosphorylase, provided the concentration of inorganic phosphate (Pi) is greater than 1 mM. The enzyme catalyzes an attack by Pi on the last (α1-4) glycosidic linkage joining the last two glucose residues at the nonreducing end, generating glucose-1-phosphate and a polymer one glucose unit shorter (n-1). Phosphorolysis preserves some of the energy of the glycosidic bond in the phosphate ester glucose-1-phosphate. Starch phosphorylase acts repeatedly until it approaches an (α1-6) branch point, where its action stops as is true with α- and β-amylase. Glucose 1-phosphate produced by starch phosphorylase is converted to glucose-6-phosphate by phosphoglucomutase, which catalyzes the reversible reaction. The glucose-6-phosphate thus formed can enter glycolysis or another pathway such as the pentose phosphate pathway and is degraded further.
4. Debranching enzyme (3.2.1.10): The enzyme also called limit dextrinase works on α1-6 glycosidic bonds. It helps both α-amylase and β-amylase as well as starch phosphoryase to continue their action on starch and the production of maltose.
5. α-1-4 Glucosidase (3.2.1.20): Yet again a hydrolytic enzyme which converts maltose disaccharides to glucose monomers by cleaving α1-4 bonding between maltose using water.

These changes are largely found in every fruit tissue collectively called starch to sugar conversion and associated with ripening of most climacteric fruits. The taste of fruit changes when it ripens due to the accumulation of sugar. Thus starch concentration decreases while the sugar concentration increases consequently during ripening of fruit. Even in nonclimacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality.

16.4.2 TCA Cycle and Glyoxylate Cycle Are Responsible for Changes in Organic Acids

Fleshy fruit acidity is an essential component of organoleptic quality. It is due to the presence of organic acids such as malic and citric acids in most ripe fruits. These organic acids are stored in cell vacuoles and cause the sourness of fruits. The predominant organic acid in ripe fruit varies from species to species. Malic acid is dominant in apple, loquat, and pear, whereas citric acid is dominant in citrus fruits. At the initial ripening stage, the fruits are slightly tart or sour due to the presence of these acids. In most fruits, there is a decrease in acidity due to their utilization in the respiratory process during fruit ripening. Malic and oxaloacetic acid (OAA) of cytosolic origin can be converted into tricarboxylates, mostly citric acid. The citric acid thus formed can be transformed into dicarboxylic acids through different pathways: TCA cycle, glyoxylate cycle, γ-aminobutyrate (GABA) shunt, and cytosolic acetyl-CoA catabolism to secondary metabolites. All these conversion reactions can lead to altered acidity of fruit cells. Tricarboxylic acid cycle, the major pathway of cellular respiration can consume citric acid (tricarboxylic acid) and converts it into malate or oxaloacetate (OAA) (both dicarboxylic acids); thus considerably reducing acidity. Malate can be converted to pyruvate by oxidative decarboxylation by a cytosolic NADP-malic enzyme (NADP-ME; 1.1.1.40). NADP-ME is generally involved in lowering the malate content during ripening of many fruits. Oxaloacetic acid (OAA) can be oxidized to malate by an NAD-specific malate dehydrogenase (MDH; 1.1.1.82) or to phosphoenolpyruvate (PEP) by PEP carboxy kinase/PEPCK (4.1.1.32; using ATP) with the elimination of CO_2. Pyruvate also can be converted to PEP using pyruvate orthophosphate dikinase (2.7.9.1; using ATP). The PEP formed thus can be channeled to glucose via gluconeogenesis and finally to sucrose. This causes a reduction of malate content and a consequent decline in acidity during fruit ripening. Glyoxylate cycle (occurring in glyoxysomes, a specialized peroxisome) uses acetyl-CoA from β-oxidation of fatty acid metabolism and finally provides succinate or citrate. Succinate is transformed to malate through the TCA cycle. Citrate released in cytoplasm can be converted to succinate through GABA shunt or by cytosolic citrate lyase (2.3.3.8) to (ATP-dependent) OAA. This is the case for most fruits. In other fruits, such as mango, strawberry, and some cherries, the concentration of acids decreases during ripening but the amount of these compounds per fruit increases to their commercial harvest. The overall decrease in their concentration is due to the dilution effect because of an increase in the size of

the fruits. In banana and lemon, both the organic acid concentration and content per fruit increase throughout ripening because of the action of a cytosolic enzyme PEP carboxylase which converts PEP to OAA and consequently an increase in acidity.

16.4.3 Sucrose Synthesis and Degradation

Sucrose is the most abundant disaccharide containing glucose and a fructose molecule as fruit sugars. It is the major form of sugar translocated from photosynthetic to nonphotosynthetic tissues via phloem. Its synthesis occurs in cytoplasm of photosynthetic cells in the following way:

$$\text{UDP} - \text{glucose} + \text{Fructose} - 6 - \text{phosphate} \xleftrightarrow{\text{Sucrose phosphate synthase (2.4.1.14)}} \text{Sucrose} - 6 - \text{phosphate} + \text{UDP}$$

$$\text{Sucrose} - 6 - \text{phosphate} + H_2O \xleftrightarrow{\text{Sucrose phosphate phosphatase (3.1.3.24)}} \text{Sucrose} + \text{Pi}$$

Sucrose is translocated from cytosol of photosynthetic cells to nonphotosynthetic tissues (roots, tubers, and seeds) and may be stored temporarily as sucrose or further converted to starch. For this, sucrose is catalyzed to its monomers by sucrose synthase.

$$\text{Sucrose} + \text{UDP} \xleftrightarrow{\text{Sucrose synthase (2.4.1.13)}} \text{Fructose} + \text{UDP} - \text{glucose}$$

Furthermore, the activated precursor for starch biosynthesis is ADP-glucose, so UDP is replaced with ADP and ADP-glucose can then enter starch synthesis via starch synthase.

$$\text{UDP} - \text{glucose} + \text{PPi} \xleftrightarrow{\text{UDP} - \text{glucose} - \text{pyrophosphorylase (2.7.7.9)}} \text{Glucose} - 1 - \text{phosphate} + \text{UTP}$$

$$\text{Glucose} - 1 - \text{phosphate} + \text{ATP} \xleftrightarrow{\text{ADP} - \text{glucose} - \text{pyrophosphorylase (2.7.7.27)}} \text{ADP} - \text{glucose} + \text{PPi}$$

16.5 ROLE OF CELL WALL-DEGRADING ENZYMES IN FRUIT RIPENING AND SOFTENING

Textural changes that lead to softening of fruits are accompanied by the loss of neutral sugars, solubilization, and depolymerization of the polysaccharides of the cell wall, and further rearrangements of their associations, due to the combined action of several cell wall-modifying enzymes, acting in pectic, cellulosic, and hemicellulosic fractions. It is generally recognized that the degradation of the polysaccharide components of the cell wall and reduction of cell-to-cell adhesion, as a result of middle lamella degradation,

are the foremost factors causing softening of the fruit. Major polysaccharides of the cell wall, like pectin, cellulose, and hemicellulose are transmuted during ripening to their simple counterparts. Pectins are thechief components of primary cell wall and middle lamella that contribute toward fruit texture and quality. Degradation of these pectins while ripening leads to fruit softening due to removal of polymeric or single-sugar side chains, methyl-ester or acetyl groups from homogalactouronic acids, cleavage of polymeric backbones, along with associated loosening of hydrogen bonding between cellulose microfibrils and glycans. Fruit softening is a physiological phenomenon accompanied by numerous biochemical changes occurring in the cell wall. Various enzymes responsible for cell wall degradation and softening of fruit during ripening can be categorized into four principal groups:

1. Pectinases (including depolymerization and de-esterification);
2. Cellulases;
3. Hemicellulases;
4. Other texture-softening enzymes.

16.5.1 Pectinases

Pectins rank third as cell wall constituents after cellulose and hemicellulose, and form approximately 35% of the dry weight of dicot cell walls. Fruits and vegetables contain considerable amounts of pectin that contribute to the strength of their tissues. Unripe fruits are usually hard due to the presence of pectin in the primary cell wall. The main component of pectin backbone is galacturonic acid residue linked by α-1-4 linkages with neutral sugars, notably arabinose, galactose, and xylose, present in pectin side chains. On the other hand, rhamnose is present in minute amounts. The carboxyl groups of galacturonic acid are partially esterified by methyl groups and partially or fully neutralized by sodium, potassium, or ammonium ions. Pectinases break down pectins into simpler molecules like galacturonic acids. The pectinases group of enzymes can further be classified into depolymerizing (polygalactouronaes [endo- and exo-type] and pectin lyases) and saponifying enzymes (pectin methyl esterases), each having a discrete function as given below:

- Pectin methylesterase (PME; 3.1.1.11): This removes methyl ester groups from galacturonic acid (GalA) residues leaving charged free carboxylic acid groups at the sixth position. The de-esterified pectin becomes a suitable substrate for both polygalactouronases (PGs).
- Endo-polygalacturonase (EndoPG; 3.2.1.15): Endo-PGs split α-1,4-D-galacturonan linkages in homogalactouronan (HG) segments. They act on galactouronic acid residues which are generally nonesterified substrates. The enzyme attacks randomly on its substrate and produces a number of GalA oligosaccharides.
- Exopolygalacturonase (ExoPG; 3.2.1.15): Exo-PG attacks the substrate from the nonreducing end and removes terminally linked

α-1,4-D-galacturonan residues from HG chains. The enzyme requires nonesterified GalA units as its preferred substrate.

- Pectate lyase (PL; 4.2.2.10): A lyase acts on sites α-1-4 linked GalA residues which are even esterified and at internal sites. The reaction proceeds via β-elimination and does not require water.
- Rhamnogalacturonan hydrolase (RGH; 3.2.1.171): Rhamnogalacturonan (RG) hydrolase acts on the α-D-1,4- GalA-α-L-1,2-Rha linkage using water in the RG-I (rhamnogalatouronan-I) backbone. Rhamnose remains at the nonreducing side. The enzyme's activity ceases for acetyl-esterification of the RG-I backbone.
- β-Galactosidase (3.2.1.23): Exo-acting enzymes that remove the galactan side chain of RG-I.
- α-Arabinosidase (3.2.1.99): Exo-acting enzymes that remove the arabinan side chain of RG-I.
- Other important enzymes for pectin breakdown include rhamnogalacturonan rhamnohydrolase (RGRH), rhamnogalacturonan galacturono hydrolase (RGGH), endo xylogalacturonan hydrolase (XGH), each with specific activities in pectin breakdown.

16.5.2 Cellulases

Cellulose is the most abundant polysaccharide on Earth. It is a linear polymer of β1-4 glucose residues present in the primary cell wall of plants, including fruits and vegetables. During ripening, it is degraded to a certain extent in conjunction with pectin degradation. Major cellulases which can degrade cellulose include endo-glucanase (3.2.1.4), exoglucanse (3.2.1.91), and β-glucosidase (3.2.1.21). Endo 1,4-β-glucanase attacks randomly at multiple internal sites in the amorphous regions of cellulose fibers and cleaves β1-4 glycosidic bonds to form crystalline cellulose. Exoglucanase/cellobiohydrolase acts on crystalline cellulose and converts it into glucose monomers, dimers, or oligomers from one end of the cellulose chain. β-Glucosidase hydrolyzes the β-1-4 linkage between dimers and oligomers to produce glucose units. However, the role of cellulases alone is of less significance in fruit ripening and cellulose microfibrils do not appear to be degraded during ripening in most fruits, except a few like avocado.

16.5.3 Hemicellulases

Hemicelluloses represent a group of heterogeneous polysaccharides which originate through biosynthetic routes other than that of cellulose. These are present in considerable amounts (25−30%) and form a part of secondary cell wall structure along with lignins. These are composed of most of the neutral sugars like mannose, glucose, arabinose, xylose, rhamnose, galactose, etc. Hemicelluloses include xylans, arabinoxylan, glucronoxylan, glucomannan, and xylomannan. While fruit ripening, following enzymes help in degrading hemicelluloses to simple sugars:

- Endo-1,4-β-glucanase (EGase; 3.2.1.151): EGase cleaves the glucan backbone of xyloglucan chains at internal sites.
- Endo-1,4-β-xylanase (EXase; 3.2.1.155): The enzyme is responsible for cleavage of xylan backbone of glucuronarabinoxylan or unsubstituted xylan at internal sites.
- Endo-1,4-β-mannanase (EMase; 3.2.1.78): Cleavage of the mannan backbone of mannan, galactomannan, or glucomannan at internal sites randomly is carried out via hydrolysis by EMase.
- Xyloglucan endotransglycosylase (XET; 2.4.1.207): The XET catalyzes internal cleavage of glucan backbone of xyloglucan and rejoins the end to another xyloglucan molecule. Its action is of utmost importance during germination, fruit ripening, as well as during cell wall expansion. Xyloglucan remains linked with cellulose in primary cell walls.

16.5.4 Other Texture-Softening Enzymes

In addition to the above, some other texture-softening enzymes may also be present in ripening fruits. These may include glycosidases, α-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, α-xylosidase, and β-xylosidase. A nonenzymatic protein called expansin has also been reported, which aids in loosening of hydrogen bonding between cellulose microfibrils and matrix glycans. It might allow enzymes resident in the cell wall space access to substrates that were normally unavailable. Loosening of the xyloglucan–cellulose network by expansin may be required for normal fruit softening. Suppression of a tomato ripening-related expansin resulted in firmer fruit throughout ripening, while three-fold overexpression of the protein caused a striking increase in softening.

16.6 FLAVOR AND AROMA CHANGES

Fruit aroma is a vital indicator to reflect the quality of fruit. Flavor is the delicate and complex perception that combines taste, smell, and mouth feel. In general, the typical flavor of a fruit is not present at harvest and develops during ripening. Ripe fruits have intense aroma and flavor. This development of flavor encompasses the generation of volatiles to alter aroma, the production of sugars and their interconversions, decline in acidity through altered organic acid metabolism, and a reduction in astringency through lower levels of tannins and phenolics. These aromatic volatile compounds are highly diverse in nature and comprise esters and lactones, alcohols, acids, aldehydes, ketones, acetals, phenols, ethers, terpenes, apo-carotenoids, etc., to participate in this aromatic perception. In addition, some sulfur compounds also contribute to the flavor of fruits such as melons. Flavor and aroma changes in fruits and vegetables are mediated by a certain set of pathways associated with typical enzymes which are described here under.

16.6.1 β-Oxidation of Fatty Acids

β-Oxidation is primarily involved in the degradation of fatty acids. In plants, it happens to be in mitochondria, though some fatty acids are metabolized in peroxisomes. β-Oxidation is carried out through four enzymatic reactions by three proteins, namely acyl-CoA oxidase, a multifunctional protein containing domains for four enzymatic activities (2-*trans*-enoyl-CoA hydratase, l-3-hydroxyacyl-CoA dehydrogenase, δ-3-hydroxyacyl-CoA epimerase, and Δ3,Δ2-enoyl-CoA isomerase), and l-3-ketoacyl-CoA thiolase. During β-oxidation, acylation of fatty acids occurs in cytosol. Each cycle is repeated with the oxidative removal of two carbon atoms in the form of acetyl-CoA from the carboxyl end of a fatty acid until it is completely oxidized. The β-oxidation of long-chain fatty acids produces acetic, butanoic, and hexanoic acids, which can be further reduced to their corresponding alcohols by alcohol dehydrogenase (ADH; 1.1.1.1) in nicotinamide adenine dinucleotide (NAD^+)-dependent reaction. The alcohols are further esterified by the alcohol acyltransferase (AAT; 2.3.1.84) enzyme. Since acetyl-CoA is the main acyl-CoA produced in β-oxidation, most of the esters are acetate esters. Pear and apple aromas are two classical examples of aroma executed by the β-oxidation pathway. This pathway gives rise to major aroma compounds, namely γ-decalactone and γ-dodecalactone in peach and nectarine, δ-octalactone in pineapple, and γ-octalatcone in coconut.

16.6.2 Lipoxygenase Pathway

The first dedicated step in straight-chain ester formation is attributable to lipoxygenase (LOX; a dioxygenase) through the stereospecific oxidation of unsaturated free fatty acids. Lipoxygenase (1.13.11.12) catalyzes the oxygenation reaction of polyunsaturated fatty acids comprising a *cis*-1,4-pentadiene conformation to yield a conjugated diene (*cis*, *trans*) hydroperoxide (HPO). Its main substrates include linoleic (18:2) and linolenic (18:3) acids, which in turn emanate from triacylglycerols, phospholipids, and glycolipids due to the action of acyl hydrolases. Here linoleic or linolenic acid reacts with molecular oxygen. Most of the type-1 LOXs produce 9-hydroperoxide, and type-2 LOXs create exclusively 13-hydroperoxide derivatives from polyunsaturated fatty acids. These hydroperoxy fatty acids are highly reactive and hazardous for the plant and thus further metabolized by other enzymes such as hydroperoxide lyase (HPL; 4.2.9.9), allene oxide synthase (4.2.1.92), peroxygenase (1.11.2.1), and divinyl ether synthase (4.2.1.121). These compounds are converted by HPL to aldehydes such as hex-3-enal and hex-2-enal. The HPL enzyme is related to the cytochrome P450 CYP74B/C family and converts hydroperoxides to short-chained aldehydes (six or nine carbon atoms). The resultant aldehydes are afterward reduced to the corresponding alcohols by the enzyme ADH. ADH (1.1.1.1) is an oxidoreductase that catalyzes the reversible reduction of aldehydes to corresponding alcohols with NADH and NADPH as cofactors. The resulting alcohols serve as the natural substrates for the AAT (2.3.1.84) enzyme, which transfers an acyl group mediated through an

oxygen-dependent reaction from acyl-CoA to the alcoholic −OH group, thus generating an ester. The esters generally contribute "fruity" and "sweet" flavor. Aroma-producing enzymes are upregulated by ethylene during ripening.

16.6.3 Amino Acid Degradation

Standard branched chain amino acids, isoleucine (Ile), leucine (Leu), and valine (Val), give rise to volatile aromatic compounds, that is, aldehydes, alcohols, acids, and esters in ripening fruits. These aromatic compounds are the second most important source of volatile compounds for the aroma of fruits and vegetables. Amino acids can undergo an initial deamination or transamination by branched-chain amino transferase (2.6.1.42) enzyme leading to the formation of the corresponding α-keto acid (2-oxo-3-methyl pentanoate and 2-oxo-3-methyl butanoate). However, decarboxylation followed by reductions, oxidations, and esterifications ultimately results in the formation of aldehydes, acids, alcohols, and esters.

16.6.4 Terpenes as Flavor and Aroma Compounds

The flavor and aroma of certain fruits, such as particular grape varieties, are governed by low-molecular-weight volatile aldehydes and monoterpenes (C10). Carotenoid-derived volatile terpenoid compounds serve as major parts of flavor and aroma in many fruits and vegetables.

16.7 TERPENES/TERPENOID PATHWAY

The terpenes, or terpenoids, constitute the largest class of secondary metabolites, having more than 30,000 products. The diverse substances of this class are generally insoluble in water and biosynthesized by acetyl CoA or glycolytic intermediates. All terpenes result from amalgamation of five-carbon compounds that have a branched carbon skeleton of isopentane. The basic structural elements of terpenes are sometimes entitled isoprene units as terpenes can decompose at higher temperatures to form isoprene. Thus, terpenes are denoted as isoprenoids. Hemiterpenes, monoterpenes, and sesquiterpens like low-molecular-weight terpenoids generally remain volatile in nature and contribute to the typical flavor and aroma of certain plant species. Notable among these are isoprene, eucalyptol, geraniol, linalool, pinene, myrcene, gingiberine, cryophyllene, β-farnesene, etc. Terpenes are classified by the number of five-carbon units they comprise.

All terpenoids are produced by the universal five carbon precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Terpenoid biosynthesis comprises mostly of head to tail addition of IPP to DMAPP, which are derived from two alternate biosynthetic pathways localized in different subcellular compartments. A major pathway operating in cytosol for IPP and DMAPP synthesis is known as the mevalonic acid (MVA) pathway, originating in the condensation of three acetyl CoAs. Another pathway operates in plastids known as the 2-C-methylerythritol-4-phosphate (MEP) pathway,

leading to the formation of IPP and DMAPP from pyruvate and glyceraldehyde-3-phosphate, originated from carbohydrate metabolism. Although the subcellular compartmentalization of two pathways has allowed both to operate independently, yet there exists metabolic crosstalk between the two pathways.

The MVA pathway occurring in cytosol starts with the condensation of two acetyl-CoA molecules to yield acetoacetyl-CoA by the enzyme acetyl-CoA acetyl transferase (2.3.1.9). Another molecule of acetyl-CoA joins with acetoacetyl-CoA in the presence of enzyme hydroxymethyl glutaryl-CoA synthase (2.3.3.10) to form β-hydroxy-β-methyl-glutaryl-CoA (HMG-CoA). The latter is acted upon by an enzyme HMG-CoA reductase (HMGR; 1.1.1.34) and gets into mevalonic acid. The enzyme uses two $NADPH + H^+$; CoASH gets released and $>C=O$ is reduced to CH_2OH (methylene) group. The HMGR is a highly conserved enzyme in eukaryotes and catalyzes the rate-limiting step of IPP biosynthesis in the MVA pathway. In higher plants, the enzyme HMGR is encoded by a multigene family of the nuclear genome. HMGR activity is highly responsive toward an array of environmental and physiological signals including light inhibitors, growth regulators, phosphorylation, metabolic feedback, wounding, etc. Mevalonic acid produced is subsequently converted to isopentenyl pyrophosphate (IPP), the first stable 5C compound. IPP is isomerized to its isomer, dimethylallyl pyrophosphate (DMAPP) by an isopentenyl diphosphate isomerase (5.3.3.2). Both IPP and DMAPP are the activated building blocks of terpene biosynthesis that join together to produce larger molecules. First, IPP and DMAPP react to give geranyl pyrophosphate (GPP), with the aid of an enzyme geranyl di-phosphate synthase/dimethylallyl transferase (GDS; 2.5.1.1) by eliminating a pyrophosphate. The consequential 10-carbon precursor, GPP, gives rise to nearly all the monoterpenes, the reaction being catalyzed by terpene synthase (TPS; 4.2.3.47). GPP can then bind to another IPP molecule to generate the 15-carbon compound, farnesyl pyrophosphate (FPP). The reaction is catalyzed by enzyme fornesyl diphosphate synthase (FDS; 2.5.1.68). The parental precursors (GPP and FPP) enter into several structural modifications through oxidation, reduction, isomerization, hydration, conjugation, and/or other transformations, ultimately contributing to a myriad of terpenoids. The FPP acts as the precursor of nearly all the sesquiterpenes and the reaction is catalyzed by a TPS. The addition of one more molecule of IPP mediated by geranylgeranyl diphosphate (GGDP) to FPP gives rise to the 20-carbon compound geranylgeranyl pyrophosphate (GGPP), the precursor of the diterpenes. Also, FPP can dimerize to give the squalene which gives rise to triterpenes (C_{30}) by squalene synthase and steroids, while dimerization of GGPP yields phytoene which vintages the tetraterpenes (C_{40}).

An alternative MEP pathway occurring in plastids is involved in the formation of IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (GAP). The pathway comprises seven enzymatic steps (Fig. 16.2). The first committed step of the pathway is catalyzed by thiamine pyrophosphate-dependent DOXP

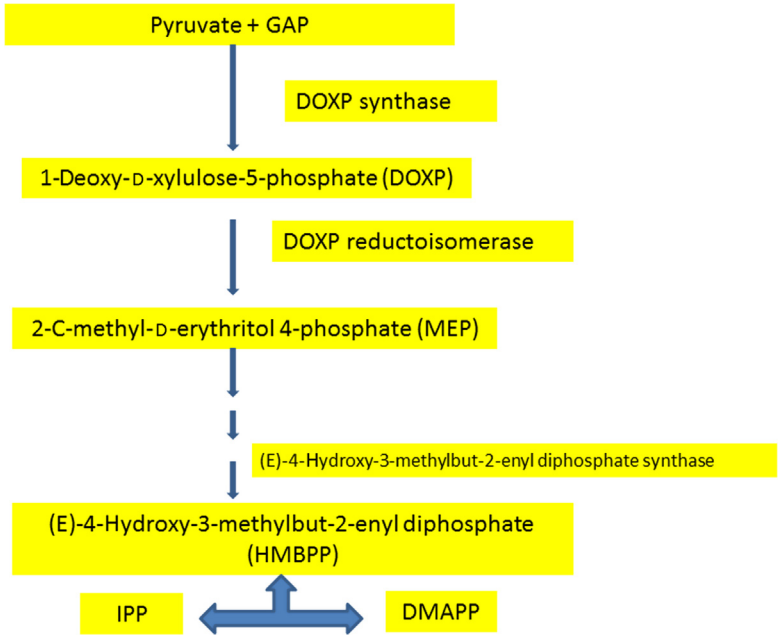

FIGURE 16.2

Plastidial MEP pathway of terpenoid biosynthesis. *Nagegowda, D.A., 2010. Plant volatile terpenoid metabolism: biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS Lett. 584, 2965–2973.*

synthase (DXS; 2.2.1.7) via a transketolase-like decarboxylation reaction from pyruvate and GAP to yield 1-deoxy-D-xylulose-5-phosphate (DOXP). The enzyme requires Mg^{2+} or Mn^{2+} for its activity. The DOXP is then transmuted into 2-C-methyl-D-erythritol 4-phosphate (MEP) by DOXP reductoisomerase/MEP synthase (DXR; 1.1.1.267). The metabolite MEP is the first stable intermediate and consequently owes its name to this pathway. MEP generation occurs through an intramolecular rearrangement cum reduction catalyzed by enzyme DXR using NADPH as coenzyme. A series of enzymatic reactions ultimately give rise to IPP and DMAPP, which can be ultimately converted to various terpenoids as discussed earlier.

16.8 COLOR CHANGES AND ASSOCIATED METABOLIC PATHWAYS

Ripening is associated with color change in many fruits. These color changes in ripened fruits and vegetables are due to chlorophyll (green in capsicum), phaeophytin (in cucurbits), carotenoids, flavonoids, and anthocyanins. Specific secondary metabolites are often inculcated in only one plant species or a taxonomically related group of species, on the other hand, the basic primary metabolites are ubiquitous to the plant kingdom. Each plant secondary

metabolite contributes toward significant biological and physiological functions impacting on plant survival. Bananas, apples, plums, berry-fruit, and stone-fruit are some noteworthy examples where color remains a principal indicator of ripeness. The gold, orange, and red colors of many fruits, such as tomato and citrus, are formed by enzymes in the carotenoid biosynthetic pathway. Carotenoids are divided into two classes: the hydrocarbon carotenes, for example, lycopene (red) and β-carotene (orange); or the oxygen-containing xanthophylls, for example, lutein (yellow). Other red and purple pigments in grapes and boysenberries are due to anthocyanins, an offshoot of the phenylpropanoid pathway. Also, loss of chlorophyll or removal of central magnesium ion to give pheophytin contributes to color changes while ripening in fruits and vegetables.

A myriad of enzymes are responsible for color changes through three to four basic pathways of secondary metabolism. In addition to color changes, these secondary metabolic pathways also yield metabolites/products destined for various other functions ranging from aroma, flavor, aesthetics, defense, rigidity, pollination, reproduction, and many more. Color changes while ripening can be attributed to these three secondary metabolic pathways:

1. Chlorophyll degradation;
2. Carotenoid biosynthetic pathway;
3. Phenylpropanoid biosynthetic pathway.

16.8.1 Chlorophyll Degradation

Chlorophyll (Chl) is the most abundant pigment in plants and has Mg^{2+} ions in its center with four pyrrole rings, one of which is bonded to a phytol tail. The degradation of Chl during ripening is the most miraculous process where total green color disappears. For example, the ripening phase of many fruits, such as banana (*Musa acuminata*) and tomato (*Lycopersicon esculentum*), embraces massive degradation of Chl. The process of Chl breakdown is a multistride, perfectly controlled process taken care of by six Chl catabolic enzymes and a metal-chelating substance (MCS). Final catabolites of chlorophyll degradation are transported via specific transporters to the central vacuole. Since the Chl molecule has light-absorbing properties, it may act as a potential cellular phototoxin during high light intensity. The overexcited photosynthetic apparatus can transfer energy to oxygen, which in turn results in the production of reactive oxygen species. Chlorophyll breakdown ensues to be a prerequisite to detoxify this potential phototoxic pigment inside vacuoles in order to permit the remobilization of nitrogen from Chl-binding proteins. Chlorophyll is degraded to a group of colorless linear tetrapyrroles called phyllobilins through a pathway common to higher plants. The pathway can be divided into two parts: (1) early reactions with colored pigments as substrates, ending with the generation of a "primary" colorless breakdown product termed pFCC (blue primary fluorescing chlorophyll catabolite) and (2) in second phase, this pFCC is either retained or gets converted into their respective

nonchlorophyll catabolites (NCCs) inside the vacuole via modifying reactions using the nonenzymatic isomerization.

The pathway of Chl breakdown is known as the pheophorbide A oxygenase (PAO)/phyllobilin pathway because here pheophorbide (i.e., magnesium- and phytol tail-free chlorophyll) arises as a core intermediate. The PAO pathway drives in senescing leaves and during ripening of mostly fruits. The pathway is compartmentalized between chloroplast and vacuoles of above tissues. The first phase from chlorophyll to pFCC happens to be in the chloroplast and is common to all plants. The conversion of pFCC generally ensues in vacuoles and results in species-specific modification of Chl breakdown products. During fruit ripening, chloroplasts get converted to chromoplasts. Conversion of Chl "b" to Chl "a" is a crucial step in Chl catabolism and is catalyzed by two enzymes in a two-stepped process: NADPH-dependent Chl(ide)[chlorophyllide] *b* reductase (CBR; 1.1.1.294) and ferredoxin-dependent 7-hydroxymethyl Chl(ide) *a* reductase (HCAR; 1.17.7.2). Both give rise to Chl "a" and the reaction takes place at thylakoid membrane. Chl(ide) b reductase is the only enzyme of Chl catabolism thought to be a bound enzyme. Chl "a" is converted to chlorophyllide Chl(ide) by enzyme chlorophyllase (3.1.1.14). Chlorophyllase, a membrane-bound esterase, catalyzes the hydrolytic cleavage of ester bond to yield hydrophobic thylakoid-anchoring phytol chain and chlorophyllide (porphyrin ring), which retains the typical green color.

$$\text{Chl b} \xleftarrow{\text{Chl b reductase}} \text{C7} - \text{hydroxy chlorophyllide a} \xleftarrow{7 - \text{hydroxymethyl Chl(ide) a reductase}} \text{Chl a}$$

Further, a low-molecular-weight compound named MCS removes the Mg^{2+} ion from chlorophyllide to yield pheophorbide "a." However, the structure of this MCS and exact mechanism are still unknown. Pheophorbide a oxygenase (PaO; 1.14.15.17), a chloroplast envelope-bound enzyme, catalyzes the cleavage of the porphyrin ring, resulting in the red chlorophyll catabolite (RCC), which is further reduced by the enzyme RCC reductase to a nonphototoxic pFCC (still green). The pFCC is transported from the senescing chloroplasts/chromoplasts to the vacuole, where the net acidic pH of the vacuole converts pFCC to NCCs and are stored indefinitely. From senescent chloroplasts, these catabolites are directed to the vacuole through primary activated transport processes.

16.8.2 Carotenoid Biosynthetic Pathway

Carotenoids, naturally occurring pigments, occupy second slot in terms of abundance, having more than 750 members. The polyene backbone of carotenoids comprises 40 carbons along with conjugated double bonds, while ring structures are present at the ends. This conjugated double-bond system permits carotenoids to engross light of the visible region and consequently vintage colorless to yellow, orange, and red colors with variations reflected in many fruits, flowers, and vegetables. Notable examples include β-carotene

from carrots and sweet potatoes, lycopene from tomatoes and watermelon, capsanthin and capsorubin from red peppers, and lutein from marigold flowers. The vibrant yellow, orange, and red colors of many horticultural crops are credited to high levels of carotenoid accumulation in their chromoplasts.

As carotenoids fall into a subgroup of terpenoids, the former are derived from two isoprene isomers, IPP and its allylic isomer DMAPP. The IPP and DMAPP used for carotenoid biosynthesis in plants originate in the plastidic MEP pathway (Fig. 16.2). Two enzymes of the MEP pathway of terpene biosynthesis, DXS and DXR, are imperative to flux regulation toward carotenoid biosynthesis. In *Arabidopsis*, both enzymes are encoded by single genes and it is noted that both serve as rate-determining enzymes, possibly being regulated in feedback manner. The overexpression of both in *Arabidopsis* seedlings increased carotenoid production. Both IPP and DMAPP, after undergoing a number of subsequent steps and a sequential series of condensation reactions, yield the precursor of carotenoid biosynthesis, GGPP. Synthesis of carotenoids takes place in all types of differentiated plastids but accumulation happens to be at the high levels in green tissue chloroplasts vis-à-vis in the chromoplasts of roots, fruits, and flower petals. Carotenoid biosynthesis starts up with the head-to-head condensation of two GGPP molecules catalyzed by phytoene synthase (PSY; 2.5.1.32) which leads to generation of 15-*cis*-phytoene, the first colorless carotenoid. There are multiple PSY genes in horticultural crops exhibiting tissue-specific expression, for example, PSY1 in fruits and PSY-A in all tissues including fruits. Phytoene (colorless, C40 compound) undergoes a series of desaturations by phytoene desaturase (PDS; 1.3.99.31) and ζ-carotene desaturase (ZDS; 1.3.5.6) to introduce *cis*-double bonds, accompanied by isomerization reactions being carried out by ζ-carotene isomerase (Z-ISO; 5.2.1.12) and carotenoid isomerase (CRTISO; 5.2.1.13) to convert the *cis*-configuration back into the *trans*-configuration. The reactions of desaturases and isomerases finally yield red-colored all-*trans*-lycopene, the principal red pigment of tomato and watermelon. It is also the first colored carotenoid of the pathway. A total of four double bonds (7,9,7′,9′) are introduced by both desaturases by catalyzing two symmetric dehydrogenation reactions converting 15-*cis* phytoene to tetra-*cis*-lycopene. A carotenoid isomerase (CRTISO) isomerizes *cis*-double bonds at 7, 9 and 7′, 9′ positions of tetra-*cis*-lycopene to all-*trans*-lycopene. The isomerase activity of CRTISO employs reduced flavin adenine dinucleotide as cofactor (FADred) to catalyze net electron transfer without any redox change. In plants, all-*trans*-lycopene is the preferred substrate for the cyclases. The cyclization of lycopene is a decisive step in carotenoid metabolism, creating a branch point in the pathway and spawns carotenoid diversity distinguished by different cyclic end groups in the form of a β-ring and/or ε-ring. Two different enzymes, lycopene β-cyclase (β-LCY; 5.5.1.19) and lycopene ε-cyclase (ε-LCY; 5.5.1.18), generate these rings. β-LCY carries out cyclization of two β-rings of all *trans*-lycopene (red-colored) and converts it into β-carotene

through γ-carotene intermediate. On the other hand, ε-LCY mediates cyclization of one β- and one ε-ring, thus forming the b,ε branch of carotenoids. The latter is first converted to δ-carotene and finally to α-carotene. Both α- and β-carotenes are orange in color. Carotenoids containing only hydrocarbons are clustered as carotenes. These consist of phytoene (colorless) and all sorts of carotenes (lycopene, α- and β-carotenes). The addition of oxygen by hydroxylases and epoxidases to cyclic carotenes produces xanthophylls (lutein and xanthin) and their derivatives, which are termed carotenoids. β-Carotene is hydroxylated by β-carotene hydroxylase (CHYB; 1.14.13.129) into β-cryptoxanthin and zeaxanthin. Zeaxanthin can then finally be converted to neoxanthin by the action of enzymes zeaxanthin epoxidase (ZEP; 1.14.13.90), violaxanthin de-epoxidase (1.10.99.3), and neoxanthin synthase (5.3.99.9). However, α-carotene is contracted by two different cytochrome P450 type hydroxylases, named CYP97A and CYP97C. CYP97A hydroxylates β-ring to yield zeinoxanthin and finally to lutein (3,3′-dihydroxy-α-carotene), while CYP97C hydroxylates ε-ring to generate α-cryptoxanthin which ultimately gets converted to lutein by the same enzyme. Lutein and other xanthins are yellow in color. Lutein is abundant in marigold and daffodil flowers and in dark green leafy vegetables. In red pepper and tiger lily, zeaxanhin can be further converted by capsanthincapsorubin synthase (CCS; 5.3.99.8) into capsanthin and capsorubin (k-cyclic carotenoids with an unusual cyclopentane ring), the main carotenoids that engender the characteristic red and orange colors of these species. The CCS enzyme is a member of the lycopene cyclase family and shares high sequence similarity with LCYB enzyme. Carotenoids are catabolized enzymatically by a family of carotenoid cleavage dioxygenases/oxygenases to produce apo-carotenoids.

16.8.3 Phenylpropanoid Biosynthetic Pathway

Phenylpropanoids are a diverse group of compounds derived from the carbon skeleton of phenylalanine that are involved in plant defense, structural support, and survival. Phenylpropanoid metabolism sits at the interface of primary and secondary metabolism. Phenylpropanoid-based polymers, like lignin or condensed tannins, contribute substantially to the stability and robustness of gymnosperms and angiosperms toward mechanical or environmental damage, like drought or wounding. The magnificent diversity of phenylpropanoids is due to the proficient modification and amplification of a very limited set of core structures, derived from the shikimate pathway. The importance of lignin is underscored by the fact that it is estimated to be the second most abundant biopolymer on Earth after cellulose. Color producing phenylpropanoids include flavonoids, isoflavonoids, anthocyanin, and anthocyanidins.

Shikimate (shikimic acid) pathway: The phenylpropanoid pathway ensues in plastids of plant cells and begins by the shikimic acid pathway which is the biosynthetic pathway of the aromatic amino acids in microorganisms, plants, and fungi, but is absent from animals. On the one hand, the shikimate

pathway contributes to primary metabolism in plants by providing its immediate end products (phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp)); on the other hand, it gives rise to the vast array of compounds that are ultimately derived from its end products: alkaloids, indole glucosinolates, flavonoids, hydroxycinnamic acids, lignins, and lignans, etc. In addition, the shikimate pathway intermediate chorismate serves as the starting point for the biosynthesis of physiologically important compounds like the folates and quinones. The precursors of the shikimate pathway are PEP (a three-carbon intermediate compound from glycolysis) and erythrose 4-phosphate (E4P) (a four-carbon intermediate of the pentose–phosphate pathway). The pathway instigates with the aldol condensation of PEP with E4P to form 2-dehydro-3-deoxy-D-arabino-heptonate-7-phosphate (DAHP) and inorganic phosphate. This primary reaction is catalyzed by 2-dehydro-3-deoxy-D-arabino-heptulosonate-7-phosphate aldolase (DAHP synthase; 2.5.1.54). Here an inorganic phosphate of E4P is removed by DAHP synthase. The enzyme regulates the amount of carbon entering the shikimate pathway and is known to be controlled at a transcriptional level in plants. The DAHP is converted to shikimate by the subsequent action of enzymes 3-dehydroquinate synthase (4.6.1.3; ring closure), dehydratase (4.2.1.10), and shikimate-3-dehydrognase (1.1.1.25; $NADPH + H^+$-dependent). Shikimate is the first committed product of this pathway, thus giving rise to the pathway name. It is further converted to chorismate which ultimately gives rise to Trp, Tyr, and Phe.

The phenylalanine/hydroxycinnamate pathway: The phenylalanine/hydroxycinnamate pathway starts with metabolism of Phe and is called general phenylpropanoid metabolism. The reactions involving formation of hydroxycinnamates and their activated forms (CoA thoesters and 1-O-acylglucosides) fall under the purview of the phenylalanine/hydroxycinnamate pathway (Fig. 16.3). The first enzyme of the hydroxycinnamate pathway is phenylalanine ammonia lyase (4.3.1.24), which catalyzes the nonoxidative deamination of Phe to *trans*-cinnamate (first phenylpropane) structure. The *trans*-cinnamate is further reduced to p-coumarate (4-coumarate) by the action of an NADPH-dependent cinnamate-4-hydroxylase (1.14.13.11). This p-coumarate (alternatively called hydroxycinnamate) is activated by hydroxycinnamoyl-CoA ligase (6.2.1.12) to *p*-coumaryl-CoA (or hydroxycinnamoyl-CoA) in a reaction analogous to fatty acid activation. Here, an ATP binds hydroxycinnamate to form hydroxycinnamoyl-AMP. The energy released by cleavage of pyrophosphate helps the addition of CoASH to form hydroxycinnamoyl-CoA. Various products of this pathway, that is, *trans*-cinnamic acid, *para*-coumaric acid, *para*-coumaryl CoA, caffeate, sinnapate, and ferulate are called simple phenolic compounds or phenylpropanoids as they comprise a benzene ring attached to a three-carbon side chain (C6–C3). The hydroxycinnamates can undergo following four different types of reactions to form a plethora of products.

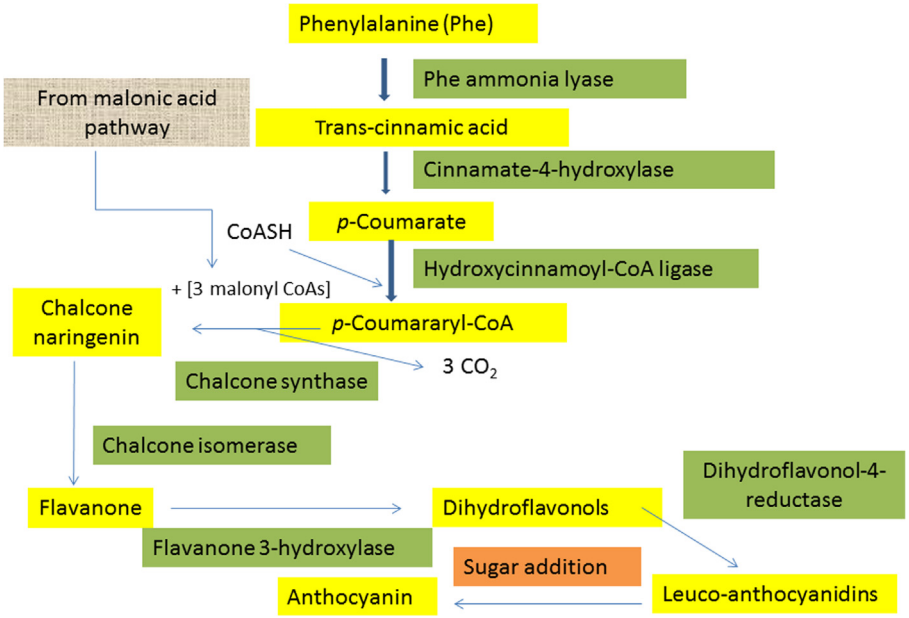

FIGURE 16.3

The phenylalanine/hydroxycinnamate pathway for biosynthesis of anthocyanin. *Taiz, L., Zeiger, E., 2010. Plant Physiology, second ed. Sinauer Associates, Sunderland, MA, pp. 347–376.*

1. Degradation: This is side chain shortening in a C3 unit by removal of an acetate molecule leading to the formation of benzoic acid derivatives (C6–C1 type).
2. Reduction: This reaction is NADPH-dependent and culminates in the production of lignin precursors, hydroxycinnamyl alcohols, and hydroxycoumarins.
3. Conjugation: Conjugation of a hydroxyl- or amino group-bearing molecule with hydroxycinnamates to produce esters or amides with simultaneous elimination of water. Phenolic hydroxyl groups get attached to glycosides. For example, the hydroxycinnamate is acylated at the first position by an activated glucose molecule (UDP-glucose) to form 1-O-hydroxycinnamoyl glucose.
4. Condensation: This is a sort of side-chain elongation where malonyl CoAs are attached to hydroxycinnamates, CO_2 is liberated, and the acetate unit gets joined and further the output products are flavonoids.

The phenylpropanoid group is one of the most diverse groups, having great variations in their structures and functions. The color-producing phenylpropanoid products are flavonoids and anthocyanins produced by condensation reactions.

Synthesis of flavonoids, anthocyanins, and anthocynidins: Flavonoids are colored compounds imparting colors ranging from red, yellow, scarlet, violet, and

blue and so on. There are more than 5000 different known flavonoids. Flavonoids are C15 aglycone skeletons in the arrangement C6−C3−C6 with two aromatic rings connected by a three-carbon bridge. Interestingly, this structure originates with two separate biosynthetic pathways. The bridge and one aromatic ring (ring B) are founded by a phenylpropanoid unit biosynthesized from *p*-coumaryl-CoA (refer to the formation of *p*-coumaryl CoA from Phe, above). The six carbons of the other aromatic ring (ring A) originate from the condensation of three acetate units via the malonic acid pathway. An acetyl-CoA carboxylase (6.4.1.2; biotin-dependent enzyme chloroplastic enzyme) joins acetyl-CoA with a molecule of CO_2 using energy released from ATP hydrolysis. Its activity is controlled at the transcriptional level as well as by covalent modification. Flavonoid biosynthesis involves the stepwise condensation of *para*-coumaroyl CoA with three malonyl CoA residues (of the malonic acid pathway) in a reaction catalyzed by chalcone synthase (2.3.1.74) to yield the first intermediate called chalcone or naringenin chalcone. Here, a molecule of CO_2 is eliminated and acetate is added at one time in a defined manner and eliminating both CoAs. This reaction is a rate-limiting step for flavonoid biosynthesis and offers a key regulatory step for channeling *p*-coumaryl-CoA to flavonoids. Its activity is modified in a feedback manner by flavonoid pathway products such as naringenin and chalcone naringenin. Also, control is implemented at the level of gene transcription. The enzyme works in alkaline pH. This series of condensations is analogous to the cycle of two-carbon addition during fatty acid biosynthesis. The construction of the flavonoid skeleton starts with *para*-coumaroyl CoA and the growing acyl chain is not reduced or dehydrated. Flavonoids derived by modification of chalcones are classified into different groups based primarily on the degree of oxidation of the three-carbon bridge or to, for example, the central pyran ring. Chalcone is then isomerized to flavanone by chalcone isomerase (5.5.1.6) where cyclization of the central carbon chain occurs to a heterocyclic or pyran ring structure (L-flavanone). Flavanone can be converted to isoflavone by the action of an NADPH-dependent dehydratase type reaction catalyzed by isoflavone synthase (1.14.13.136). However, a dehydratase reaction (flavone synthase; 1.14.11.22) gives rise to flavone. Induction of an −OH group at the third position of the pyran ring by flavanone 3-hydroxylase (1.14.11.9) creates dihydroflavonol (3-hydroxyflavanone). The dihydroflavonol again can be divulged to flavonol (flavonol synthase; 1.14.11.23) or leucoanthocyanidin. Flavanones to isoflavone, flavone, dihydroflavonol, and flavonol production-mediating enzymes are oxidoreductases. The dihydroflavonol when acted upon by dihydroflavonol-4-reductase (1.1.1.219; NADPH-dependent) leads to the formation of anthocyanidins. The leucoanthocyanidins can give rise to further anthocyanins and tannins. The majority of flavonoids exist naturally as glycosides having sugars attached to them. Various flavonoids carry out diverse functions in the plant, in addition to pigmentation and defense.

Anthocyanins are responsible for most of the red, pink, purple, and blue colors observed in plant parts. Anthocyanins are glycosides that have sugars at

position 3 and sometimes elsewhere. Anthocyanins are called anthocyanidins when they are devoid of their sugar moiety. Anthocyanins are water-soluble pigments, being synthesized in the cytosol while stored in cell vacuoles. Enzymes involved in hydroxylation, methylation, or glycosylation can alter the basic ring structure of anthocyanins. However, the color of anthocyanin is influenced by many factors, including the number of hydroxyl and methoxyl groups in ring B of the anthocyanidin, the presence of aromatic acids esterified to the main skeleton, and the pH of the cell vacuole in which these compounds are stored.

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CHAPTER 17
Vitamins

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17.1 INTRODUCTION

Fruits and vegetables are the main source of health-promoting nutrients, including vitamins, dietary fibers, and phytochemicals. Vitamins (Table 17.1) are key bioactive compounds, absolutely necessary in the human diet. As humans cannot synthesize vitamins de novo, they depend on fresh fruits and vegetables to meet their daily requirements to support a balanced diet. Currently, there are more than 18 phytonutrient compounds that have been classified as vitamins: the fat-soluble (vitamin A, D, E, and K), and the water-soluble (vitamins B1, B2, B3, B5, B6, B7, B9 or folic acid, B12, and C) ones (Table 17.1).

Vitamin deficiencies have been related to a plethora of diseases than can be even lethal in some cases (Fitzpatrick et al., 2012). Considering the fact that in the developing world many people do not have access to a balanced and healthy diet, vitamin deficiencies are among the major health problems in these countries where billions of people suffer from hunger and/or malnutrition. For instance, vitamin A deficiency can lead to blindness, and increased mortality among children, particularly in sub-Saharian Africa. According to the World Health Organization, an estimated 250 million preschool children are vitamin A-deficient, and over 250,000 of them become blind every year

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Table 17.1 RDA, Solubility, and Antioxidant Capacity of the 13 Plant-Derived Vitamins

#	Vitamin	Compound	RDA	Solubility	Antioxidant Capacity
1	Provitamin A	α-Carotene	900 RAE[a]	Lipid-soluble	$^1O_2$ quencher
		β-Carotene		Lipid-soluble	$^1O_2$ quencher
		β-Cryptoxanthin		Lipid-soluble	$^1O_2$ quencher
2	B1	Thiamine	1.2 mg	Water-soluble	OH^- scavenger
3	B2	Riboflavin	1.3 mg	Water-soluble	None
4	B3	Niacin	14–16 mg	Water- soluble	None
5	B5	Pantothenic acid	5 mg	Water-soluble	None
6	B6	Pyridoxal	1.3–1.7 mg	Water-soluble	$^1O_2$ scavenger
		Pyridoxine		Water-soluble	$^1O_2$ scavenger
		Pyridoxamine		Water-soluble	$^1O_2$ scavenger
7	B7	Biotin	30 μg	Water-soluble	None
8	B9	Folic acid	400 μg	Water-soluble	$^1O_2$ scavenger
9	B12	Cobalamin	2.4 μg	Water-soluble	None
10	C	Ascorbic acid	75–90 mg	Water-soluble	$H_2O_2/OH^-/^1O_2$ scavenger
11	D	Calciferol	15–20 μg	Lipid-soluble	
12	E	Tocopherols	15 mg	Lipid-soluble	$^1O_2$ quencher
		Tocotrienols		Lipid-soluble	$^1O_2$ quencher
13	K1	Phylloquinone	90–120 μg	Lipid-soluble	$LOO^-/LO^-/OH^-$ scavenger

[a]*Retinol activity equivalents.*

(http://who.int/nutrition/topics/vad/en/). Other well-known vitamin-related disorders include scurvy (vitamin C), beriberi (vitamin B1), pellagra (vitamin B3), and anemia (vitamin B6) (Asensi-Fabado and Munné-Bosch, 2010). In developed countries, the disorders related to vitamin deficiencies are generally not a major problem, either due to the adequate supply of fresh fruits and vegetables, or due to the biofortification of plant-derived food through conventional or molecular breeding strategies. Yet, based on the World Health Organization (http://www.who.int/nutrition/topics/vad/en/), it is believed that the average consumption of fruits and vegetables falls far short of the recommended dietary allowance (Table 17.1).

An enriched dietary intake of fresh fruits and vegetables has been considered as a key element of public health policy over the years, as it has been linked to reduced risk for a wide range of oxidative-stress-related diseases such as cancers, cardiovascular diseases, diabetes, Alzheimer's, cataracts, and, overall, so-called "aging." For example, carotenoids may provide protection against cardiovascular diseases, chronic liver disease, diabetes, and some forms of cancer (prostate and breast), while vitamin C has been associated with lower risk of heart diseases, stroke, and cancer. Similarly, vitamin E protects against diabetes, cancer, and neurological diseases, reduces the risk for Alzheimer's disease, and maintains the cardiovascular system. On the other hand, the deficiency of folates in the human diet can trigger diseases like neural tube defects, megaloblastic anemia, Alzheimer's, cardiovascular and coronary diseases, as well as several cancers (Blancquaert et al., 2015).

However, the exact mechanism by which the consumption of fruits and vegetables reduces the risk of certain human diseases has not yet been fully understood. Hence, in spite of the fact that the health problems of vitamin deficiencies have been well documented, the positive effects of increased vitamin intakes as multivitamin supplements against major diseases is disputable to a great extent. This is mainly because multivitamin supplements cannot replace a healthy diet rich in fruits and vegetables, suggesting that the enhancement of the nutritional value of fresh produce should be a clear target for producing superior fruits and vegetables.

Apart from being essential for human health, vitamins are also important regulators of cellular metabolism for plant development due to their redox chemistry and their role as cofactors in numerous reactions crucial for plant survival. Most of them, including carotenoids (provitamin A), ascorbic acid (AsA or vitamin C), tocopherols (vitamin E), and recently vitamin B1 (thiamine), vitamin B6, vitamin B9 (folic acid), and vitamin K1 (phylloquinone) have a strong antioxidant capacity, within the plant cell (Table 17.1; Fitzpatrick et al., 2012).

Exploring and understanding the regulation of individual vitamins in heterotrophic tissues (fruits and vegetables) has been a topic of widespread attention, particularly in the context of developing novel cultivars with higher nutritional contents, resistance to stress factors, and improved postharvest behavior. Current technological approaches may help plant scientists to alleviate vitamin deficiencies by improving the nutritional quality of fresh produce. In this regard, unraveling plant vitamin metabolism including biosynthesis and catabolism, as well as transport, and storage in plants, in combination with the use of modern genomic tools, can enable the identification of vitamin-rich alleles that can further be introduced to elite cultivars through modern breeding approaches. This chapter will attempt to illustrate the multiple roles of the main groups of vitamins such as vitamins A, C, E, and B9 (folic acid) in plants, as well as the significance of exploiting the occurrence and diversity of individual vitamins within the plant kingdom. Current efforts to enrich vitamin levels in horticultural fresh produce are also discussed. In view of the fact that a great number of studies over recent years have reinforced the hypothesis that high levels of antioxidant compounds (such as vitamin C) improve shelf-life, this chapter also focuses on the postharvest occurrence of the key vitamins in fresh produce.

17.2 THE MULTIPLE ROLES OF VITAMINS IN FRUITS AND VEGETABLES

Carotenoids are lipid-soluble pigments that are responsible for the yellow, orange, and red colors of several fruits and vegetables. Basically, they consist of long, aliphatic, conjugated double-bonded systems and are usually composed of eight isoprene units with the molecular formula $C_{40}H_5$, which mainly serve as light-absorbing chromophores. More than 600 carotenoid

compounds have been identified so far, classified into two major groups: carotenes (α- and β-carotene, and lycopene), containing only C- and H-, and their oxygenated derivatives, called xanthophylls (e.g., lutein, cryptoxanthin, violoxanthin, and zeaxanthin).

Carotenoids exert diverse functions in plants, participating in a wide range of physiological processes, including plant growth and development, as well as in responses to environmental stimuli (Liu et al., 2015). One of the major roles of some carotenoids, such as lutein and violoxanthin, is to absorb light energy and to augment light harvesting, that is, in turn, transferred to chlorophylls to be utilized in the photosynthetic process. At the same time, they may act as photoprotectants for other pigments (e.g., chlorophylls), but also as attractants for pollinators and seed dispersers. Additionally, xanthophylls, acting as powerful antioxidants, can quench the excited status of singlet chlorophyll in photosystem II under excessive light energy, and thereby prevent the accumulation of reactive oxygen species (ROS) in plastids.

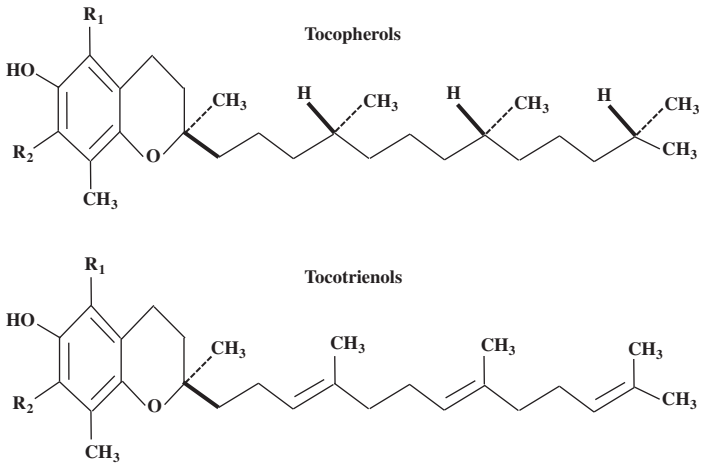
In fruits and vegetables, carotenoids may serve as quality parameters, and their increased accumulation is of outmost importance in their visual and quality properties attracting consumers. Specifically, carotenoids confer distinctive attractive colors such as yellow, orange, and red, but they also act as precursors of a wide range of volatile flavor components in flowers, fruits, and leaves. Last but not least, carotenoids serve as precursors of abscisic acid (ABA) and strigolactones, acting as key regulators of plant growth and stress responses. Apart from their roles within the plant kingdom, α- and β-carotene, as well as cryptoxanthin, also exert provitamin A activity, providing the precursors for the biosynthesis of vitamin A. Evidently, they are considered as the most important carotenoids in the human diet.

AsA or vitamin C is the most abundant antioxidant compound present in all plant tissues. It has a predominant role in the antioxidant defense machinery owing to its remarkable ability to scavenge ROS, serving as an excellent sensor of the cellular redox state (Asensi-Fabado and Munné-Bosch, 2010). AsA is an extremely effective antioxidant as it can easily donate electrons to free radicals. It is a multifaceted trait with a wide range of functions linked to oxidative stress, such as the scavenging of hydrogen peroxide (H_2O_2) in the ascorbate–glutathione (AsA–GSH) cycle, the direct scavenging of other ROS such as hydroxyl radical, superoxide, and singlet oxygen, the regeneration of α-tocopherol, and the electron donation to photosystems I and II in isolated thylakoids under stress conditions (Mellidou et al., 2018). It further acts as a cofactor for violaxanthin de-epoxidase, the enzyme that catalyzes the de-epoxidation of violaxanthin to zeaxanthin in the xanthophyll cycle. In order to orchestrate the antioxidant machinery against ROS within the cell, AsA acts in concert with GSH and other enzymatic antioxidants of the AsA–GSH cycle in chloroplasts, mitochondria, peroxisomes, and cytosol. As a result, it is considered as a key regulator in cellular homeostasis and signaling. AsA is also

found in significant amounts in the apoplast, where it governs signal perception and transduction of the oxidative stimuli, and in this regard, it is believed to regulate multiple stress responses to environmental changes. Due to its antioxidant function in cellular homeostasis, AsA plays a fundamental role during leaf senescence by acting as a signaling molecule or by serving as a cofactor for several hormone biosynthetic enzymes, such as gibberellins (GAs), ethylene (ETH), and ABA. Apart from its antioxidant properties, AsA controls multiple other cellular processes associated with normal plant growth and development, such as cell division and expansion, shoot apical formation and root development, flowering time, but also to the modulation of hormone (ETH, GA, and ABA) homeostasis.

Apart from the photosynthetically active tissues, AsA can also be found at high levels in heterotrophic tissues such as fruits, where its mode of action can be other than serving as an antioxidant scavenger. Being an enzyme cofactor in several biosynthetic pathways such as those of ETH, GA, and anthocyanins, it is evident that AsA has a key role in fruit development and ripening. In climacteric fruits, apoplastic AsA released by membrane permeabilization early in fruit ripening promotes the enhanced pectin solubilization (nonenzymatic) and the depolymerization of polysaccharides. Furthermore, AsA can serve as a precursor for other organic acids highly abundant in some fruits (e.g., grapes, kiwifruit) such as oxalic acid, L-threonic acid, and L-tartaric acid. In this regard, fruit species that are rich in the aforementioned organic acids are usually poor in AsA. Other functions of AsA in fruits are related to its antioxidant properties during seed development, maturation, germination, and storage, where it is essential to limit oxidative stress (Mellidou et al., 2018), but also to the inhibition of seed formation in tomato (Bulley et al., 2012). At postharvest, AsA plays a vital role in maintaining fruit quality, as has been shown in apple (Mellidou et al., 2012b) and tomato (Ioannidi et al., 2009). Additionally, the development of the physiological disorder called internal flesh browning has been linked to the oxidation degree of the AsA pool in apple (Mellidou et al., 2014). In addition, the ability of AsA to preserve fruit quality may be associated with its inhibitive effect over the polyphenol oxidase activity that oxidizes diphenols to quinones and leads to browning in several fruits and vegetables after wounding.

Vitamin E (tocochromanols), a lipophilic antioxidant, consist of tocopherols and tocotrienols, which are compounds synthesized by photosynthetic organisms. Vitamin E consists of eight distinct forms organized in two chemical groups (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols, respectively). These differ significantly in their vitamin E activity in vivo, with α-tocopherol showing the highest activity on a molar basis and being the most common in nature (Fitzpatrick et al., 2012). Tocopherols and tocotrienols are seen as promoting health and are found in fruit and vegetables, especially in oily seeds, olives, nuts, peanuts, avocados, and almonds (Kanellis and Manganaris, 2014; Mène-Saffrané and Pellaud, 2017).



	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

FIGURE 17.1
Structures of tocopherols and tocotrienols.

Vitamin E is a small group of natural products that have in common a chroman-6-ol ring system. The number and positions of methyl groups on this ring system determine the four different tocopherols and tocotrienols that are found in nature, namely α, β, γ, or δ (Fig. 17.1) (Dellapenna and Mène-Saffrané, 2011). All the isomers have aromatic rings with a hydroxyl group that can donate hydrogen atoms to reduce ROS (Kanellis and Manganaris, 2014). Tocopherols have three chiral centers with naturally occurring tocopherols being of the R,R,R configuration, while chemically synthesized tocopherols are racemic mixtures of eight stereoisomers. Tocotrienols have a single chiral center that occurs exclusively as the D-isomer in nature and as a racemic mixture in synthetic tocotrienols.

The main role of tocopherols as antioxidants is the scavenging of lipid peroxy radicals, which are the reason for generating lipid peroxidation. The antioxidant activity of tocopherols as free radical scavengers is due to the ability to offer their phenolic hydrogen to lipid-free radicals given the specific requirements of the molecule. Furthermore, recent studies have shown that tocopherols participate in cellular signaling in plants, which indicates the role of tocopherols in plant development and stress tolerance. Tocopherols also play a significant role in plants by protecting them so as not to lose excessive energy in photosynthetic membranes. Tocopherols detoxificate O_2 and other ROS and therefore protect the plants from wasting excessive energy in chloroplasts. Furthermore, in plants α-tocopherol strongly interacts with membrane lipids making them more rigid.

The term folates, in general, is used for a group of compounds that consist of a pteridine nucleus, *p*-aminobenzoic acid, and an L-glutamic acid. The simplest of these compounds is the vitamin B9 that has only one copy of those three structural units and has been named pteroyl glutamic acid (PGA). PGA is the

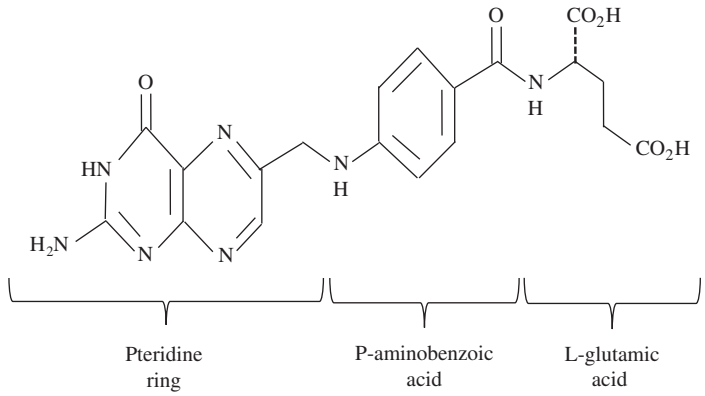


FIGURE 17.2
Pteroyl glutamic acid.

most important member of this family and it is known as folic acid (FA) (Fig. 17.2). FA is a synthetic, stable, and oxidized form of the vitamin B9 and is an increasingly common source of dietary folate that often is present in dietary supplements and fortified foods and, when it is transported into the cells, is converted to tetrahydrofolate (THF) and is chemically identical to natural food folates. It is a water-soluble compound that plays a major role in DNA biosynthesis and methylation cycles. In this regard, FA plays an important role in plant growth and development as it enables plants to synthesize DNA and RNA molecules, and functions as a regulator in the plant's metabolic pathways by enabling the metabolism of carbohydrates, lipids, and the synthesis and catabolism of protein amino acids (Gorelova et al., 2017). Folic acid can improve the plant's resistance to biotic stresses, while its metabolism is modulated by abiotic stresses (Wittek et al., 2015), indicating an active role in the plant's stress responses.

17.3 VITAMIN OCCURRENCE AND DIVERSITY

The overall consensus is that vitamins B, C, and K are ubiquitous within the plant kingdom, whereas those exerting provitamin A (mainly β-carotene) and tocopherols, albeit absolutely necessary in all photosynthetic tissues, are not uniformly dispersed among different plant species (Asensi-Fabado and Munné-Bosch, 2010). Below, we discuss vitamin diversity in different plant tissues, organelles, and horticultural species, as well as throughout fruit development and at postharvest.

17.3.1 Within Plant Tissues/Cellular Compartments

Carotenoids are usually present at relatively low concentrations in plant species. Both photosynthetic and nonphotosynthetic tissues of horticultural crops can produce multiple forms of carotenoids. For example, β-carotene accumulates at high levels in leaf tissues, whereas α-carotene and β-cryptoxanthin are

mostly found in fruits (Kanellis and Manganaris, 2014). In the green parts of plants, generally, the darker the green color, the higher the carotenoid content (Yahia et al., 2018a). Flowers represent a good source of β-carotene and β-cryptoxanthin. By contrast, tubers and roots do not contain β-carotene or β-cryptoxanthin, respectively (Asensi-Fabado and Munné-Bosch, 2010). In green leaf tissues, carotenoids govern essential functions in the photosynthetic apparatus, while in heterotrophic tissues, they are responsible for the vivid yellow, orange, or red colors, as well as the flavors that attract insects and animals for pollination and seed dispersal. In order to fulfill these roles, carotenoids are synthesized in all forms of plastids, but mostly in the chloroplasts of photosynthetic tissues, and in the chromoplasts of fruits, roots, and flowers.

Vitamin C is the most abundant cellular antioxidant within the plant kingdom. However, there is a great diversity of AsA content in various plant tissues, presumably due to its tissue-specific functions. In principle, AsA accumulates at photosynthetically active tissues such as leaves, but it can also be found at high concentrations in heterotrophic tissues such as fruits. The general perception, however, is that developing tissues such as young leaves, meristems, flowers, young immature fruits, root tips, and tubers are richer in AsA (Mellidou et al., 2018). Foliar tissues may have higher demands for AsA to counteract oxidative damage caused by increased accumulation of ROS due to elevated rates of photosynthesis; this in turn triggers higher need for reduced power and consequently a net increase in AsA pool size. Within the fruit, the peel, serving as the interface between the flesh and the environment, accumulates more AsA than the flesh, probably due to its higher need for antioxidants to alleviate the direct effect of (a)biotic stress factors, but also as a result of the greater exposure to direct light. On the other hand, AsA can be found in nearly all the cellular compartments, with the highest concentrations being observed in chloroplasts, peroxisomes, and cytosol, while the apoplast contains less AsA than the symplast due to the enhanced AsA oxidation.

The content and composition of vitamin E varies vastly in plant tissues. Photosynthetic tissues generally contain low levels of vitamin E ($<50\,\mu g/g$) compared with seeds and particularly oil seeds, which contain 10−20 times this level. High levels of tocotrienols are found in the fruits and seeds of some species, whose oils have well-known health properties. Tocopherols have been found in photosynthetic bacteria, fungi, algae, plants, and animals, even though animals cannot synthesize them. They have also been found in seeds, flowers (e.g., sepals and petals), roots, tubers, cotyledons, hypocotyls, stems, and particularly in leaves of higher plants. Plant tissues show variations in tocopherol content with total concentrations ranging from exceptionally low levels in potato tubers to very high levels in leaves and seeds.

α-Tocopherol, the most abundant form of vitamin E, is found in the chloroplasts of leaves and is synthesized in the chloroplast envelope from homogentisic acid and isopentenyl diphosphate. Furthermore, α-tocopherol is found in

the lipoprotein particles of the chloroplast stroma, called plastoglobuli, and in thylakoid membranes, where it exerts its functions. Most of the α-tocopherol synthesized is partitioned between the chloroplastic envelope and the thylakoids, and is stored in plastoglobuli only in some cases, particularly during periods of oxidative stress and senescence. Tocotrienols are not usually found in the green parts of higher plants, although negligible amounts of α-tocotrienol have been found in the leaves of some species. However, tocotrienols are found in high amounts in seeds and the pericarp.

Folates play a crucial role in plant embryo development. In leaf tissues, folate synthesis and accumulation are elevated in light exposure conditions, indicating the need for folates in the leaf metabolic pathways via the one-carbon transfer reactions in the photosynthetic tissues. Within the cell, folates can be found in mitochondria, plastids, cytosol, and vacuoles but are synthesized only in mitochondria. In the cell, the folate is always found in the reduced form of THF, which is the only enzymatically active compound involved in passing different one-carbon compounds like formyl (−CHO), methylene ($-CH_2-$) and methyl ($-CH_3$). THF is involved in the photorespiratory pathway, which includes the use of three different organelles in the plant cell, the chloroplast, the mitochondrion, and the peroxisome, and is an absolute requirement for the synthesis of the purine and thymidylate, as well as the glycine to serine conversion, a step in the photorespiration pathway that takes place in the mitochondria.

17.3.2 In Different Fruits and Vegetables

As a general rule, fruits are not as good a source of carotenoids as vegetables, with the exception of pineapple, mango, citrus, and peach (Kanellis and Manganaris, 2014). Among vegetables, carrots, sweet potato, red peppers, and tomato contain considerable amounts of different forms of carotenoids (Table 17.2). Although increased consumption of tomato is considered as a valuable source of lycopene and β-carotene in the human diet, the levels are not sufficient to meet the daily RDA dose required for optimal health benefit. In this context, a novel approach was tested to increase the endogenous levels of lycopene in tomato. This approach involved ripening-specific expression of polyamines spermidine and spermine in tomato fruits, which resulted in two- to threefold enrichment of lycopene as compared to conventional tomatoes (Mehta et al., 2002). Polyamines are ubiquitous biogenic amines and have been found to have a profound influence on fruit metabolism and shelf-life (Mattoo et al., 2006; Fatima et al., 2016).

Additionally, due to the diverse fruit pigmentation (yellow, orange, orange-red, and red), tomato serves as an excellent model to study carotenoid metabolism. For instance, red ripe tomatoes are rich in lycopene, while yellow tomatoes are poor in β-carotene, lacking any trace of lycopene. In this regard, a plethora of mutants with diverse skin and flesh pigmentation have been developed over recent years. Red pepper predominantly synthesizes the

Table 17.2 Fruits and Vegetables With High Vitamin Levels

Provitamin A (Carotenoids)	Vitamin C (AsA)	Vitamin E	Folic Acid
Squash	Acerola	Almond	Peas
Carrot	Blackcurrant	Broccoli	Beans
Sweet potato	Pepper	Spinach	Broccoli
Tomato	Kiwifruit	Avocado	SpinachAsparagus
Red pepper	Broccoli	Melon	Orange
Broccoli	Cauliflower	Lettuce	Banana
Dark leafy vegetables	Spinach		
Pineapple	Strawberry		
Mango	*Citrus* spp.		
Melon			

characteristic carotenoid capsanthin, while yellow-fruited cultivars mainly produce lutein and α- or β-carotene, without any trace of capsanthin. Carrot, sweet potato, broccoli, and many dark leafy vegetables such as spinach, are also rich in carotenoids, and in particular in β-carotene and lutein. Melon (the orange-fleshed cultivars such as cantaloupe) and watermelon primarily accumulate β-carotene and lycopene, respectively, as the principal carotenoids. Within *Citrus* spp., mandarin, orange, and clementine accumulate several carotenoids such as β-cryptoxanthin, violaxanthin, lutein, and zeaxanthin, while grapefruit accumulates primarily phytoene and phytofluene. Tropical and subtropical fruits (e.g., mango and papaya) are typically considered as better sources of carotenoids than temperate fruits, which contain more anthocyanins. Although banana contains relatively low amounts of carotenoids, its high consumption, especially in the developing countries of Africa and Asia, makes it a valuable source of carotenoids, indicating the need for carotenoid biofortification in breeding programs.

Fruits of acerola, blackcurrant, pepper, kiwifruit, and broccoli are very rich in vitamin C, while cauliflower, strawberry, spinach, and citrus also represent a significant source of AsA (Table 17.2). Popular species in the human diet, such as tomato and apple, contain relatively medium-to-low amounts of AsA, and thus significant efforts to enhance their AsA content have been undertaken in recent decades. In most species, including tomato, apple, kiwifruit, and potato (Bulley et al., 2012), the main biosynthetic pathway derived from L-galactose (see below) is the main reason for AsA accumulation, while the pathway proceeding via L-galacturonic acid seems to play a major role in strawberry and citrus species (Mellidou and Kanellis, 2017). Significant differences can also be found between different genotypes within the same species. For example, in tomato, AsA concentrations may vary from 10 to 25 mg/100 g fresh weight (FW), while in apple and kiwifruit the variability can be even higher, reaching up to 10-fold differences among cultivated varieties (Mellidou et al., 2012a; Gest et al., 2013). As a general rule, large fruits and

vegetables are poorer in AsA than small-sized ones, mainly as a result of the dilution effect in the first case. An interesting observation is that the wild ancestors of the cultivated genotypes tend to obtain higher amounts of AsA, at least in tomato fruit, presumably due to the domestication process that has led to the loss of diversity of AsA contents in favor of fruit size and total yield (Gest et al., 2013).

Vitamin E, and in particular α-tocopherol, is more abundant in oil seeds, olives, nuts, peanuts, avocados, and almonds (Kanellis and Manganaris, 2014). The percentage of α-tocopherol in total tocochromanols may significantly vary within different cultivars in olives (Bodoira et al., 2015; Georgiadou et al., 2015, 2016) and carrots (Luby et al., 2014).

17.3.3 During Development and Ripening

Biosynthesis of carotenoids in ripening fruit is regulated by control mechanisms that are distinct from those in photosynthetic tissues. Hence, carotenoid production is strictly under developmental and ETH control (in climacteric fruit), as a part of the overall ripening process being initiated with the concomitant chlorophyll loss and de novo biosynthesis of carotenoids. Examples of this phenomenon are fruit species such as mango, apricot, acerola, and tomato (Yahia et al., 2018a). Particularly, in tomato, the concentration of carotenoid increases tremendously after the breaker stage, mainly due to the biosynthesis of lycopene. Phytohormones such as ETH, indolo-acetic acid, and ABA are also considered as principal regulators of carotenoid biosynthesis during ripening. In contrast, in ripe fruits in which anthocyanins are responsible for their pigmentation (e.g., strawberry, apple, and olive), or in those that retain their green color, such as kiwifruit, carotenoid accumulation decreases with ripening. On the other hand, leaf senescence is characterized by degradative processes resulting in reduced levels of carotenoids most evident in leafy vegetables.

AsA accumulation in fruits may change remarkably during growth and development in a species- and genotype-specific manner. Generally, young fruits possess significant amounts of AsA to support cell division and expansion, owing to their higher biosynthetic capacities. Thereafter, and during fruit maturation, the trend of AsA accumulation can vary tremendously within different genetic backgrounds, but also due to the different growing conditions and cultural practices (Mellidou et al., 2018).

Several studies have focused on the content of vitamin E of olive fruit during several developmental stages within a year (Bodoira et al., 2015; Georgiadou et al., 2015) and across multiple years (Georgiadou et al., 2016). The concentrations of α-tocopherol generally declined, both within and across years (Georgiadou et al., 2016). High amounts of tocochromanol content have been observed during the early stages of olive fruit development, followed by a decrease as fruit development progressed (Bodoira et al., 2015). Overall, olive fruit contained significantly higher concentrations of tocopherols and tocotrienols until the breaker stage compared with later stages, thus suggesting that

the color change phase might be of critical importance in vitamin E content of olive fruit (Georgiadou et al., 2015). Available data have shown that olive oil production increases and reaches its highest levels toward the end of the mesocarp development, associated with the initiation of color change (Bodoira et al., 2015). Similarly, in climacteric fruits, such as mango and tomato, vitamin E content increased during ripening, implicating a role of ETH as a regulatory molecule in its biosynthesis.

17.3.4 At Postharvest

Biosynthesis of some carotenoids (lycopene and carotenes) may continue in most fruits during storage, as a consequence of the postharvest ripening process, provided that they are kept intact and at optimum storage conditions. At low storage temperatures, it is evident that this process can be delayed, and carotenoid accumulation may be retarded. As they are lipid-soluble, carotenoids are not significantly lost into water-soluble media during processing or storage in cans. However, during storage, carotenoids are vulnerable to heat, light, and oxygen, and stable during freezing and freeze-drying in the absence of oxygen (Yahia et al., 2018a). At postharvest, the loss of carotenoids and the change in their composition are primarily due to improper storage conditions. Several studies show that the principal causes of postharvest-related loss of carotenoids are their enzymatic and/or nonenzymatic oxidation, causing the loss of provitamin A activity, as well as the isomerization of *trans*- to *cis*-forms, especially under heat treatment. Lycopene is less sensitive to oxidation and overall more bioavailable in its *cis*-form after cooking. Evidently, there is a greater bioavailability of processed tomatoes than of fresh ones. Cutting, shredding, chopping, and pulping of fruits and vegetables induce carotenoid oxidation, leading to lower accumulation. Heat treatment in blanching may also provoke some carotenoid losses, but the inactivation of carotenoid degradation enzymes will prevent greater losses. Beside these general rules, it should be noted that a great diversity in the degree of stability during storage has been recorded within different fruits and vegetables, even when the same processing storage conditions are employed.

Studies on postharvest physiology support the notion that AsA levels decrease rapidly after harvest and this loss is an index of quality deterioration. Proper postharvest handling processes, storage conditions, and duration are, therefore, of paramount importance to maintain their esthetic as well as nutritional quality. At harvest, the delay between harvesting and cooling or processing can lead to water loss, product decay, and deterioration in nutritional quality, including the rapid loss of vitamin C. On the other hand, low storage temperatures decrease the metabolic rate and fungal growth, and are thereby considered to preserve nutritional quality. During storage, AsA is reduced to a great extent in a plethora of horticultural species, including tomato, apple, potato, strawberry, broccoli, zucchini, potato, and cabbage (Mellidou et al., 2018). This is consistent with a decrease in the redox state of the AsA pool

size (e.g., an increase in the oxidation degree). In most fruits and vegetables, AsA concentrations reduce not only at both high- and low-temperature conditions, but even at optimum storage temperature rates. The extent of AsA loss depends upon both storage temperature and genotype. Significant losses of AsA have also been reported after bruising, trimming, and cutting, as well as upon physiological disorders in numerous horticultural species. The development of the internal browning (physiological and not after cutting) disorder in apples and pears after prolonged storage has been reported to occur when AsA levels decline below a certain threshold. Furthermore, the risk of developing the disorder has been associated with enhanced oxidation of the AsA pool.

Modified (MA) and controlled atmospheres (CA) are common storage techniques that use low O_2 and/or high CO_2 to delay microbial decay and senescence, and thereby prolong the shelf-life of fresh commodities. This is presumably due to a lower respiration rate and ETH production rates that can lead to reduced softening, pigment degradation, and lignification, as well as sugar interconversions (Yahia et al., 2018b). As a general consensus, fruits stored under MA or CA conditions have better AsA retention than fruits stored in normal air. Thus, MA packaging, using a wide range of film materials, has been reported not only to effectively reduce chilling injury, but also to positively affect vitamin C retention during storage.

Application of 1-methylcyclopropene (1-MCP), an antagonist of ETH action, on AsA accumulation is quite puzzling, and the effect seems to be species- and dose-dependent. Within this context, 1-MCP has been recorded to decrease AsA concentrations in apple after long-term storage, or to increase AsA levels when applied to high concentrations in spinach and peach (Mellidou et al., 2018). The positive effect of 1-MCP in certain species may be attributed to the prevention of AsA oxidation that occurs during storage. Another common postharvest practice, mainly in citrus fruits, is degreening via the application of ETH that accelerates chlorophyll degradation and carotenoid accumulation by upregulating transcription levels of multiple biosynthetic genes (Yahia et al., 2018a). Owing to the wide range of studies aiming to decipher AsA contents after postharvest treatments using different genotypes, results are often conflicting. For instance, ETH degreening in lower doses resulted in lower or even higher vitamin C contents in some grapefruit and orange varieties.

The processing, storage, and cooking of fresh, frozen, and canned fruits and vegetables can all have various effects on vitamin E. In term of canning, very few studies have been contacted because most canned fruit and vegetables are not important sources of this vitamin. Sweet potatoes, spinach, and tomato products are the exceptions, with processed tomatoes and products exhibiting the highest levels of vitamin E. All cooking methods lead to a significant release of α-tocopherol in fresh broccoli, while in frozen broccoli no dramatic change was noticed. Cooking of fresh red pepper does not show considerable change in α-tocopherol content, whereas the same in frozen red pepper

presented no significant changes. It must be noted that microwave cooking, broiling, and roasting resulted in high tocopherol losses.

The oxidative cleavage of the C9−N10 bond of the folate is an irreversible reaction that has, as a result, the degradation of the folate and the formation of the pteridine and the *p*-aminobenzoylglutamate as degradation products. In postharvest studies in leaves and fruits, folate breakdown has been estimated at a rate of about 10%/day, a high rate when compared to the folate breakdown rate in mammalian cells that is normally about 0.5%/day. The significant high rate of folate breakdown in plant tissues is of high significance concerning nutritional values in the postharvest period.

17.4 GENETIC CONTROL OF VITAMINS IN FRUITS AND VEGETABLES

As a consequence of the indispensable roles of vitamins in plant growth and development, as well as in human health, several efforts have been made toward understanding the regulation of their metabolism in plant tissues. Enhanced accumulation of vitamins is also of outmost importance for plant survival under unfavorable environments, as some of them exert significant antioxidant functions, which help cells to maintain their redox homeostasis. Evidently, the development of tolerant crops is among the cutting-edge research topics in plant molecular breeding. A useful approach that has been extensively employed over the last few decades in order to unravel their genetic regulation is the transgenic modifications of the expression of several structural genes participating in metabolic pathways related to vitamins. In spite of the fact that plants enriched in individual vitamins (including vitamins A, C, and E) have been successfully produced, to date, relatively few attempts have been made toward simultaneously increasing different vitamins. Some positive examples include the simultaneous increase of zeaxanthin and tocopherol in potato tubers downregulating zeaxanthin epoxidase, and the high concentration of β-carotene and tocopherol upregulating phytoene desaturase (Asensi-Fabado and Munné-Bosch, 2010). By contrast, negative examples have also been reported, such as the high vitamin E and low vitamin C levels when tocopherol cyclase was overexpressed. Notably, most of the transgenic approaches involve leaf material of model plants (*Arabidopsis* or tobacco), while far fewer studies focused on increasing vitamin levels in fruits, except probably in tomato. In plants, biosynthesis of some vitamins (such as carotenoids and E) is limited to a single compartment (i.e., plastids), but the synthesis of others, including water-soluble vitamins C and B, is spread over different compartments (cytosol and mitochondria) (Fig. 17.3). Therefore, the cellular compartmentation of structural genes, especially those from large gene families (e.g., ascorbate peroxidase), can be associated with wide functional diversity and merits further attention. Below, an overview of the recent advances on the genetic regulation of individual metabolic pathways of vitamins is presented.

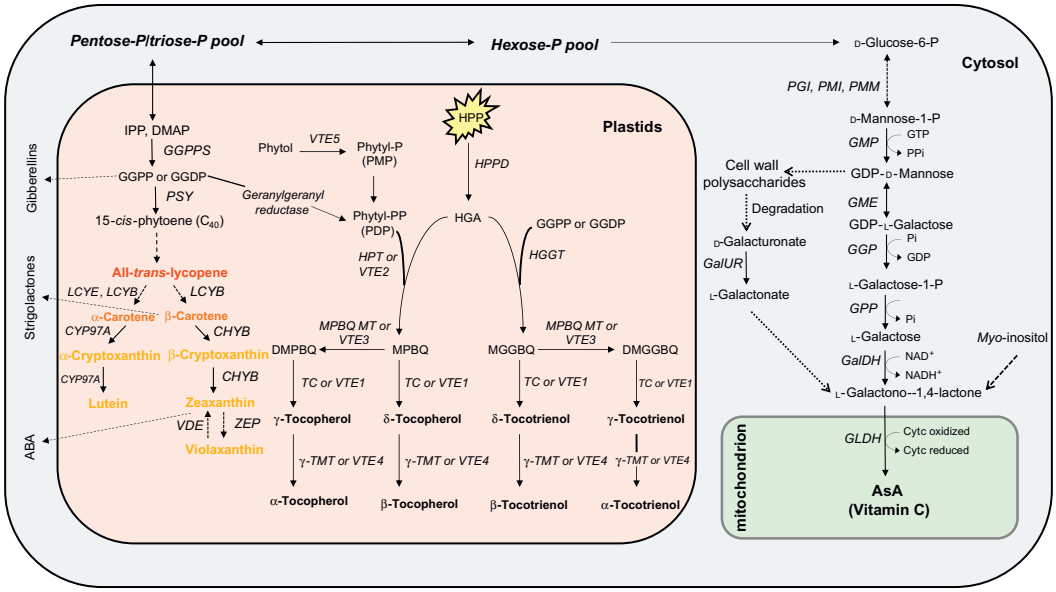


FIGURE 17.3

Crosstalk of biosynthetic pathways of vitamins in higher plants. The cut arrows indicate simplified reactions with missing steps. *Carotenoids: IPP*, Isopentenyl diphosphate; *DMAPP*, Dimethylallyl diphosphate; *GGPP*, Geranylgeranyl diphosphate; *GGPPS*, GGPP synthase; *PSY*, Phytoene synthase; *LCYE*, Lycopene ε-cyclase; *LCYB*, Lycopene β-cyclase; *CHYB*, β-carotene hydroxylase; *CYP97C*, Cytochrome P450-type monooxygenase 97C; *ZEP*, Zeaxanthin epoxidase; *VDE*, Violaxanthin de-epoxidase; *CCD*, Carotenoid cleavage dioxygenase; *NCED*, 9-*cis*-epoxycarotenoid dioxygenase. Metabolites are bolded and colored according to their compound colors. *Vitamin C: PGI*, Phosphoglucose isomerase; *PMI*, Mannose-6-phosphate isomerase; *PMM*, Phosphomannomutase; *GMP*, GDP-D-mannose pyrophosphorylase; *GME*, GDP-D-mannose 3′ 5′ epimerase; *GGP*, GDP-L-galactose-phosphorylase; *GPP*, L-galactose-1-P phosphatase; *GalDH*, L-galactose dehydrogenase; *GLDH*, L-galactono-1,4-lactone dehydrogenase; *GalUR*, D-galacturonate reductase. *Vitamin E:* The enzymes/genes are: *HPPD*, p- or 4-hydroxyphenylpyruvate dioxygenase; *HPT or VTE2*, homogentisate phytyltransferase or vitamin E2; *geranylgeranyl reductase; VTE5*, phytol kinase or vitamin E5; *Phytyl phosphate kinase; HGGT*, homogentisate geranylgeranyl transferase; *MPBQ MT or VTE3*, 2-methyl-6-phytyl-1,4-benzoquinol methyltransferase or vitamin E3; *TC or VTE1*, tocopherol cyclase or vitamin E1; *γ-TMT or VTE4*, γ-tocopherol methyltransferase or vitamin E4. The metabolites are: Phytol; *phytyl-P or PMP*, Phytyl phosphate; *phytyl-PP or PDP*, Phytyl diphosphate; *GGPP or GGDP*, geranylgeranyl pyrophosphate or geranylgeranyl diphosphate; *HPP*, p- or 4-hydroxyphenylpyruvic acid; *HGA*, homogentisic acid; *MPBQ*, 2-methyl-6-phytylbenzoquinol; *DMPBQ*, 2,3-dimethyl-6-phytyl-1,4-benzoquinol; *MGGBQ*, 2-methyl-6-geranylgeranylbenzoquinol; *DMGGBQ*, 2,3-dimethyl-6-geranylgeranyl-1,4-benzoquinol; α-, β-, γ-, δ-tocopherols; α-, β-, γ-, δ-tocotrienols.

Currently, as there are multiple research programs on carotenoid enrichment, and in particular β-carotene, aiming at tackling vitamin A deficiency, understanding the genetic regulation of their accumulation in fruits and vegetables is of outmost priority. Significant progress has been made towards characterizing the structural genes of carotenoid biosynthesis in several species, with the identification of mutants with altered carotenoid contents providing new insights into carotenoid regulation. Carotogenesis occurs in plastids and is dependent on precursors from the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. Apart from precursor substrate availability,

carotenoid accumulation is regulated by the transcription of biosynthetic genes such as phytoene synthase (*PSY*) and geranylgeranyl diphosphate synthase (*GGDPS*), as well as genes encoding phytoene desaturases and carotene isomerases (Fig. 17.3). As the MEP pathway is also associated with the synthesis of terpenoids, vitamin E, and hormones (GAs, ABA, and strigolactones), there is an intricate interactive network of interconnected pathways and metabolites in plants that has gained much attention. Based on these considerations, a critical bottleneck in the pathway controlling the flux to carotenoid biosynthesis rather than GAs is the *PSY* gene that catalyzes the head-to-head condensation of two GGDP molecules to produce the first colorless carotenoid, 15-*cis* phytoene. This step through *PSY* has been widely used in carotenoid pathway engineering to increase carotenoid accumulation in tomato fruits (Liu et al., 2015).

Taking into account that a rapid carotenoid accumulation is triggered with the onset of ripening and the boost in ETH production, it is evident that regulatory genes and proteins related to ETH may also be important. In this context, ETH has been reported to regulate *PSY* transcription (Liu et al., 2015), while the ETH-response transcription factor ERF6 plays a vital role in carotenoid accumulation in tomato fruit (Lee et al., 2012). The MADS-domain ripening regulators, TAGL1 and FRUITFULL 1 and 2, have also been found to regulate the ETH pathway and carotenoid accumulation during ripening (Vrebalov et al., 2009). One of the most abundantly expressed, ripening-specific genes in tomato fruit, E8, has been also involved in regulating the levels of multiple volatiles, probably by enhancing the ETH content (Tieman et al., 2017).

Another key branch point of the pathway is the cyclization of lycopene through lycopene β-cyclase (*LCYB*) or through lycopene ε-cyclase (*LCYE*), which leads either to β-carotene, zeaxanthin, violaxanthin, or neoxanthin, providing precursors for the synthesis of ABA and strigolactones, or to α-carotene and lutein, respectively (Fig. 17.3). In tomato, there are two *LCYB*: *LCYB1* is abundant in vegetative tissues, and *LCYB2* is significant in fruits and flowers (Yuan et al., 2015). Overexpression of a bacterial *LYCB* in tomato resulted in high levels of β-carotene (D'Ambrosio et al., 2004), whereas, silencing of *SlLCYB2* in tomato caused low levels of β-carotene, high levels of lycopene, and simultaneously reduced carotenoid-derived volatile content (Vogel et al., 2010).

As a next step in the biosynthetic pathway, the addition of O_2 by hydroxylases and epoxidases to carotenes produces xanthophylls. There are two different types of hydroxylases: the CHYB type, which hydroxylates the β-ring of cyclic carotenes, producing β-cryptoxanthin and zeaxanthin, and the cytochrome P450 type, forming α-cryptoxanthin and lutein (Yuan et al., 2015). In turn, zeaxanthin can be converted to violaxanthin, which can be back-converted to zeaxanthin in a reaction catalyzed by violaxanthin de-epoxidase, and AsA as a cofactor. The so-called violaxanthin cycle is essential for plants to adapt to different light conditions. In some species, such as red peppers, their

characteristic pigment arises from the conversion of violaxanthin to capsanthin by capsanthin capsorubin synthase.

Apart from biosynthesis, carotenoid accumulation can also be controlled by their catabolism, which is essential to maintain metabolic equilibrium in photosynthetic tissues. The degradation products of carotenoids, termed apocarotenoids, include isoprenoids with pivotal functions in plant−environment interactions, volatile aromatic compounds, as well as the phytohormones ABA and strigolactones (Fig. 17.3) (Beltran and Stange, 2016). Apocarotenoids can be produced nonenzymatically in reactions initiated by ROS or by enzymatic degradation of carotenoids. The carotenoid catabolic enzymes include multiple isoforms of carotenoid cleavage dioxygenases (CCDs) that contribute to the production of isoprenoid volatiles or strigolactones, and 9-*cis*-epoxycarotenoid dioxygenases (NCEDs) that are associated with ABA. Recently, genome-wide association studies in tomato identified a plethora of loci affecting carotenoid-derived volatiles, such as apocarotenoids, responsible for tomato aroma (Tieman et al., 2017). In strawberry, transcript levels of *CCD* have been related to ripening with a concomitant decrease in lutein content, while suppression of *NCED* in tomato produces fruits with high levels of lycopene and decreased ABA biosynthesis (Liu et al., 2015).

Plant endogenous AsA levels are regulated by the fine orchestration of net biosynthesis, recycling, and degradation, as well as inter- and intracellular transport, and feedback regulation through inhibition of the activity of the last enzyme in the biosynthetic pathway (Mellidou and Kanellis, 2017). Within this context, AsA can be increased primarily via enhancing its biosynthesis, promoting its recycling via the so-called AsA−GSH cycle, and reducing its breakdown, but its content also depends on the subcellular localization of structural enzymes, and inter- and intracellular AsA translocation. In principle, the pathway proceeding from glucose via L-galactose is considered as the dominant route of AsA biosynthesis in higher plants.

Although several structural genes from this pathway have been proposed to be key genetic regulators of AsA in different species, conclusive evidence suggests that it is less possible that the initial steps of the pathway through phosphomannose isomerase (PMI), phosphomannose mutase (PMM), and GDP-D-mannose pyrophosphorylase (GMP) (Fig. 17.3) exert a control point over AsA accumulation. The same holds true for GDP-D-mannose-3,5-epimerase (GME). Despite the correlation between *GME* transcripts and AsA content observed in a few studies (e.g., apple and blueberry), there is growing evidence that *GME* is not the rate-limiting step in AsA biosynthesis, but it may have diverse roles in pollen development, seed production, and vegetative growth. Currently, a plethora of studies provide important clues on the pivotal role of GDP-L-galactose phosphorylase (GGP), the enzyme catalyzing the first committed step of the pathway (Fig. 17.3) as the key control point in the AsA biosynthetic pathway in several species, such as tomato, apple, and kiwifruit (Bulley and Laing, 2016; Mellidou and Kanellis, 2017). Regarding the following steps in the

main biosynthetic pathway proceeding via L-galactose- 1-P phosphatase (GPP), L-galactose dehydrogenase (GalDH), and L-galactono-1,4-lactone dehydrogenase (GLDH), none of them was found to exert a remarkable effect over the AsA pool. Transgenic studies overexpressing these structural genes from the AsA biosynthetic pathway have so far relatively limited success in heterotrophic tissues, with the exception of overexpressing *GGP* in tomato fruits that resulted in a twofold increase of AsA content (Bulley et al., 2012). This fact reinforces the notion that it may be necessary to interfere with whole regulatory networks using more than one structural gene or transcription factor in order to raise AsA beyond the current levels. Alternative routes proceeding either via D-galacturonic acid which is used for the synthesis of L-galactonic acid derivatives via D-galacturonate reductase (GalUR), or via *myo*-inositol may also contribute to increased AsA accumulation in specific tissues or maturity stages, as well as certain species (strawberry, citrus, grapes). These alternative pathways may serve to complement synthesis from the main route, particularly under abiotic stress conditions.

For a balanced AsA pool, biosynthesis acts in concert with recycling that proceeds via the AsA−GSH cycle (Fig. 17.3). In this cycle, AsA can be enzymatically regenerated from its oxidized forms, with the contribution of GSH reductase, dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR). As AsA scavenges H_2O_2 in the first reaction of the cycle catalyzed by AsA peroxidase, it is evident that increasing AsA accumulation through manipulating recycling could provide greater protection against ROS through increasing its biosynthesis. The role of MDHAR and DHAR in controlling AsA contents has been clearly demonstrated in tomatoes (Stevens et al., 2007) and apples (Mellidou et al., 2012b), respectively, using QTL studies. However, transgenic efforts to enhance AsA contents via overexpressing these two genes yielded no astonishing results. However, the potential value of enhancing AsA recycling rate merits further exploitation, mainly as it can be an effective tool toward increasing tolerance to abiotic stress.

Similar to the regulation of carotenoid biosynthesis, phytohormones have also been implicated in controlling AsA contents. In this context, both ETH synthesis (through ACC oxidase) and signaling (through ETH receptors) seem to be involved in AsA accumulation, although the exact mechanism remains to be elucidated. An outstanding example of ETH-AsA interconnection is the AsA-rich tomato mutants *Never-ripe* that fail to ripe normally, suggesting a possible adverse link between AsA accumulation and ethylene perception.

Vitamin E biosynthetic genes have been identified using a combination of genetic and genomic tools in *Arabidopsis*, and subsequently, in many other plant species such as *Nicotiana tabacum* L. and *Lactuca sativa* L. and in several fruit species including tomato (Quadrana et al., 2013), apple (Seo et al., 2011), and mango (Singh et al., 2011), while very little information exists regarding fruit tree crops, including olive (Georgiadou et al., 2015; Georgiadou et al., 2016).

The origins of tocochromanols are from 4-hydroxyphenylpyruvic acid (HPP) and homogentisic acid (HGA), geranylgeranyl diphosphate, phytol, and phytyl phosphate (phytyl-P), which come from either the shikimate acid pathway, methylerythritol phosphate pathway, or chlorophyll degradation, respectively. The phytyl diphosphate (phytyl-PP) is derived either by GGDP after decrease by geranylgeranyl reductase or by phytol after phosphorylation by phytol kinase (*VTE5*). HPP is decreased to HGA by 4-hydroxyphenylpyruvate dioxygenase (*HPPD*), which serves as a shared precursor for the biosynthesis of tocopherols and tocotrienols (Fig. 17.3). HGA is further decarboxylated and then condensated with a phytyl diphosphate (Phytyl-PP) into 2-methyl-6-phytylbenzoquinol (MPBQ) by *homogentisate phytyltransferase* (*VTE2*). The MPBQ is either catalyzed into 2,3-dimethyl-6-phytyl-1,4-benzoquinol (DMPBQ) by 2-methyl-6-phytyl-1,4-benzoquinol methyltransferase (*VTE3*) and then to γ-tocopherol by tocopherol cyclase (*TC* or *VTE1*), or to δ-tocopherol directly by *TC*. The γ- and δ- tocopherols are changed into α- and β- tocopherols by γ-tocopherol methyltransferase (γ-*TMT* or *VTE4*). Another alternative is HGA metabolized into 2-methyl-6-geranylgeranylbenzoquinol (MGGBQ) by homogentisate geranylgeranyl tranferase (*HGGT*) and then to δ- and subsequently β-tocotrienol catalyzed by *VTE1* and *VTE4*, correspondingly. The biosynthesis of γ- and subsequently α-tocotrienol requires an additional step; the conversion of MGGBQ into 2,3-dimethyl-6-geranylgeranyl-1,4-benzoquinol (DMGGBQ) in addition to the reactions catalyzed by *VTE1* and *VTE4* (Fig. 17.3; Georgiadou et al., 2015).

Regulation of tocochromanol biosynthesis in fruits has not been fully researched. A common precursor for tocochromanols and carotenoids is *GGDP*. This means that if the carotenoid biosynthetic pathways in the fruit are changed then probably the tocochromanol levels in this organ will change. This was found in tomato fruit that presented the overexpression of a fruit *PSY*, which accumulated more α-tocopherol, although the mechanisms that caused the increased levels of vitamin E content in the fruit are not clear. What may have caused this is the light regulation of tocopherol accumulation in the fruit through the transcriptional activation of tocopherol biosynthetic genes (*geranylgeranyl reductase* and *VTE4*), as observed in the *DE-ETIOLATED1* (*DET-1)* defective tomato transgenics which accumulate 2- to 10-fold more tocopherol. However, the *DET-1* mutation, which disturbs the light signal transduction pathway, in addition has an impact on the plastid biogenesis, carotenoid biosynthesis, and other secondary metabolites. This indicates the plausibility that tocopherol changes are due to a more general effect on plastid compartment size or fruit metabolism (Baldet et al., 2014).

The peak pattern of the tocochromanols content around the breaker stage indicates a tight correlation with the expression profile of *VTE5*, which was upregulated during the period of mesocarp development followed by marked downregulation at the breaker stage and throughout ripening (Georgiadou et al., 2015). These results are in agreement with those reported in tomato fruit (Quadrana et al., 2013), which present a decrease in *VTE5* gene

expression associated with tomato ripening. This decline, directly restricts phytol diphosphate [Phytyl-PP (PDP)] input supply toward VTE biosynthesis (Quadrana et al., 2013) and correlates with the low concentrations of tocopherols and tocotrienols in the olive fruit during ripening. These results suggest that *VTE5* is particularly important in the biosynthesis of vitamin E in olive fruit and is thus proposed as a marker gene in relevant studies.

Two methods have been used to increase the content of vitamin E in crops, that is plant breeding and metabolic engineering strategies (Fitzpatrick et al., 2012). Levels of vitamin E can vary immensely between plant species and within species (i.e., among varieties and cultivars), which means that the nutritional value can be improved by the exploitation of micronutrient enrichment traits. The exploitation of this variability depends on the combination of comprehensive molecular mapping and high-throughput phenotyping methods, to use an efficient selection process. Approaches to increase the concentration of vitamins should consider the possibilities offered by the manipulation of environmental factors (temperature, light exposure, mild abiotic stresses) through agronomic practices. Treatments with plant growth regulators and/or elicitors are also an approach to increase the contents and stability of vitamins in vegetables. The application of moderate stresses through agronomic practices (without effects on production) is another way to improve the nutritional quality of products with low cost.

Metabolic engineering is based on the modification of genes which are involved in the biosynthesis, stability, recycling, transport, catabolism, or regulation of vitamin E levels in plants. The main source of vitamin E in the diet of humans is oilseed crops, metabolic engineering of vitamin E in *Arabidopsis*, canola, and soybean has been done by expressing both *Synechocystis* bifunctional prephenate dehydrogenase (*tyrA*), a feedback insensitive enzyme, and the plant *HPPD* genes. The result was an up to 1.8−2.6-fold increase in tocochromanol levels in the seeds. With a similar strategy, based on the stable coexpression in tomato of the yeast *PDH* and *Arabidopsis HPPD* genes controlled by the tomato phosphoenolpyruvate carboxylase 2 (*SlPPC2*) fruit-specific promoter, lead to a threefold increase in tocotrienol content in tomato fruit (Baldet et al., 2014).

The manipulation of *VTE4* gene is considered the most effective method for improving vitamin E content in plants, as it converts both δ- and γ-tocopherol to β- and α-tocopherol, respectively. The *VTE4* overexpression and naturally increased α-tocopherol accessions show the highest vitamin E increases so far regardless of the host or the origins of the transgene. By changing preexisting tocopherols into forms that are higher in biological potency, the vitamin E activity improves. For example, oilseed crops (that is soybean, rapeseed, cotton, and oil palm) accumulate γ-tocopherol, which is converted into α-tocopherol by *VTE4* overexpression, which in turn enhances vitamin E activity in a tissue. This conversion has been completed in many other plants such as shiso, lettuce mustard, maize, tobacco, and sunflower. As a result, most

VTE4 overexpressing crops present 5−10 times higher vitamin E activity than untransformed plants. Similarly, *Brassica napus VTE4* homolog was able to rise the α-tocopherol content 50 times in transgenic *Arabidopsis* seeds using a transgenic approach (Fritsche et al., 2017). In crops with δ-tocopherol, *VTE4* overexpression converts it into β-tocopherol, which is 16.6 times more potent at 50% of the vitamin E activity of α-tocopherol. This method, which has no negative impact on growth and fertility, is one of the most effective for vitamin E biofortification of crops (Mène-Saffrané and Pellaud, 2017).

The synthesis of the folates is split between three subcellular compartments. Guanosine triphosphate (GTP) is used for the formation of the pterin, which is then joined to *p*-aminobenzoate in mitochondria to produce dihydropteroate, which is subsequently glutamylated and reduced. The *p*-aminobenzoate moiety seems to be of plastidial origin, as in plants the first enzyme of *p*-aminobenzoate synthesis and specifically of the aminodeoxychorismate (ADC) is ADC synthase (ADCS), which is localized in the chloroplasts (Basset et al., 2004).

THF biosynthesis starts in the mitochondrion and requires the use of GTP for the generation of pterin compounds with 6-hydroxymethyldihydropterin being the starting point of the THF biosynthetic pathway. The activation of 6-hydroxymethyldihydropterin involves the use of ATP and a bifunctional enzyme, a hydroxymethyldihydropterin pyrophosphokinase (HPPK)-dihydropteroate synthase, which is located in the mitochondrion. A glutamate compound is then attached to the carboxyl moiety of the *p*-aminobenzoic acid (*p*-ABA), in an ATP-dependent reaction that results in the formation of the dihydrofolate, via the activity of a dihydrofolate synthetase. NADPH then provides the electron needed for the reduction of the dihydrofolate to THF by the enzyme dihydrofolate reductase (DHFR). DHRF in higher plants is a bifunctional enzyme that bears DHFR and thymidylate synthase (TS) activity. Its catalytic domain has a double function that involves the reduction of the dihydrofolate that is originating from the de novo synthesis of the dihydrofolate originating from the oxidation of the THF during the activities of the TS.

Folate fortification in dietary products via metabolic engineering or breeding is a prominent strategy for addressing folate deficiency, especially in underdeveloped and developing countries (Blancquaert et al., 2015). Through breeding the enhancement is achieved based on the inheritance of certain trait loci providing the favorable characteristics to the offspring from the parents. Although this method is time-consuming and is limited by the genetic characteristics available in the parental lines' germplasm, QTL can be used in marker-assisted breeding to attain a sufficient increase in folate biosynthesis (Strobbe and Van Der Straeten, 2017). Boosting the folate biosynthesis can be achieved via metabolic engineering. In tomato fruit, high levels of folate have been achieved by overexpression of genes involved in its biosynthesis (Blancquaert et al., 2015). Approaches targeting single genes in the folate biosynthesis pathway do not have significantly increased folate concentrations.

The increase is often insignificant, and in some cases overexpression of such genes, like ADCS, an enzyme initializing the formation of the *p*-ABA in the plastids, has the reverse results. For a significant increase in folate, strategies that target more than one gene are implemented and the success of a strategy in one species does not mean that it will have the same results in another. Stimulating the pathways of the pterin and *p*-ABA production via the combined use of the transgenes *GTPCHI* and *ADCS* significantly increased the rates of folate in tomato fruit. In contrast the same strategy was ineffective when implemented in *Arabidopsis* or potato tubers (Strobbe and Van Der Straeten, 2017). Increasing the content of folate in a crop is one side of the equation, as it is equally important to increase the stability of the compound postharvest, as well as during the process and cooking. In plants, the folates are predominantly present in the form of polyglutamylated derivatives, a tail of glutamates sequentially added in the THF by folylpolyglutamate synthetase. The polyglutamylation affects the stability of folate positively and the maintenance of the polyglutamate tail increases the folate stability postharvest. The accumulation and maintenance of the tail is achieved by knocking-down the γ-glutamyl hydrolase (GGH), which is responsible for the removal of the tail from the folate. The suppression of the GGH results in high levels of polyglutamate folates and in combination with overexpression on the folylpolyglutamate synthetase increases the stability of the folate in GA-engineered rice during postharvest storage conditions (Blancquaert et al., 2015; Strobbe and Van Der Straeten, 2017). The transgenic biofortified rice also meets folate requirements even after cooking losses of 45% (100 g of rice contains 500 mg of folates) (Blancquaert et al., 2015).

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CHAPTER 18
Flavors and Aromas

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18.1 INTRODUCTION

18.1.1 Definitions of Odor, Aroma, Flavor, Taste, and Chemical Feelings

Sensory attributes of foods are perceived in the following order: (1) appearance, (2) odor/aroma, (3) consistency and texture, and (4) flavor (aromatics, taste, chemical feeling).

Odor can be defined as the perception of volatile compounds by the olfactory system when they go through the nasal membranes, whilst *aroma is the odor of a food*. However, other definitions can be given for odor and aroma; for example, odor can be the perception of the volatile compounds with the food outside the mouth, while aroma can be is defined as the perception of the volatile compounds with the food inside the mouth.

Food flavor is the sum of perceptions resulting from stimulation of the senses by the food at the junction of digestive and respiratory tracts. Within this complex property, three subconcepts are included: (1) aromatics (volatiles perceived by the olfactory system in the mouth), (2) tastes (soluble substances perceived by the gustatory system in the mouth), and, (3) chemical feelings (stimulation of nerve ends in the buccal and nasal cavities, such as astringency and pungency).

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18.2 COMPONENTS OF THE FLAVOR AND AROMA OF FRUITS AND VEGETABLES

Flavor and aroma of fruits and vegetables are the core contribution to consumer acceptance. Sweet, salty, sour, bitter, and umami are considered basic tastes, but in fruit and vegetables, the common basic tastes are sweet, sour, and bitter. Soluble sugars and organic acids provide sweet and sour taste, respectively, while others such as phenolic compounds, triterpenes, or some aldehydes provide bitterness. The main compounds responsible for the other main tastes, salty and umami, are salts and glutamic salts, respectively. On the other hand, odor is more difficult to study because it is influenced by a large number of different volatile compounds, and chemical sensations. These sensations are produced by chemical irritants that stimulate trigeminal nerve ends; for example, astringency (causing the skin to contract or tighten) is caused by tannins and pungency (burning sensation) by capsaicin, piperine, etc.

18.2.1 Taste Components

Sweet, sour, and bitter are the main tastes found in fruits and vegetables. If appearance leads consumers' first buying choice, taste attributes are the key to the acceptance of the purchased product and loyalty in future purchases. These sensory attributes are due to different chemical compounds which is highly relevant for food companies.

Sugars are the main chemical compounds responsible for sweet taste. The composition and content of each individual sugar can be affected, among other factors, by the cultivar and the fruit maturity stage at harvest. The most common sugars in fruits and vegetables are sucrose, glucose, and fructose. Glucose and fructose are monosaccharides, and glucose a disaccharide (glucose-fructose). Here are some examples of the major sugar compound in some fruits:

- Glucose: table grapes;
- Fructose: strawberry, blueberry, mango, tomato, and pomegranate;
- Glucose: apricot, plum, and peach.

Organic acids are the chemical responsible for sourness in fruit and vegetables. As reported for sugars, the acid profile of fruits and vegetables changes depending on different factors and so does the sour perception in the mouth. Sour taste depends on the concentration of hydrogen ions (H^+ or more precisely H_3O^+) and the ability of the acid to dissociate. The most common organic acids are malic, citric, tartaric, quinic, oxalic, fumaric, and succinic acid. Some examples of the major organic acids found in specific fruits are given include the following:

- *Tartaric acid*: table grape and pineapple;
- *Citric acid*: orange, lemon, pineapple, strawberry, blueberry, mango, and tomato;
- *Malic acid*: apricot, plum, nectarine, pomegranate, apple, and cherry;
- *Benzoic acid*: cranberry, raspberry, plum, prunes, and lingonberry.

The relative proportions of each sugar and organic acid, with its unique taste, greatly contributes to the changes of the overall taste. For example, citric acid masks the perception of sucrose and fructose, while malic acid enhances sucrose perception.

Bitterness is possibly the most unpleasant taste for humans, which is due to the bitter taste of poisonous compounds and our instinct to reject them. However, most of the bitter compounds found in plants have bioactive properties in our organism. Several compounds can be perceived as bitter, such as ions, peptides, alkaloids, polyphenols, terpenes, and glucosinolates, but *polyphenols are mainly responsible for the bitterness in fruits and vegetables*; for instance, pomegranate and quinces are fruits rich in bitter polyphenol compounds.

Salty and umami tastes are rarely found in fruits and vegetables because they are generated by salts and glutamic salts, respectively. Salt structure is made by a chemical bond of a cation and an anion, with the cation being responsible for the salty taste. Sodium chloride (NaCl) is the structure that produces the common salty taste, although there are other salts that also produce salt taste: LiCl, KCl, $MgCl_2$, etc. Glutamate, or glutamic acid, is mainly responsible for the umami taste. It is an amino acid which can be part of some salts, such as sodium glutamate, which is considered the most common substance responsible for the umami taste.

18.2.2 Odor and Aroma Components

Besides appearance, odor is the main attribute controlling consumer purchasing decisions. *The mixture of many volatile compounds is responsible for aroma and odor*, so the qualitative and quantitative composition of a fruit or vegetable determines its particular aroma. In addition, each volatile compound has a defined sensory descriptor that can be perceived by the human nasal olfactory system.

Volatile compounds can be classified by their chemical structure, and some of them are species-specific. The main families of compounds are: esters, alcohols, aldehydes, terpenes, hydrocarbons, and others. Some examples of volatile compounds in different fruits and vegetables are given in Table 18.1.

18.2.3 Chemical Feeling Factors and Their Components

There are some compounds, called "chemical irritants," that cause perceptions of burning, heat, astringency, cold, or pungency in the mouth, nose, and eye mucosa. Those sensations are due to the stimulation of trigeminal nerve ends. Normally, high concentrations of these compounds are needed to stimulate the trigeminal nerve, but there are some exceptions, such as capsaicin (chili peppers), whose threshold of perception is usually very low. The most common chemical feelings perceived in fruit and vegetables are astringency and pungency.

Table 18.1 Examples of Key Volatile Compounds in Some Fruits and Vegetables

Compound	Descriptors	Fruits and Vegetables
Esters		
Ethyl acetate	Fruit, brandy, pineapple	Apple, mulberry, pomegranate
Ethyl butanoate	Apple, fruity, pineapple	Apple
Ethyl hexanoate	Apple, banana, pineapple	Apple, pomegranate
Propyl acetate	Fruity, pear	Apple
Butyl acetate	Red apple	Apple, apricot
Hexyl acetate	Red apple, pear	Apple, apricot, peach, pomegranate
Alcohols		
1-Propanol	Alcoholic	Apple, pomegranate
Eucalyptol	Citrus, herbaceous, fruity	Tomato
1-Pentanol	Fusel like, mild	Apple, tomato, pomegranate
1-Hexanol	Sweet alcohol, muddy	Apple, tomato, pomegranate, oyster mushroom, cauliflower
trans-2-Hexen-1-ol	Green fruit, caramel	Apple, tomato
2-Methyl-1-propanol	Sweet	Apple
cis-3-Hexenol	Fresh, green grass	Tomato, pomegranate, cabbage, broccoli, cauliflower
Linalool	Lemon, orange, citrus	Tomato, apricot, mandarin, peach
α-Terpineol	Lilac	Tomato, apricot, pomegranate, mandarin
Aldehydes		
Hexanal	Fatty, green, grassy, powerful, penetrating	Tomato, apricot, mulberry, pomegranate
trans-2-Hexenal	Sweet, almond, fruity, green	Tomato, apricot, mulberry, pomegranate, oyster mushroom, cabbage, broccoli, cauliflower
Nonanal	Apple, coconut, grape, lemon	Mulberry, tomato, pomegranate, peach
Decanal	Floral, citrus, sweet	Tomato, peach, pomegranate
Geranial	Lemon	Tomato
Benzaldehyde	Bitter, almond, fragrant, cherry	Mulberry, apricot, oyster mushroom, cabbage, broccoli, cauliflower
cis-2-Nonenal	Fatty, waxy	Mulberry
3-Methylbutanal	Fruity, peach, cocoa	Mulberry, apricot, pomegranate, oyster mushroom
2-Methylbutanal	Fresh, fruity, herbal	Mulberry, apricot
Phenylacetaldehyde	Apple, apricot, berry, cherry, grape	Cabbage, broccoli, cauliflower
Terpenes		
Limonene	Lemon, orange, citrus	Mulberry, tomato, pomegranate, mandarin, broccoli, cauliflower
α -Pinene	Sharp, pine	Tomato, pomegranate, mandarin
β-Pinene	Woody, pine	Pomegranate, mandarin

(*Continued*)

Table 18.1 (Continued)

Compound	Descriptors	Fruits and Vegetables
β-Myrcene	Sweet, balsamic, plastic	Pomegranate, mandarin, peach
α-Terpinene	Lemon	Pomegranate, mandarin
Lactones		
ϒ-Octalactone	Butter, caramel, musty	Peach
Hydrocarbons		
Dodecane	Woody	Pomegranate
n-Tetradecane	Creamy	Oyster mushroom
Acids		
Hexanoic acid	Cheesy, fatty, sour	Mulberry, pomegranate, tomato
Octanoic acid	Coconut, creamy, herbaceous	Mulberry, pomegranate
Acetic acid	Sour	Mulberry, pomegranate
Sulfides		
Dimethyl disulfide	Green	Cabbage, broccoli, cauliflower
Dimethyl trisulfide	Sulfurous	Cabbage, broccoli, cauliflower
2,4,5-Trithiahexane		Cabbage, broccoli, cauliflower
Isothiocyanates		
Allyl isothiocyanate	Onion	Cabbage, cauliflower
But-3-enyl-isothiocyanate		Cabbage, cauliflower
2-Phenylethyl isothiocyanate	Green	Cabbage, broccoli, cauliflower
Cyanides		
3-Methylthiopropyl cyanide		Cabbage, cauliflower
2-Phenylethyl cyanide		Cabbage, broccoli, cauliflower

Astringency is due to chemical compounds producing a sensation of dryness in the mouth caused by the precipitation of salivary proline-rich proteins. Typical astringent compounds are low-molecular-weight polyphenols and some tannins. Astringency is linked with bitterness as several astringent phenols activate bitter taste receptors. Thus, astringent compounds are bitter and it is very usual to detect astringency and bitterness in the same fruit or vegetable. Some of the most common astringent phenols are caffeic acid (cocoa), catechin (tea), kaempferol (onion), ferulic acid (cocoa), gallic acid (tea), luteolin (cocoa), quercetin (tea), etc.

Pungency is described as a burnt feeling. It is not common in fruits, but it is typical in *Capsaicum* species. Furthermore, pungent components from hot peppers are under study as functional compounds, mainly capsaicinoids. The capsaicinoids family includes compounds such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, homocapsaicin, and others. Pungency is also caused by some spices (black pepper) and it is provided by piperine, chavicine, or piperittine, which are alkaloids.

18.3 SYNTHESIS OF THE MAIN BIOCHEMICAL COMPOUNDS BEHIND THE FLAVOR OF FRUITS AND VEGETABLES

18.3.1 Taste Compounds of Fruits and Vegetables

Basic tastes are felt in the mouth because taste stimulus molecules are solubilized in saliva and reach taste buds in the tongue. Taste buds are formed by a variable amount of cells which are responsible for sending the stimuli to the brain. There are different types of cells in taste buds:

- *Type I cells*: responsible for salt transduction. Ions pass through ion channels in cell membranes. They communicate by synapsis.
- *Type II cells*: responsible for sweet and bitter compounds, as well as glutamate. They have G-protein-coupled receptors on their microvilli that respond to substances. Molecules act as "keys" that fit the "locks" formed by shapes of the G-protein-coupled receptor portions outside the membrane. These cells make a cell-to-cell communication (they do not have synapses).
- *Type III cells*: responsible for sour transduction. Ions pass through ion channels in membranes. They communicate by synapsis.

As previously mentioned, sweet, sour and bitter are the main basic tastes of fruits and vegetables. The sweet taste is directly related to the concentration of sugars, which can be found as glucose, fructose, sucrose, sugar alcohols, starch, and can be converted into lipids. The variability in the sugar content depends on the activity of the main metabolic pathways such as glycolysis, tricarboxylic acid cycle, and respiration. Relative sweetness changes depending on the sugar structure, making us feel different sweet intensities with the same quantity of different sugars. If we take sucrose as a reference (sweetening power 1), fructose power is 1.73 and glucose power is 0.74.

A sour taste is due to organic acids, which are widely distributed in nature. Malic, citric, and tartaric acids are the main compounds responsible for the sour taste of fruit and vegetables, and each of these acids has a unique taste that contributes to the overall flavor. In general, *young/unripe fruits have high contents of organic acids, which decrease during pre- and postharvest maturation and ripening due to their conversion into sugar (gluconeogenesis)*. Most of the organic acids are intermediates in the metabolic pathways of the tricarboxylic acid

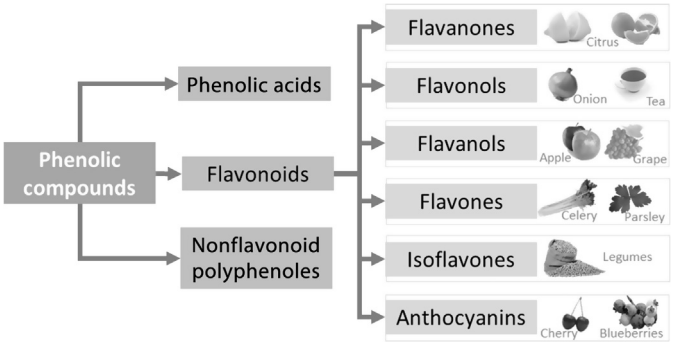

FIGURE 18.1
Classification of the polyphenolic compounds found in fruits and vegetables.

cycle. The phosphoenolpyruvate carboxylase and NAD-malate dehydrogenases are the enzymes involved in *malic acid* synthesis, while the NADP-malic enzyme is responsible for the malic acid degradation. The enzymes responsible for *citric acid* synthesis are citrate synthase and mitochondrial aconitase, while the cytosolic aconitase and NADP-isocitrate dehydrogenase are involved in citric acid catabolism. *Tartaric acid* is the main organic acid in grapes, and can be biosynthesized from carbohydrates and other organic acids.

The bitterness of fruits/vegetables is mainly related to the occurrence and content of polyphenols. The major group of polyphenolic compounds found in fruits is phenolic acids (hydroxybenzoates and hydroxycinnamates) and flavonoids (Fig. 18.1). The main precursor of most fruit phenolic compounds is the aromatic amino acid, L-phenylalanine. The biosynthesis of phenylalanine occurs through the shikimate or arogenate pathway, and can be later deaminated by the enzyme phenylalanine ammonialyase (key enzyme in phenolic biosynthesis) which catalyzes the nonoxidative deamination of L-phenylalanine to form *trans*-cinnamic acid and a free ammonium ion. This reaction is the first step in the biosynthesis of coumarins, flavonoids, lignans, and other phenolic compounds.

18.3.2 Odor and Aroma Compounds of Fruits and Vegetables

The biosynthesis of flavor (aroma, basic tastes, and chemical feeling factors) compounds is reliant on primary and secondary metabolites derived from photosynthesis. The odor and aroma of fruit are formed by tens to hundreds of compounds generated during all phases of growth and fruit maturation. *Terpenoids* are the largest and most structurally varied family of plant secondary metabolites. Terpenoids are very important for the quality of fruits and vegetables and have commercial interest because of their use as flavorings and fragrances in foods and cosmetics. All terpenoids are derived by repetitive fusion of branched five-carbon units based on an isopentane skeleton. In

some species they are very important for the characteristic flavor and aroma, such as limonene and valencene in citrus fruits. As an example, linalool (acyclic monoterpene alcohol) is one of the main important volatile compounds determining the flavor quality of tomatoes, peaches, plums, or pineapples, as well as several essential oils: coriander and sweet orange (S-linalool); and in lavender, laurel, and sweet basil, among others (R-linalool). Linalool synthase uses geranyl diphosphate synthase as a substrate, which is an intermediate compound in the pathway that leads to the formation of carotenoids and other pigments.

The green and grassy notes associated with many unripe fruits and vegetables are provided mainly by the C6 volatiles compounds, such as hexanol, hexanal, *cis*-3-hexenal, *cis*-3-hexenol, or *trans*-2-hexenal. Their precursors are C18 fatty acids linolenic and linoleic acids, which are the substrates for 13-lipoxygenase to produce hydroperoxides. The C6 aldehydes are subsequently released by hydroperoxide lyase(s). Finally, the enzyme alcohol dehydrogenase 2, a member of the short-chain dehydrogenase/reductase (SDR) family of enzymes, converts the aldehydes to alcohols (Fig. 18.2).

Volatile apocarotenoids contribute to the flavor of valuable foods, such as saffron. Some examples are β-ionone, 6-methyl-5-hepten-2-one, and geranylacetone, which have characteristic floral and fruity notes. These compounds can be directly generated in fruits by the action of carotenoid cleavage dioxygenases (Fig. 18.2), and are synthesized only at the latest stage of ripening (carotenoids are located within chloroplasts through most of fruit development and chromoplasts during ripening).

Another category of volatile compounds includes those synthesized from fatty acids or their immediate precursors (α-keto acids), such as isobutyl

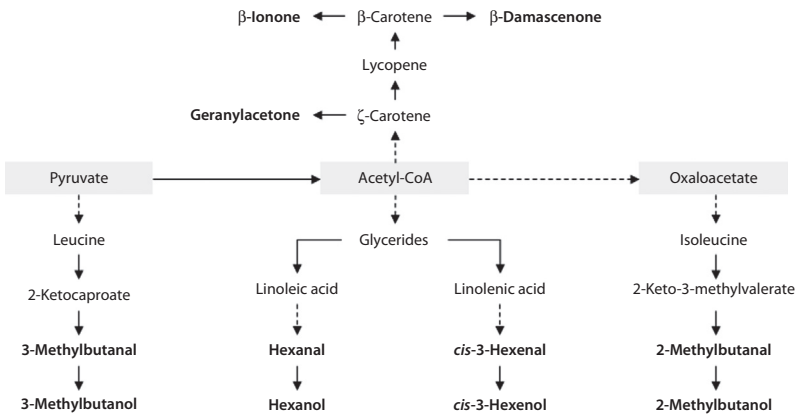

FIGURE 18.2

Summary of metabolic pathways leading to flavor-associated volatile synthesis (dashed lines indicate multiple step reactions; flavor volatiles are shown in bold).

acetate, 3-methylbutanal, 2-methylbutanal, and their corresponding alcohols (3-methylbutanol and 2-methylbutanol). The route to synthesize these compounds is the enzymatic decarboxylation of the α-keto acids to generate aldehyde volatiles, followed by the action of an SDR enzyme to generate the corresponding alcohol (Fig. 18.2). Other compounds are synthesized from phenylalanine by aromatic amino acid decarboxylases, obtaining volatile compounds such as phenylacetaldehyde or 2-phenylethanol, which are responsible for floral aromas in many fruits and vegetables.

Volatile compounds present in fruits and vegetables are perceived by humans thought the olfactory sense. It has been observed that highly volatile substances are perceived faster than others. Likewise, as the molecular weight decreases, the perception is also higher, whereas one of the main factors to be considered is the detection threshold of the person who smells. Not all volatiles are detected by humans, with their concentration and volatility being key factors in their detection.

Sensory analysis is used to analyze volatile compounds. Descriptive sensory analysis by a trained panel is commonly used to identify the perceptible volatile compounds. In order to allow proper identification, reference compounds are presented to panelists during training. It is very important to properly isolate samples during handling, storage, and analysis to avoid contamination with volatiles from other samples or artefacts (compounds initially not present in the fresh product).

18.3.3 Chemical Feeling Compounds

The chemical feeling compounds responsible for the *astringent feeling (astringency)* are mainly *polyphenols* and their relative concentrations depend on many factors. The biosynthesis of phenylalanine and its transformation on *trans*-cinnamic acid is the main pathway for the formation of polyphenols in fruits/vegetables. There are other specific pathways for the formation of astringent compounds, such as *tannins*, which are mainly responsible for the astringency in grapes, which are synthesized through the gallic acid pathway.

Capsaicinoids are plant secondary metabolites, with capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide), being the main compound responsible for the pungency of fruit/vegetables, primarily peppers. There are two main pathways for the synthesis of capsaicinoids: (1) dependent on the pathway of the phenylpropanoid (phenylalanine), and (2) dependent on the branched chain fatty acid pathway (derived from valine or leucine).

18.4 CHANGES TO THE MAIN BIOCHEMICAL COMPOUNDS DURING RIPENING AND STORAGE

Ripening is the physiological, biochemical, and sensory changes that occur from the last stages of growth and development of fruit and vegetables to

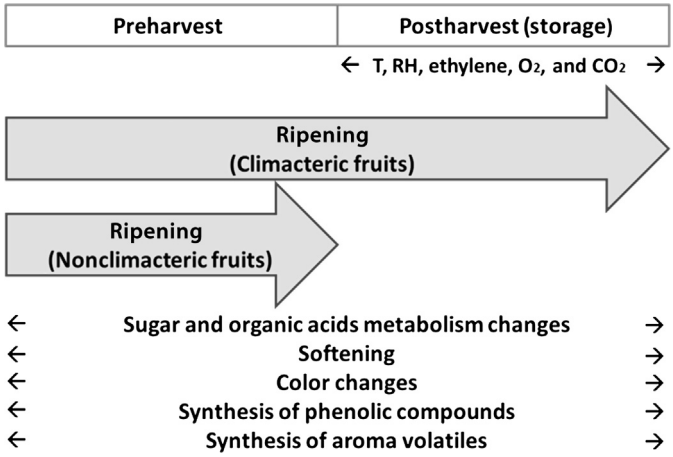

FIGURE 18.3

Relationship among ripening, changes during ripening, and storage (*T*, storage temperature; *RH*, relative humidity).

the earliest stages of senescence. Depending on the mechanisms of the regulation of the ripening process, fruits and vegetables can be divided into two groups:

- *Climacteric*, the phytohormone *ethylene is the coordinator of the ripening process*, increasing respiration and the production of ethylene (fig, banana, tomato, and apple); and
- *Nonclimacteric*, these keep the rate of ethylene production almost unchanged (pomegranate, grape, artichoke, cucumber, and pineapple). As nonclimacteric fruit and vegetables do not respond to ethylene, they present limited postharvest flavor development.

The ripening process can be preharvest (climacteric or nonclimacteric), and/or, postharvest during adequate storage (climacteric). Storage factors that influence taste and flavor are: maturity stage, temperature, edible coating, related humidity, ethylene, and O_2 and CO_2 concentrations. Biochemical and physiological changes depend on the specie, variety, and storage factors, but also major changes occur during ripening: in sugars, organic acids, phenolic compounds, aroma volatiles leading to flesh softening (Fig. 18.3).

18.4.1 Changes to Taste Compounds

At the beginning of ripening, there is often an increase in sugars, such as sucrose, via translocation from photosynthetic leaves and/or hydrolysis of starch reserves in fruit and vegetables degraded by amylase activities, leading to an increase in total soluble solids (TSSs). During the advancement of ripening, glucose and fructose increase by the action of invertase enzyme through

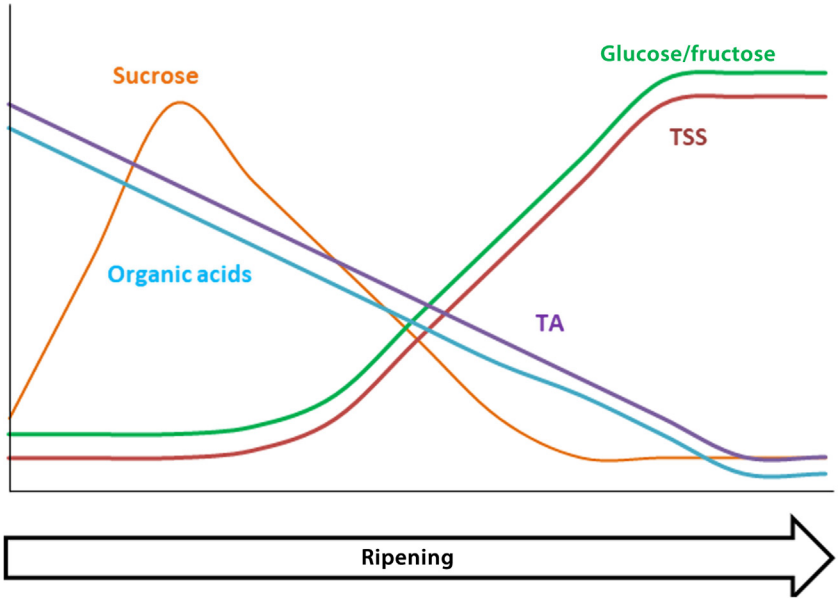

FIGURE 18.4
Main changes with ripening time on the main taste compounds and maturity parameters in fruits and vegetables (*TSS*, total soluble solids; *TA*, titratable acidity).

glycolysis (Fig. 18.4). The amount of sucrose can also be increased due to gluconeogenesis of organic acids. On the other hand, during fruit ripening, there is a common decrease in organic acids that are converted into sugars during respiration. The accumulation of sugars (TSS) and the decrease of total acidity (TA) during ripening yields mature fruits with ripe organoleptic sensory attributes. The ratio between soluble solids and acidity (TSS/TA) is used as the ripening index, and is related to the overall consumer appreciation.

The variation in total phenolic compounds and antioxidant capacity during preharvest ripening has been studied in several vegetables and fruits: plum, sweet cherry, pepper, pomegranate, and tomato, among others. The general trend observed was an increase in these compounds which generate bitter taste, except in tomato fruits. On the other hand, a decrease has been reported during inappropriate postharvest ripening in climacteric fruits due to the oxidation of polyphenols by polyphenol oxidase.

There are scientific evidences that aldehydes decrease significantly during the ripening of several fruits and vegetables, such as apricots, nectarines, kiwis, and apples. On the other hand, the application of edible coatings during postharvest in nonclimacteric fruits, such as oranges, may increase the content of aldehydes (e.g., acetaldehyde) and esters (e.g., ethyl acetate) due to the anaerobic conditions and degradation of amino acids, increasing bitter taste.

18.4.2 Changes to Odor and Aroma Compounds

Aroma compounds are divided into two groups: (1) primary volatiles, related to the smell of the fruit, and, (2) secondary volatiles, generated during eating.

The unique flavor of each fruit or vegetable is not present at early stages of growth, but appears during ripening due to the accumulation of volatiles, which can be increased up to 1000 times (Fig. 18.5). Fruit volatiles include compounds from different chemical families, including alcohols, aldehydes, esters, ketones, furanones, and terpenes, which determine the fruit aromatic characteristics.

The production of volatile compounds is related to their precursors, such as sugars and fatty acids. For instance, the sucrose content is related to the production of volatile compounds, especially with furanones. Moreover, generally, the synthesis of esters and alcohols increases during ripening because of lipid degradation.

18.4.3 Changes to Chemical Feeling Compounds

18.4.3.1 ASTRINGENCY CHANGES

During preharvest and postharvest ripening, astringency decreases probably due to the polymerization of tannins, increasing the proportion of higher-molecular-weight compounds in ripe fruit (formation of C–C links between the most reactive positions of monomers). On the other hand, some catabolism of tannins can also take place during ripening, reducing astringency. Polymerization and catabolism depend on specie, variety, and fruit part. For

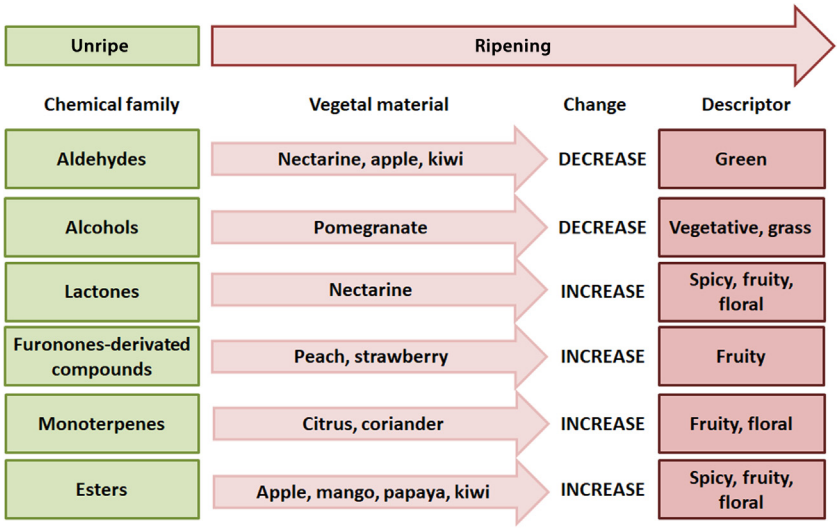

FIGURE 18.5

Main changes on volatile compounds in different fruits and vegetables during ripening.

instance, during ripening, the concentration of soluble tannins decreases in persimmon fruits, whereas it increases in pomegranate fruits (just in arils, not in skin).

18.4.3.2 PUNGENCY CHANGES

The capsaicin content of Jalapeño peppers increases during ripening up to the maximum concentration approximately 45 days after fruit set, decreasing thereafter, including fruit senescence. Other substances responsible for the pungency are piperine and other nitrogen-containing compounds such as piperidine, chavicine, and piperittine, which are mainly present in unripe black pepper. The pungency of black pepper is reduced during ripening.

18.5 MEASUREMENTS OF FLAVOR COMPONENTS AND THEIR PRECURSORS

Taste components, such as sweetness, sourness, and astringency of fruits and vegetables can be easily estimated by measuring: sugar profile (high-pressure liquid chromatography coupled with refraction index detector, HPLC-RI), acidity (by titration or by organic acid profile by HPLC-UV, using an ultraviolet detector), total phenolic content and alkaloids and glucosides (astringency and bitterness).

Volatiles determination is far more complex, and both qualitative and quantitative information are required to characterize flavor- and aroma-related compounds. In addition, volatile compounds are present in low amounts (from μg/kg to mg/kg). Several factors affect the ability of chemical compounds to be perceptible and affect flavor and aroma. Some factors can be related to the chemical-physical properties of the compound itself: chemical structure, molecular weight, functional groups and position, polarity and concentration, among others; whereas others are external, such as matrix composition and structure, temperature, and others. All those factors need to be considered when aiming to quantify volatile compounds; such compounds need to be released from the matrix and diffuse into the environment. The selective extraction of odorants is mainly based on their volatility or solubility. Solvent extraction techniques rely on product solubility, headspace analyses on volatility, and combined methods such as simultaneous distillation-extraction (SDE) to try to analyze both soluble and volatile compounds. Many odorous compounds are present in low amounts (μg/kg) and pose difficulties for accurate determination and although many techniques can be used, most of them are insufficiently reliable to provide a comprehensive description of compounds responsible for flavor and aroma. There are several reasons for flavor and aroma being difficult to be accurately determined, not only compound properties, but also external factors. It is well known that the matrix–flavor partition coefficient changes during food consumption given that temperature, humidity, degree of solubilization, and structure change during mastication, when secondary compounds are generated as well. However, although difficult and

noncomprehensive, flavor components and their precursors are being routinely analyzed and reported in laboratories. A wide set of techniques are available and in continuous development and testing to enhance the quality and reliability of determinations, increase analytical throughput, decrease analytical costs, and provide better guides to users. In this section, a view of actual methods used for the determination of flavor components and their precursors in fruit and vegetables will be provided, and their main strengths and weaknesses will be listed.

Volatiles from intact, cut, or macerated fruit can be collected using headspace techniques and analyzed directly or after concentration using various trapping technologies. In addition, volatile compounds can be extracted from homogenized fruits using various distillation and solvent extraction techniques. Then, *the most useful analytical technique to identify and quantify fruit aroma compounds is gas chromatography-mass spectrometry (GC-MS)*. However, aroma extraction methods, as well as sample preparation (intact fruit, cut, macerated), affect the profile and concentration of the extracted volatile compounds. Fruit volatiles are usually collected by headspace techniques, and also by distillation and solvent extraction techniques. Volatile compounds need to be further identified and quantified by chromatographic methods, usually by GC-MS. The electronic nose (E-nose) is also being applied to study the aroma of fruits. It is a nondestructive method that allows the analysis of aroma intensity, but it cannot, however, identify nor quantify particular aroma compounds. Finally, to relate the contribution of volatile compounds to fruit aroma and flavor, human olfactory analysis is required, because humans can smell volatile compounds at ppb levels or lower. Thus, the combination of sensory analysis of fruit flavor with instrumental analysis provides greater insights into the impact of volatile compounds on flavor than either alone.

18.5.1 Instrumental Methods

Methods for sample preparation, separation, and the quantification of volatile aromatic compounds are classified as follows.

18.5.1.1 SAMPLE PREPARATION

Headspace techniques [static (SH) and dynamic (DH)] allow direct determination of flavor compounds without disrupting food structure, nor using solvents. In SH, samples are inserted into sealed vessels and are allowed to equilibrate with the vapor phase (headspace) that is afterward injected into a gas chromatograph (GC). Non- and low-volatile compounds, and solvent peaks are not injected into the GC, avoiding problems. Those are interesting advantages added to the simple sample preparation and the possibility of being automated. The main disadvantage is that they fail to detect some compounds; the partition of volatiles between air and the matrix (water, lipids) affects headspace composition, and the detection power mainly depends on

exposure time and sample temperature, as heating is limited (up to 150°C) in SH; thus, this technique can be insufficient to determine compounds present in low amounts.

Regarding DH, also called purge-and-trap analysis, the sample is thermostated in a chamber and an inert gas passes through at a defined flow rate. Compared to SH, the detection power is enhanced down even to μg/kg. This technique allows the use of sorbents and traps for the collection and concentration of compounds of interest. The proper selection of traps and sorbents is essential for good results. It needs more complex equipment and is more costly than SH, but has been proved useful for the analysis of volatiles in vegetables.

Solid-phase microextraction (SPME) is an evolution of headspace techniques, although it can also be used in a direct extraction mode. SPME achieves sampling, isolation, concentration, and enrichment in one step (Fig. 18.6). For headspace-SPME, the sample in an isolated and inert chamber is equilibrated in the presence of a fiber coated with one or more sorbents. The fiber is protected in a tube. The fiber is slid outside the tube to be exposed to the chamber, and then retracted by sliding in to be carried to the injector port. The instrument is simple, with no use of solvent and of reduced size, which allows its use as a portable device and it also has the advantage of low environmental impact. The extraction of compounds depends on their partition coefficient among the matrix (which can be modified: pH, ionic strength, organic solvent content) and the sorbents, as well as: agitation method, exposure time, and temperature of the sample. Their sensitivity and detection power are similar to

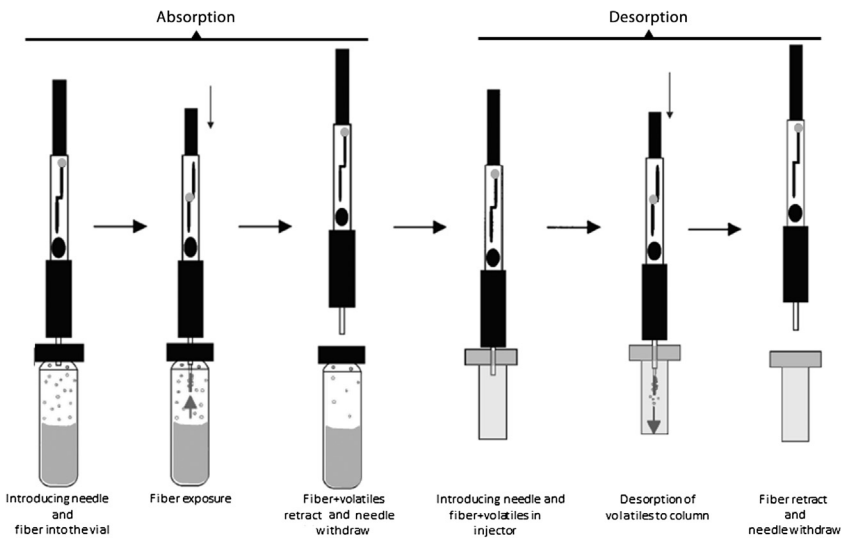

FIGURE 18.6
Schematic summary of how HS-SPME works.

that of liquid extraction, although there is a lower extent of extraction. Trapped compounds are released from the fiber by exposing the fiber in the injection port of a GC following fiber manufacturer recommendations. Compounds are further separated, and determined by GC connected to a flame ionization detector (FID) or a mass spectrometry detector (MS). Nowadays, the most effective fiber for volatile analysis in fruit, juices, and wines is that coated with three polymeric organic phases CAR-DVB-PDMS (carbowax-divinylbenzene-polydimethylsoloxane). New fiber developments are also promising, and novel developments are expected. SPME-GC-MS is a well-established method for the analysis of volatiles in vegetable foods with increasing applications in food samples (nutrients, adulteration, etc.) and it is the most used microextraction technique for plant volatiles being reported as the method of analysis in 75% of the scientific literature published from 2008 to 2012.

The stir bar sorptive extraction (SBSE), as SPME, can be used directly as a stirring bar or exposed in the headspace. Extraction of volatiles from the stirring bar takes a longer time than SPME fiber desorption. The SBSE has been proved to be an effective technique for monitoring metabolism of flavor compounds and precursors in plant and wine analysis.

Other sample preparation techniques are *simultaneous steam distillation-extraction* (SDE) and *hydrodistillation* (HD). In studies on traditional tomato cultivars, it has been reported that SPME better estimated tomato odor, whereas SDE and HD were better correlated to tomato aroma. Other authors also stated that both SPME and SDE can provide complementary information. Also, liquid/liquid microextraction methods are available and have promising applications in foods; however, currently, they are not popular among the scientific community.

Miniaturization, solvent reduction or elimination, and automation of sampling techniques are the main target for equipment developers and analysts as these enhancements may improve precision and throughput of the techniques.

18.5.1.2 SEPARATION AND IDENTIFICATION: GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY/OLFACTOMETRY

It is well known that reliable and quality separation by GC requires proper sample preparation and proper selection of stationary phase, carrier gas, injection technique, oven conditions, and detection technique (detector). Column manufacturers and scientific literature provide a whole set of commercially available columns and reference conditions. Regarding detectors, FID and MS are the most commonly used, although several new developments are also available and are being evaluated for the determination of volatiles in fruits and vegetables. The FID detector senses and measures the currents generated by ions formed during combustion of compounds (previously separated by the column) in a hydrogen (H_2) flame, generation of which is proportional to the concentration of the compound in the sample gas stream. The MS

detectors have higher detection power (10^{-12}) than the FID ones (10^{-11}), and also provide information on the fragmentation pattern of each component and so inform on their molecular weight, structure, and functional groups. MS detection can be carried out in *full scan mode* (FSM), which is highly reproducible and is basically used for compound identification, or *selected ion monitoring* (SIM), which is highly valuable for trace analysis and must be used for compound quantification. For identification of compounds by MS, usually more than one approach is used: mass spectra and its comparison with libraries (databases of spectra) together with comparison of retention time of standards or indexes calculated based on retention times of hydrocarbons (retention index), among others. The most common ionization mode is electronic impact (EI), further developments of detectors based on MS have been made, such as chemical ionization and laser ionization, and are also used for the analysis of fruit volatiles. Among chemical ionization, proton transfer reaction (PTR-MS), can analyze compounds at very low concentration (μg/kg). Regarding laser ionization, resonance-enhanced multiproton ionization (REMPI) combined with time of flight (TOF) as REMPI-TOF-MS provides high selectivity and may gain relevance in online monitoring of volatiles. Very recently, PTR-TOF-MS has been successfully used for aroma determinations of commercial peaches.

Gas chromatography can also be coupled to an *olfactometry port* (also known as a sniffing port, GC-O). In this set up, the column ends in a quartz Y-shaped joint, one branch connected to the detector and the other to the sniffing port. An analyst is needed to describe the nature and intensity of the odor. The analyst switches a potentiometer when it perceives the odor to generate the signal to be stored by the software. Recent developments of GC-O include charm analysis (CA) and aroma-extract dilution analysis (AEDA). Both are similar and samples are diluted until the subject does not detect the flavor and so allows for threshold determination. However, when GC-O is used, compounds are smelt as they come out the column separately, and so interaction between chemicals (synergism, masking) is lost. Some other drawbacks of the method are that usually measurements rely on only one subject (a higher number of subjects would be convenient) and that compounds eluting close to each other may hinder proper evaluation.

Other detectors such as infrared spectrometry (GC-IR) and Fourier transform infrared spectrometry (GC-FTIR) are being used in forensic, drugs, and perfume analysis. A quite interesting technique is isotope-ratio mass spectrometry GC-IRMS, which is being used for authentication of organic foods, food origin, forensic drugs, and has been successfully used for apple aroma studies. Multidimensional separation is becoming increasingly popular and involves more than one column with different detectors, such as ion-trap MS/MS, rapid scanning Q-MS or TOF-MS; in this way, the analytical potential of traditional GC-MS is enhanced. Currently, they are still complex, time-consuming, and costly; however, they may be expected to be increasingly used and improved.

18.5.1.3 ELECTRONIC NOSE

Electronic nose (E-nose) devices have been used for the evaluation of volatiles from fruit and vegetables to assist in fruit grading. An E-nose does not identify individual compounds and so provides complementary data to that of GC and sensory testing. In an E-nose, a number of sensors interact with the volatiles, the E-nose processes the electronic output signal from all the sensors and collectively assembles them to form a distinct digital pattern (electronic aroma signature pattern, EASP), which is like a smell-print of the gas being analyzed. Such a pattern can be used to identify a fruit type or variety or can be used in ripeness or quality grading with the advantage of being a nondestructive method. Recently, the use of E-nose in fruits was reviewed and other potential applications of this technique were pointed out, such as detection of pesticide residues, postharvest fruit disease detection, or monitoring of gases released during ripening.

18.5.1.4 INNOVATIVE INSTRUMENTS

As previously stated, food consumption modifies food matrices and there is no real static equilibrium of volatiles. Thus, new strategies are being used to have a better approach to flavor and aroma release and perception by instrumental analyses. They include the analysis of the air expired from the nose and mouth during eating (a tube collects the air which is adsorbed to a trap or directly injected to a GC). By using this method, it has been proven that volatiles are released under different kinetic conditions and time release (TR) curves can be obtained. These curves can be compared with time intensity (TI) plots obtained from sensory testing during a given period of time. Thus, TI constitutes a dynamic sensory method.

Patented colorimetric sensory arrays for organic volatiles are available for several uses. They change color after reacting with a single compound or even a unique fingerprint; they can be developed for specific uses and they aim to assess food quality based on odor monitoring and may be especially useful for packed fruits.

18.5.2 Sensory Analysis Methods

Instrumental determinations of volatile profiles, although powerful, cannot replace sensory analysis of foods, which provides useful information that cannot be obtained by instrumental methods.

18.5.2.1 OLFACTOMETRY

Olfactometry is an assessment of odor intensity run by a group of experts; it does not allow compound identification, but measures a gaseous mixture in odor units (OUs) and translates the results to OU/m^3. Odor threshold and dilution, as well as data computer processing, support the methodology. It provides an estimation of odor intensity, and is a valuable method to evaluate synergistic and masking effects.

18.5.2.2 DESCRIPTIVE SENSORY ANALYSIS

Among the sensory methods, descriptive sensory analysis (DSA) by trained panelists is the preferred method for the description of the human perception of flavor and aroma. Several details on this method have been given in Section 18.3.2. Panelists examine the food and describe its attributes and intensity. Sensory lexicons, naming specific sensory characteristics, are being developed for specific foods and increasingly being used for descriptive analysis. Clear definitions, adequate reference materials, and proper training enhance the robustness of the sensory testing. The main limitations of sensory testing are that it is expensive to be properly implemented (extensive panel training is needed), time-consuming, and cannot be applied "online."

The training of the panel in the most common flavor and aroma attributes is highly time-consuming. Even so, it is usual to do some preliminary orientation sessions: panelists discuss which are the best attributes to define the fruit or vegetable they are going to taste. When the lexicon is clear, definitions and reference products for each attribute are prepared and provided to the panel to be trained for the analyses. Samples have to be served in normalized individual booths with controlled illumination and temperature. Samples have to be randomly presented to each panelist in an odor-free plastic beaker. The intensity of the sensory attributes can be scored using a scale that the panel had previously agreed. It is common to use a 0−15 scale, and also 0−10, where 0 = none or not perceptible intensity, and 15 or 10 = extremely high intensity. After the sensory analysis, the results are processed by the panel leader, and finally, results from sensory testing can be correlated with the instrumental analysis.

However, for routine analysis, instrumental techniques are more practical. Correlations of instrumental results with sensory perception are difficult to establish; as an example, the same volatiles may provide different aroma or flavor sensations depending on the food matrix, given that foods are complex matrices. Also, during instrumental analysis heat-labile compounds may suffer transformations due to temperature, and sensory terms associated with different compounds may overlap and lead to confusion as well. Relations among volatile compounds and flavor and aroma perception are not linear and new data-processing tools are needed to better correlate both types of data.

18.6 CONCLUSIONS

The association between chemical compounds and the different flavor components (aroma, basic tastes, chemical feeling factors, and volatile compounds) is as follows: (1) sugars (sweet taste), (2) organic acids (sour taste), (3) phenolic compounds (bitter taste), (4) tannins (astringency), (5) capsaicinoids, piperine, chavicine, and piperittine (pungency), (6) salt (salty taste), (7) glutamic salts (umami taste), and (8) volatile compounds (odor/aroma).

Some fruits and vegetables suffer postharvest changes, especially those called climacteric, in which ripening is controlled and started by ethylene. Most of the organic acids and sugars change because they are intermediates in the metabolic pathways of the tricarboxylic acid cycle. As a result of this conversion, TSS increases, while TA decreases. Phenolic compounds decrease due to oxidation of polyphenols by polyphenol oxidase, but also more complex phenolics can be decomposed to smaller compounds. The volatile compounds, responsible for the odor and aroma of fruits also change during postharvest storage. In this way, the following general trends are observed for the main sensory attributes and the related chemical families: green (aldehydes) and vegetal (alcohols) attributes decrease, while fruity, spicy, and floral (lactones, furanones, terpenes, and esters) increase through different pathways. The compounds related to the chemical feeling factors (astringency, pungency) do not show a clear trend, and they can increase or decrease their concentration depending on specie, variety, and fruit part.

Measurements of all compounds can be carried out using both instrumental techniques and sensory evaluation. For instance, the most popular way to routinely measure the following parameters are sugars (HPLC with refraction index detector, HPLC-RI), sourness (titration and HPLC-UV), phenolic and volatile profiles (LC-MS, GC-MS, GC-FID, GC-olfactometry, and E-nose). Concerning sensory analysis, trained panels are a good tool to fully describe fruits and vegetables (descriptive sensory analysis). The combination of instrumental and sensory methods is fully recommended to give a complete picture of the flavor of fruits and vegetables.

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CHAPTER 19

Physiological Responses to Stress

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19.1 INTRODUCTION

Fresh horticultural products are living tissues subject to continuous changes after harvest. Their commodities are perishable products with active metabolism during the postharvest period. While some changes are desirable, most are not. Proper postharvest handling plays an important role in increasing food availability. In addition, all fresh horticultural crops are high in water content and thus are subject to desiccation and mechanical injury. Horticultural crops are an important source of carbohydrates, proteins, organic acids, vitamins, and minerals for human nutrition. When humans use plants or plant parts, whether for food or for aesthetic purposes, there is always a postharvest component that leads to loss. Their losses in quantity and quality affect horticultural crops between harvest and consumption. Thus, to reduce the losses, producers and handlers must understand the biological and environmental factors involved in deterioration.

Changes in environmental conditions can cause stress to plants. As stress is generally defined as any environmental factor potentially unfavorable to living organisms, except for decay, quality losses in actual postharvest produce can be directly or indirectly attributable to a combination of stress and stress-induced senescence (Lester, 2003). Stresses are serious threats to agriculture and are the primary cause of crop loss worldwide (Imahori, 2012). These

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stresses lead to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity. When exposed to unfavorable environments, plants result in some degree of stress and express a fraction of the plant genetic potential. Plants adapt to unfavorable conditions through genetically determined stress resistance. The plants can be sensitized for more rapid or more intense mobilization of defense responses leading to enhanced resistance to stress and acquire resistance to stresses. Therefore, the effects of stress on metabolism and performance have become a major focus of plant research, especially in postharvest produce.

19.2 BIOTIC AND ABIOTIC STRESSES

Biotic stress is the damage to plants by other living organisms such as bacteria, fungi, nematodes, protists, insects, viruses, and viroids. On the other hand, abiotic stress is defined as effects caused by many environmental factors, such as drought, salinity, extreme temperatures, chemical toxicity, and oxidative stress. Abiotic stresses are interconnected and cause similar damage. Plants have developed mechanisms to cope with and adapt to biotic and abiotic stresses imposed by the environment. The combinations of biotic and abiotic stresses have a positive effect on plant response. The defense response genes are activated by biotic stress or abiotic stress. With any given stimulus from the environmental factors, multiple signaling pathways that have interactions or crosstalk are activated (Fig. 19.1).

Stress acts as a stimulus or influence that is outside the normal range of homeostatic control in plants. Once stress is controlled, a new physiological state in the plant is established. Stress affects phytochemical accumulation or loss by inducing an increase or reduction in key enzyme activities of metabolic pathways. Stress-tolerance mechanisms are activated at molecular, biochemical, physiological, and morphological levels. These diverse stresses activate similar cell signaling pathways and cellular responses, such as the production of stress proteins, upregulation of antioxidants and accumulation of compatible solutes. Thus, plant stress responses are regulated by multiple signaling pathways that activate gene transcription and its downstream machinery. The complex plant response to stress involves many genes and biochemical-molecular mechanisms. The molecular control mechanisms of stress tolerance may result in the use of molecular tools and are based on the expression of specific stress-related genes (Fig. 19.2).

19.3 BIOTIC STRESS

Biotic stress which is often called decay is caused by infectious diseases that develop in harvested fruit and is usually caused by bacteria, fungi, or yeasts. Plants respond to biotic stress through a defense system. The defense mechanism is classified as an innate and systemic response. After infection, reactive oxygen species (ROS) are generated and oxidative bursts limit pathogen spread

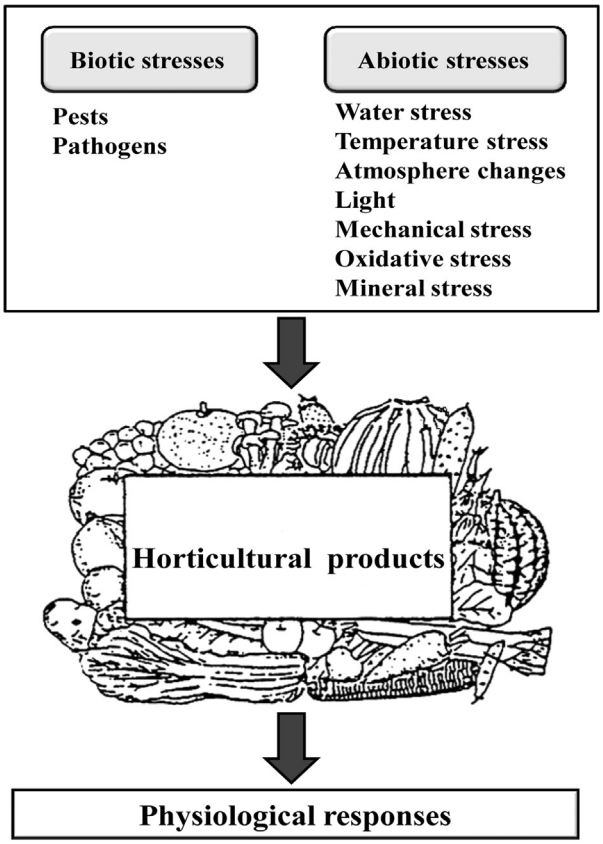

FIGURE 19.1
Schematic representation of physiological response to stress in horticultural products.

(Atkinson and Urwin, 2012). Also, in response to pathogen attack, plants increase cell lignification. This mechanism blocks invasion of parasites and reduces host susceptibility.

The defenses to biotic stress include morphological and structural barriers, chemical compounds, and proteins and enzymes. These confer tolerance or resistance to biotic stresses by protecting products and by giving them strength and rigidity. The resistance to biotic stress can be induced through specific chemical compounds such as β-aminobutyric acid (BABA) or benzothiadiazole (BTH).

Plant hormones, salicylic acid (SA), jasmonic acid (JA), and ethylene play central roles in biotic stress signaling. Several transcription factors (TFs) are mediators in multiple hormone signaling. Plant defenses against biotic stresses involve numerous signal transduction pathways. Abscisic acid (ABA) is reflected as the main hormone involved in the perception of many abiotic stresses (Cramer et al., 2011). However, ABA has a positive effect on biotic

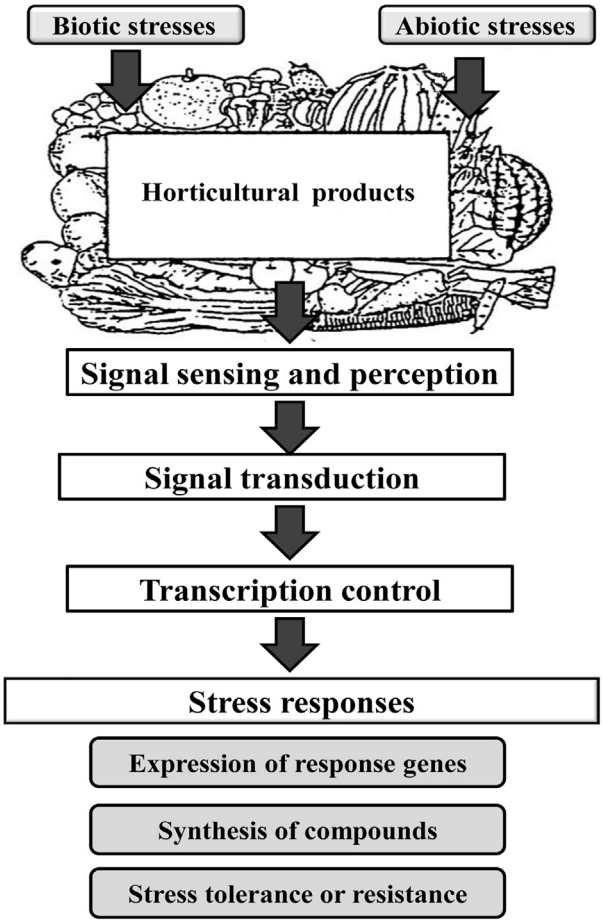

FIGURE 19.2
Schematic representation of stress responsive mechanisms in horticultural products.

stress resistance (Rejeb et al., 2014). Under abiotic and biotic stress, ABA acts antagonistically with ethylene, which induces liability of the plant against disease attack. However, under abiotic stress ABA increases and induces stomatal closure. As a result, the entry of biotic attackers through stomata is prevented. Therefore, under such situations, the plant is protected from abiotic and biotic stress (Rejeb et al., 2014). Kinase protein signals also interact with ROS and ABA leads to plant defense enhancement (Rejeb et al., 2014).

Pathogenesis-related (PR) proteins are critical for plant resistance against pathogens and when plants are attacked; their expression is strongly upregulated. It is suggested that with an increase in ABA expression of specific TFs like C-repeat binding factors (CBFs), and cup-shaped cotyledon mediated by ABA could be enhanced, which induces upregulation of PR genes (Rejeb et al., 2014).

19.4 ABIOTIC STRESS

Changes in environmental conditions can cause stress to plants. As stress is generally defined as any environmental factor potentially unfavorable to living organisms, the effects of stress on metabolism and performance have become a major focus of plant research. When exposed to unfavorable environments, plants result in some degree of stress and express a fraction of the plants' genetic potential. The plants can be sensitized for more rapid or more intense mobilization of defense responses leading to enhanced resistance to stress and acquire resistance to abiotic stresses.

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity, and oxidative stress are serious threats to agriculture and result in the primary cause of horticultural product loss worldwide. Abiotic stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity. These stresses affect phytochemical accumulation or loss by inducing an increase or reduction in key enzyme activities of metabolic pathways. These stresses are often interconnected and lead to the production of ROS. The role of ROS during abiotic stress has become a subject of considerable interest given that ROS have been implicated in processes leading to plant stress acclimation. High ROS levels can lead to phytotoxicity, whereas relatively low concentrations can be employed for acclamatory signaling. Abiotic stress signaling is an important area with respect to an increase in plant productivity. Abiotic stresses induce some changes in plants occurring at different levels, from molecular to organ, and affecting plant productivity, and affect the pathways involved in the biosynthesis of the secondary metabolites.

While ROS have the potential to cause oxidative damage to cells during environmental stresses, ROS play a key role in plants as signal transduction molecules involved in mediating responses to pathogen infection, environmental stresses, programmed cell death, and different developmental stimuli. These diverse stresses activate similar cell signaling pathways and cellular responses, such as the production of stress proteins, upregulation of antioxidants, and accumulation of compatible solutes. Thus, plant stress responses are regulated by multiple signaling pathways that activate gene transcription and its downstream machinery. The complex plant response to abiotic stress involves many genes and biochemical-molecular mechanisms. The ongoing elucidation of the molecular control mechanisms of abiotic stress tolerance, which may result in the use of molecular tools, is based on the expression of specific stress-related genes. These major tolerance mechanisms include water and ion uptake and transport such as ion transporter and aquaporins, osmoprotectants, free-radical scavengers, the protection of membranes and proteins, such as heat shock proteins (HSPs) and chaperones, late embryogenesis abundant proteins, and factors involved in signaling cascades and transcriptional control. HSPs are known to be expressed in plants not only when they experience high-temperature

stress but also in response to a wide range of other abiotic stresses, such as water stress, salinity and osmotic, cold and oxidative stress. These proteins play an important role in protecting against stress and in the reestablishment of cellular homeostasis (Wang et al., 2004).

19.5 WATER STRESS

Water stress is one of the most important abiotic factors limiting the quality of horticultural products. It induces undesirable quality such as wilting, shriveling, loss of firmness and crispness, loss of color, peel damage, and off-flavor. The significance of water loss determines the shelf-life and quality of horticultural products. Water stress adversely affects many physiological and biochemical processes in plants. Even low water loss due to transpiration induces a series of metabolic responses. A major effect of decreased water availability is diminished leaf carbon fixation due to stomatal closure, which may start at moderate plant water deficits. The reactions of plants to water stress differ significantly at various organizational levels depending upon the intensity and duration of stress as well as the plant species and its stage of development. The cell division and expansion of plants are directly inhibited by water stress. Plant cell protection homeostasis mechanisms were shown to be beneficially involved in the water stress response by delaying death and improving survival under unrealistic stress conditions.

Water stress occurs in plants when the water potential outside the plant tissue declines. The level of water potential is directly related to the plant water balance. Its gradient is a driving force of water transport. The drop-in water potential may be caused by reduced water availability by drought or low temperature or by increased osmolarity by salt. Water-deficit stress leads to severe effects in plants. Responses to water stress include loss of cell turgor, reduced water potential of plant cell and growth rate, and decreased stomatal conductance, photochemical efficiency, and chlorophyll content. Removal of water from the membrane disrupts the normal bilayer structure and results in the membrane becoming exceptionally porous when desiccated. Stress within the lipid bilayer may also result in displacement of membrane integrity, selectivity, and disruption of cellular compartmentalization and a loss of activity of enzymes, which are primarily membrane-based. In addition, cytosolic and organelle proteins may exhibit reduced activity or may even undergo complete denaturation when dehydrated. The high concentration of cellular electrolytes due to the dehydration of protoplasm may cause disruption of cellular metabolism (Mahajan and Tuteja, 2005). Plants can respond and adapt to the stress by altering their cellular metabolism and invoking various defense mechanisms under the stress conditions. Water stress leads to activation of enzymes involved in the production and removal of ROS (Mahajan and Tuteja, 2005). The ROS defense mechanism includes accumulating antioxidant enzymes, increasing the amounts of antioxidant metabolites and organic osmolytes, and a rise in the concentration of ABA.

Cell protection homeostasis mechanisms, such as antioxidant enzyme and osmolytes, were shown to be beneficially involved in the water stress response by delaying death and improving survival under fairly unrealistic stress conditions.

19.5.1 Transpiration

Transpiration of horticultural product is a mass transfer process in which water vapor moves from the surface of the plant organ to the surrounding air. Fick's law, a physical law, governs this phenomenon. The movement of any gas in or out of the plant is directly proportional to the concentration gradient across the barrier and the surface of the barrier and is inversely proportional to the resistance of the barrier to diffusion. The driving force of transpiration is the gradient of water vapor pressure between the plant tissue and the atmosphere surrounding it (Ben-Yehoshua and Rodov, 2013).

Transpiration is one of the phenomena that affect physiological deterioration of horticultural products. Transpiration not balanced by a water supply from the parent plant often results in water stress in harvested product. It affects the appearance, texture, flavor, and weight of products. The relative importance of the deterioration depends on the commodity. Transpiration is considered as the major cause of postharvest losses and poor quality in leaf vegetables, such as lettuce, spinach, and cabbage. Transpiration induces wilting, shrinkage, and loss of crispness (Ben-Yehoshua and Rodov, 2013).

19.5.2 Water Loss

Postharvest water loss results from respiration and diffusion through the surface in horticultural products. Water loss for postharvest handing and distribution leads to deterioration in horticultural products' quality. Additionally, it accelerates senescence or ripening as softening, membrane deterioration, and yellowing. The susceptibility to water loss is defined by the surface area to volume ratio in horticultural products. The range of this ratio is from as low as $0.2\ cm^{-1}$ in winter cabbage or turnip to as high as $50-100\ cm^{-1}$ in leaf vegetables (Toivonen and Hodges, 2011). There is considerable species- and cultivar-related variability. This variability arises from a combination of maturity, environmental conditions, and surface structures such as stomata, lenticels, hair, periderm, and cuticle. The water potential gradient from inside to outside the products varies throughout storage according to the temperature and relative humidity. The driving force of water loss is the vapor pressure deficit, which is the relationship between the difference in water activity of horticultural products and water activity of the atmosphere surrounding it (Ben-Yehoshua and Rodov, 2013).

Water loss causes some metabolic changes in horticultural products. An increase in osmolyte concentration in response to water loss increases the activity of polysaccharide hydrolyzing enzymes. Hence, loss of cell wall structure and concomitant increases in soluble sugars in plant tissues have been

observed, for example in carrots. Water stress results in upregulation of polygalacturonase activity. Also, water loss induces ethylene production, as a result it leads to accelerated ripening in banana fruit and accelerated senescence in bell pepper fruit (Toivonen and Hodges, 2011).

19.6 TEMPERATURE STRESS

19.6.1 Chilling Stress

As a physiological disorder, chilling injury (CI) occurs when tropical or subtropical fruits are exposed to low but not freezing temperatures. The macroscopic symptoms of CI include abnormal ripening and damaged areas at the fruit surface like pitting, scald, hard lumps in the pulp around the vascular bundles, water soaking of the flesh, and high liability to decay. These changes can be associated with integrity of the cell wall in chilling susceptible crops.

The first cell structure affected by CI is the cell membrane. At chilling temperature the cell membrane phase transits from flexible liquid crystalline to a solid gel structure, which increases cell membrane malfunction. In chilling injured crops, an increase in phospholipase D (PLD) and lipoxygenase (LOX) activities reduced integrity of cell membrane and increased CI.

Studies have revealed that CI development is dependent on energy. Longer cold storage led to a decrease in adenosine triphosphate (ATP) and adenosine diphosphate (ADP) contents and energy charges were linked with increased pitting incidence. High levels of ATP maintenance may alleviate CI. For example, cucumbers which were treated with 6-benzylaminopurine increased ATP content and prevented CI. It is also indicated that chilling stress increases ROS production, which damages membrane structure. Destruction of ROS could protect fruit from CI by inducing antioxidant properties in the fruit tissues. Moreover, CI browning symptoms have been linked to enzymatic activity, which is related to the brown pigments. Phenylalanine ammonia lyase (PAL) activity has been proposed to be the limiting factor for low-temperature-induced browning, for example, in banana fruit. Some treatments which could alleviate CI include plant growth regulators like SA, polyamines (PAs), and nitric oxide (NO).

19.6.2 Freezing Stress

Freezing temperatures are a major environmental constraint limiting the growth, development, and distribution of many plant species. The susceptibility of plants to freezing stress is responsible for the variation in sensitivity. Horticultural products vary in their susceptibility to freezing injury. Some may be able to withstand freezing stress with no apparent injury, while others are damaged by slight freezing. Freezing injury occurs at temperatures below the freezing point of water. That deterioration is observed as wilting or softening of plant parts. When a plant freezes, ice formation occurs within the plant tissues, and this ruptures cell membranes, causing loss of cellular integrity and

ultimately the death of plant tissue. The primary manifestation of damage from freezing is in the plasma membrane. Thus, membranes play a key role in the ability of a plant cell to withstand or be subject to freezing injury.

Plants survive freezing either by preventing the crystallization of ice within their tissues by supercooling of tissue water (freezing avoidance) or they allow ice to crystallize in the apoplast (freezing tolerance). Freezing avoidance involves supercooling and prevention of ice crystallization in the apoplast. Pure water can be supercooled to a certain point below 0°C without ice nucleation. Some specialized cell types and organs can use supercooling as a strategy. Freezing tolerance is a survival strategy and is achieved through several changes in the physiological response regulated by gene expression. The level of freezing tolerance is obtained through cold acclimation but is rapidly lost upon return to a warm nonacclimating temperature. This tolerance can be induced by the accumulation of osmotic substances, such as sugars, organic acid, proline, and glycinebetaine. The high abundance of sugars act as osmoregulation, whereas less abundant sugars have a role in the cryoprotective mechanism or as signaling molecules induced by cold stress.

Freezing tolerance is regulated by a number of genes that are affected by cold stress. The expression of a specific cold-responsive gene called *COR* results in various physiological and biochemical changes during the process of cold acclimation, and the intensification of the resistance of plants against freezing. The existence of the regulator *cis* element, known as CRT (C-repeat), controls the expression of *COR* gene in response to freezing. The induction of TF CBF occurs through freezing and follows the expression of regulation genes that impart freezing tolerance.

19.6.3 Heat Stress

Heat stress could affect the stability of various proteins, membranes, and RNA structures, and alter the efficiency of reactions of enzymes in the cell, which causes metabolic imbalance (Lurie and Pedreschi, 2014) and toxic compound accumulation, such as ROS. Heat-stressed unfolded proteins bind molecular chaperones to unfolded proteins, while heat stress TF (HSF) subunits trimerize, undergo phosphorylation, and bind to heat shock response promoters in the genome to activate heat stress responses (Lurie and Pedreschi, 2014). Another hypothesis involved opening a specific channel of calcium in the plasma membrane as a result of heat stress, which changes membrane fluidity, and ROS signaling, mainly the respiratory bursts, which are mediated by a plasma membrane NADP oxidase. There has been some evidence that smRNAs may also be contributed in response to heat stress (Lurie and Pedreschi, 2014). Short exposure times to high-temperature cause stress and defense protein accumulation, including HSPs, pathogen-resistant proteins (PRs), antioxidant enzymes, and polyphenols (Lurie and Pedreschi, 2014).

19.7 LOW-OXYGEN ATMOSPHERE AND HIGH-CARBON-DIOXIDE ATMOSPHERE

Exposing horticultural products to reduced O_2 and/or elevated CO_2 can either be beneficial or harmful, depending on the concentration of these gases, temperature, exposure duration, and commodity. Controlled atmosphere (CA) or modified atmosphere (MA) storage utilizing reduced O_2 and/or elevated CO_2 are known to maintain the quality and consequently extend the shelf-life of many horticultural products. The beneficial effects of CA or MA storage include delayed ripening, reduced physiological and pathological disorders, and the possibility for disinfesting products. The gas composition of CA is monitored and deviations from the set points corrected. MAs differ in that they are not actively controlled and the gas composition results from a balance between the plant gas consumption or production and gas diffusion through a permeable membrane.

To date, much research has been conducted to evaluate the effects of CA and MA storage on the quality and storability for many horticultural products and specific cultivars of each commodity. However, despite the enormous economic significance of CA or MA storage, accompanying the use of low O_2 or high CO_2 atmospheres for maintaining quality of fresh horticultural products produce during CA or MA storage is the risk that very low O_2 and/or high CO_2 atmospheres may cause damage to the produce. A better understanding of basic biochemical and physiological responses to low O_2 or high CO_2 atmospheres is needed to effectively evaluate storage conditions.

19.7.1 Low-Oxygen Atmosphere

CA and MA can prolong the storage life of fruit by decreasing postharvest decay. CA storage reduces the respiration rate and decreases the susceptibility to disease. At least 1% O_2 in storage is required to avoid anaerobic metabolism. When the concentration of oxygen is lowered, respiration of mitochondria decreases, and aerobically produced CO_2 declines with an increase in fermentation, which is related to the low-oxygen consequences and a rise in overall CO_2 production causing glycolysis increase, known as the Pasteur effect. At low O_2 concentrations, fermentation pathways are induced which decrease ATP levels in cells. During fermentation, acetaldehyde is converted to ethanol using nicotinamide adenine dinucleotide (NADH) (Imahori, 2012). Also, lactate is formed by pyruvate reduction. Consequently, fermentative metabolisms use NADH and pyruvate, and glycolysis can proceed. Ethanol and lactate are produced by most plants under low O_2. The induction of pyruvate decarboxylase, alcohol dehydrogenase, and lactate dehydrogenase are mechanisms for the accumulation of anaerobic products because of fermentative metabolism (Imahori, 2012).

Low-oxygen storage physiological disorders are commonly seen in apples and pears, when concentrations of O_2 are below the Pasteur point for prolonged periods of anaerobiosis. Low O_2 in storage rooms changes the energy status of pear fruit with unfavorable consequences in membrane phospholipids and

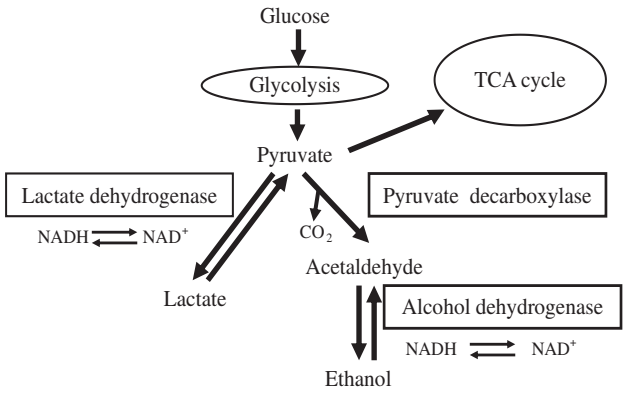

FIGURE 19.3
Fermentation pathway.

enhancement of internal disorders in "Conference" pear and "Braeburn" apple. Moreover, low O_2 levels may stimulate the proliferation of anaerobic psychrotrophic microorganisms which could be decreased using high-oxygen MA packaging (Fig. 19.3).

19.7.2 High-Carbon-Dioxide Atmosphere

CA could extend the storage life of horticultural crops and a combination of 1%−5% O_2 with moderate CO_2 is generally considered the best gas for storage. Extreme CO_2 (higher than 12%) causes physiological injuries.

High CO_2 damages products by interior pulp darkening, which occurs due to the oxidative damage, and in this condition hydrogen peroxide (H_2O_2) is produced and leads to cell membrane modifications. Moreover, it can be aggravated by concentrations of low O_2. High-CO_2-stored fruits have higher polyphenol oxidase (PPO) activity compared to fruits stored under normal CA storage and PPO activity was positively correlated to browning of fruit. The incidence of core-browning was increased in pears stored at 5% CO_2 compared with 0.7% CO_2 which was related to lipid peroxidation accumulation (Singh, 2016). The decrease in antioxidant enzyme activities might cause lipid peroxidation and browning (Singh, 2016). Moreover, for sensitivity to CO_2 injury there is a genetic basis. For example, apple fruit cultivars "Gala," "Golden Delicious," and "Jonagold" are less susceptible to CO_2 injury than other cultivars. Moreover, the increase in concentration of CO_2 negatively influenced fruit quality by increasing color changes and firmness, and pectic compounds solubilization, which might cause cell wall breakdown.

19.8 ETHYLENE AND NONETHYLENE VOLATILES

Ethylene has a significant role in aromatic volatile development of fruit. The relationship between ethylene and biosynthesis of volatiles in ripening fruit

has been stated in many fruits. In apple "Greensleeves" fruit several volatile compounds were reduced or induced using ethylene. Esters and alcohols were induced by ethylene treatment, particularly butyl acetate, hexyl acetate, and 2-methylbutyl acetate. The role of TF RIN in biosynthesis of ethylene and aroma volatiles during tomato fruit ripening has been indicated. Increased biosynthesis of volatile compounds during tomato fruit ripening depends on ethylene, as there were no ripening-associated increases in the ethylene-insensitive Nr mutant, which suggests that ethylene perennially controls the generation of aroma volatiles. Moreover, using antisense technology, a lower concentration of hexanol, *trans*-2-heptenal, *cis*-3-hexanol, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and geranylacetone were detected in antisense 1-aminocyclopropane-1-carboxylic acid synthase (ACS) 2 "Lichun" tomato. Also, "Royal Gala" apple fruit produced no detectable ethylene using antisense ACS and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), which resulted in aroma volatiles reduction. These results showed that enzymes located in the final step of ester biosynthesis, are regulated by ethylene during fruit ripening. In addition, ethylene has a significant role in the production of aromatic volatile compounds from catabolism of amino acids like valine, leucine, isoleucine, phenylalanine, and cysteine, through enhancing aminotransaminase activity and promoting the relative expression of CmBCAT1 and CmArAT1.

Storage temperatures have different effects for volatile production. For example, "Odem" mandarins which were stored at 2°C accumulated 13 volatiles, mainly terpenes, while six volatiles accumulated at 8°C, comprising five terpenes and one terpene. Peach "Hujingmilu" fruit stored at 5°C had the lowest amount of volatile compounds such as esters and lactones. Tomato fruit which was stored in a refrigerator changed the levels of 3-methylbutanal, linalool, guiacol, hexanol, trans-2-hexenal, and trans-3-hexenol. Production of seven key volatile compounds was downregulated after chilling exposure, including 6- methyl-5-hepten-2-one, β-ionone, *trans*-2-hexenal, hexanal, 2-phenylethanol, and 2-methylbutanal. C6 aldehyde and alcohol aroma volatiles were reduced after chilling tomato, which could be explained by a decrease in hydroperoxide and alcohol dehydrogenase enzymes activities in the oxylipin pathway (Fig. 19.4). Moreover, the effect of low temperature on flavor loss could also be related to the cultivar. For example, the effects of chilling temperature on the decrease in volatile compounds were more noticeable in cv Cappricia RZ than cv Amoroso RZ of tomato fruit. CA and MA storage also affect aroma production. In MA storage, accumulation of alcohols such as ethanol and acetaldehyde, which are principally related to anaerobic atmospheres or high CO_2 atmospheres, increase.

19.9 LIGHT

Appropriate light intensity and quality can influence the postharvest quality of horticultural products. Low light intensity in preharvest resulted in shortened shelf-life of greenhouse-grown cucumbers, as low light led to a reduction in

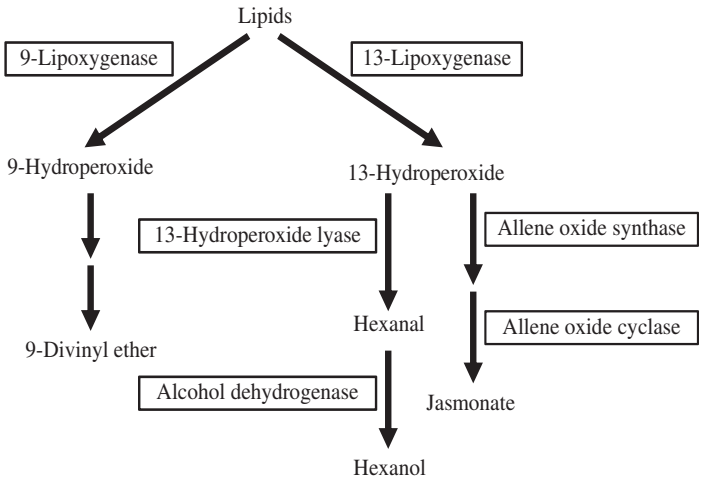

FIGURE 19.4
Oxylipin pathway.

total chlorophyll content of cucumber skin. When lettuce was grown under different light intensities, cultivation under high light intensity prolonged the shelf-life of both intact leaves and fresh-cut product. The improved shelf-life was reflected in improved chlorophyll fluorescence values. Postharvest lighting of the fresh-cut product at low light intensities considerably prolonged the shelf-life of fresh-cut product compared to storage in darkness. The prolonged shelf-life of low light-treated products is related to the higher levels of sugar, counteracting starvation processes.

Although light is not essential for the synthesis of ascorbate, the amount and intensity of light during the growing season have an influence on the levels of ascorbate formed. In addition, the fruit products exposure to light appeared to accelerate the ascorbate synthesis. Low light resulted in lower levels of ascorbate in greenhouse-grown products. Also, continuous irradiation with blue LED light (470 nm) at an intensity of 50 μmol/m^{2}s significantly increased the ascorbate in the juice sacs of three citrus varieties, Satsuma mandarin, Valencia orange, and Lisbon lemon.

Ultraviolet (UV) light causes biological stress in plants and defense mechanisms of plant tissues with the consequent production of phytoalexin compounds. Phytoalexin accumulation could be accompanied by other inducible defenses such as cell wall modifications, defense enzymes, and antioxidant activity (Imahori, 2012). The UV portion of the electromagnetic spectrum ranges from approximately 10 to 400 nm. UV radiation has been applied to produce in long (UV-A: 315–400 nm), medium (UV-B: 280–315 nm), and short wave (UV-C: 100–280 nm) dosages. The shortest wavelengths of the UV spectrum are the most energetic ones and are more effective biocides for surface sterilization of some food products. Exposure to UV leads to retardation

of plant growth and alteration in metabolic processes such as photosynthesis and respiration. UV radiation-induced damage is related to acceleration in ROS generation, leading to oxidative stress. However, plants have developed several antioxidation strategies to scavenge ROS. Enhancement of antioxidant defense in plants can increase tolerance to UV radiation. Additionally, the secondary metabolism in plants is activated together with the enzymatic antioxidant system (González-Aguilar et al., 2010).

UV radiation has been used to maintain the postharvest quality and extend the shelf-life of several fresh horticultural products (Yamauchi, 2013). Low UV doses induce the production of antifungal compounds, ripening delay, and reduction of CI (Pombo et al., 2009). The exposure to UV-C delays fruit softening, which is one of the main factors determining fruit postharvest life. UV-C decreased the activity of enzymes involved in tomato cell wall degradation and delayed fruit softening. Treatment with UV-C increases ascorbate and total phenolic contents and improved nutritional qualities of tomato fruit. UV radiation can affect physiological processes at the genetic level. In parsley, UV-B upregulates genes encoding the flavonoid biosynthetic pathway, such as chalcone synthase and PAL, which are key enzymes in anthocyanin formation. In tomato fruit, this exhibits ethylene production with ripening onset, UV-C treatment has disrupted ethylene production by decreasing the formation of ACS. Peach fruit treated with UV-C showed increased activation of genes for β-1,3-glucanase and PAL. Although treatment with UV-A or UV-B seems to be less harmful than UV-C radiation, it has the effect of increasing antioxidants such as ascorbate, α-tocophol, and polyphenol (Yamauchi, 2013).

Hormetic doses of UV-C radiation have been used as a physical treatment to extend the postharvest life of several fruits and vegetables. Low doses of UV-C radiation stimulated beneficial reactions in biological organs, a phenomenon known as hormesis (González-Aguilar et al., 2010). Hormesis has been defined as the use of potentially harmful agents at low doses to induce a beneficial stress response (Shama and Alderson, 2005). Hormetic effects manifest themselves in treated plant tissue through the action of a variety of induced chemical species. They include phytoalexins such as scoparone in oranges and resveratrol in grapes. Also induced are enzymes such as chitinases and glucanases in peaches and PAL in tomatoes. The deleterious effects of UV light on plant tissues, such as decreased protein synthesis, impaired chloroplast function, and DNA damage have been shown. However, low doses of UV could inflict repairable damage to DNA, and this slight trauma would activate repair mechanisms for radiation-induced DNA damage. Sublethal radiation may stimulate vital processes inside cells and create a positive change in the homeostasis of a plant (Shama and Alderson, 2005).

Changes in the antioxidative defense system (Fig. 19.5) were shown in several horticultural products subjected to UV radiation. UV-C radiation enhanced the activities of antioxidant enzymes including ascorbate peroxidase (APX) in

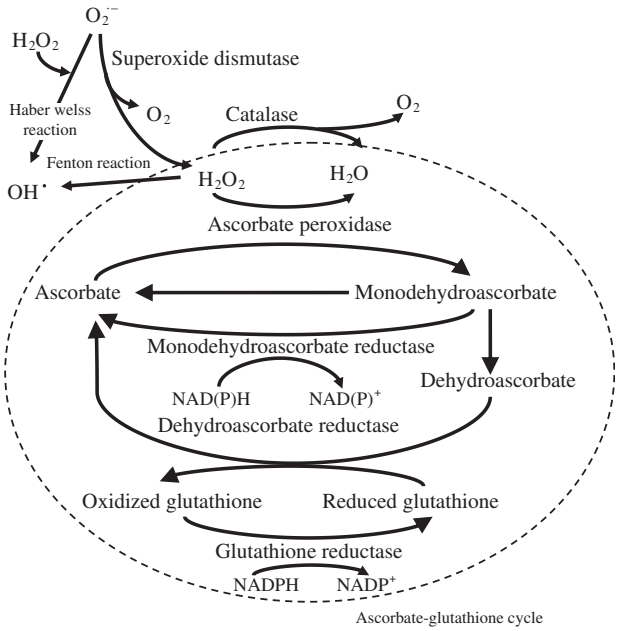

FIGURE 19.5
Antioxidative defense system.

strawberry fruit during storage. The effect of UV-C radiation is the induction of enzymes that played a role in oxidative stress, such as APX and guaiacol peroxidase in tomato fruit during ripening. Also, in UV-B-treated broccoli florets during storage, the contents in these antioxidants, such as ascorbate and glutathione, and APX activity increased (Yamauchi, 2013). These effects of UV radiation on the synthesis of antioxidant compounds and enzymes can vary depending on the hermetic doses, time of exposure, and treated horticultural products (González-Aguilar et al., 2010). When a hermetic dose of UV irradiation is absorbed by biological material, it can interact with atoms and molecules, mainly water, producing ROS by the univalent reduction of O_2 in a rapid and controlled manner. The primary ROS formed in the cell is O_2^-, which triggers a cascade of reactions that results in the formation of a variety of ROS and induction of antioxidative enzymes. A key ROS is H_2O_2 produced by superoxide dismutase (SOD) (González-Aguilar et al., 2010). Therefore, UV radiation induces production of ROS, such as O_2^-, by exciting electrons by electronic transition in appropriate photosensitizers. SOD then dismutases the produced O_2^- to H_2O_2. Accordingly, H_2O_2 scavenging enzymes such as APX efficiently catalyze the breakdown of H_2O_2. H_2O_2 may act more as a signal molecule than directly inducing oxidative damage at lower UV dose, and be responsible for the improvement of the antioxidant status of horticultural crops activating gene expression of enzymes related to the synthesis and accumulation of antioxidant capacity (González-Aguilar et al., 2010).

19.10 MECHANICAL STRESS

Horticultural products are subject to mechanical stress during harvest and handling. This stress involves cuts, punctures, and impacts leading to bruises. Cuts can induce transitory increases in respiration, wound-induced ethylene production, phenolics production, and deterioration in plant tissue (Toivonen and Hodges, 2011). Cut injuries occur during the harvest process and are more severe in machine-harvested product. The severity and size of bruises are influenced by several factors; maturity, water potential, tissue or cellular orientation at the site of the injury, shape of object imparting the bruising force, energy and angle of the impact, and temperature of the horticultural product.

Cuts are most prevalent in fresh-cut products. The effects of fresh-cut abiotic stress include discoloration, especially browning of cut surfaces due to tissue disruption and subsequent oxidative processes, increased respiration and ethylene evolution, loss of flavor and texture, weight loss and dehydration, decline in nutrient content, development of off-odors, membrane breakdown, and tissue softening. Cut-induced respiration has been associated with enhanced synthesis of enzymes involved in the respiratory pathway and to a transitory increase in aerobic respiration in fresh-cut products. Phenolic levels are increased through wound induction of PAL, the committed enzyme in phenolic biosynthesis (Hodges and Toivonen, 2008).

19.11 OXIDATIVE STRESS

All environmental and biotic stresses generate oxidative stress, which damages cell components and is induced by ROS accumulation. ROS contains activated atoms of oxygen which are not necessarily radicals. ROS generates in the mitochondrion and causes senescence of fresh products. ROS and free radicals stimulate oxidative stress by oxidation of compounds of cells. Hydrogen peroxide (H_2O_2), superoxide radical ($O\bullet^{-2}$), hydroxyl radical ($\bullet OH$), and $NO\bullet$ are important ROS for oxidative stress induction.

Abiotic stress conditions like low temperature, high temperature, and water-deficit stress, increase ROS production and cause oxidative damage to the plant. ROS accumulation may damage lipids and form toxic products like malondialdehyde (MDA) which is an indicator of the oxidative stress of plants.

Plants have antioxidant defense systems which scavenge ROS and protect cells from ROS-induced injuries. SOD removes $O_2\bullet^-$ by catalyzing it to O_2 and H_2O_2, and catalase (CAT) and peroxidase (POD) dismutase H_2O_2 into H_2O and O_2, for detoxification of ROS (Gill and Tuteja, 2010). Plants also have non-enzymatic detoxifications to scavenge ROS, including ascorbate and glutathione, and tocopherol, flavonoids, alkaloids, and carotenoids. Oxidative stress is an important phenomenon in many biological systems. However, it could be decreased by several means such as synthetic antioxidants like sulfites and diphenylamines or heat treatment or low-temperature conditioning (Hodges, 2003).

19.12 MINERAL STRESS

Optimum plant performance depends on a balanced and timely availability of mineral nutrients. Mineral nutrients influence the quality of horticultural products in many ways but particularly in physiological disorders. Some postharvest disorders of horticultural products result from imbalances of certain mineral nutrients. Calcium is the mineral most commonly associated with postharvest disorders. It plays an important role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion-exchange behavior. Also, it is suggested that calcium is a putative signaling molecule involved in the development of cross-tolerance to abiotic stresses (Toivonen and Hodges, 2011).

Potassium has a few important effects on postharvest abiotic stress susceptibility of vegetables. Improved potassium nutrition reduces susceptibility of potatoes to internal bruising in response to mechanical stresses imposed during postharvest handling (Toivonen and Hodges, 2011).

Mineral imbalances result in salt-stressed plants. These imbalances result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or are caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for the essential element. In addition to mineral imbalances, salinity affects plant physiological responses such as osmotic stress and toxic ion stress. Higher salinity causes broad adjustments in the metabolism and physiology of tomato fruit. It contributes increased sugar content in the fully mature fruit. Tomatoes grown under high salinity produce smaller fruit. Smaller fruit have higher surface area to volume ratios, and hence greater susceptibility to water stress (Toivonen and Hodges, 2011).

19.13 CONCLUSION

After harvest, horticultural products experience many types of postharvest stress during storage and and/or transport. Therefore, the maintenance or improvement of the postharvest quality and the postharvest life of horticultural products is becoming increasingly important. During harvest or postharvest treatments horticultural crops are exposed to several stresses that often lead to the accumulation of metabolites. Stress can be understood as a stimulus or influence that is outside the normal range of homeostatic control in each organism. These stresses lead to a series of morphological, physiological, biochemical, and molecular changes in horticultural products. Once stress is controlled, a new physiological condition is established, and homeostasis is reestablished. It became clear that plants can acquire resistance to stresses. The acquired resistance is often associated with enhanced mobilization of defense responses after subsequent exposure of the plant to stresses. Also, stresses are used in postharvest activities as traditional tools to extend the shelf-life of

product, focusing mainly on color, texture, and flavor quality change. Also, these stresses can be used in preharvest activities to enhance the quality and yield of products in the field. Therefore, approaches to modulate or control stresses in horticultural products can be very important for improving shelf-life and quality retention during postharvest handling of horticulture products.

Different postharvest treatments are employed to extend the shelf-life in horticultural crops of commercial interest. These treatments can be of a physical, chemical, or biotechnological property. The reluctance of consumers regarding the chemical treatment of horticultural commodities has promoted the use of physical treatments, which consist mainly of heat treatments before or during the low-temperature storage period and regulation and control of the gaseous composition around the horticultural commodities during storage. These technologies can be explained by the existence in plants of cross-resistance to different stress conditions. The exposure of plants to certain conditions of moderate stress not only induces resistance to this kind of stress, but also protects against other kinds of stress. Thus, approaches to modulate or control the stresses in plant tissues are very important to improving shelf-life and quality retention during postharvest handling of horticultural crops. The controlled stresses could be the basis for designing strategies to develop novel tools that will open the possibility of tailoring fresh fruits and vegetables with enhanced benefit properties for use of the fresh produce and processing industries. Therefore, there is need to understand how different plant tissues and their metabolic pathways respond to different biotic and abiotic stresses, applied alone or in combination with others. There is also a need to understand how different stresses trigger the specific enzymes involved in the targeted metabolism, as well as the possible interaction between different stresses and the response of the plant tissue. Such information will be invaluable in the development of postharvest treatments for practical commercial use.

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CHAPTER 20

Physiological and Biochemical Effects of Controlled and Modified Atmospheres

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20.1 INTRODUCTION: A BRIEF HISTORY AND CURRENT TRENDS IN CONTROLLED AND MODIFIED ATMOSPHERES

The main feature of modified and controlled atmosphere (MA and CA, respectively) techniques is the reduced oxygen concentration coupled with an increase in carbon dioxide levels. The difference between the two techniques relies on the fact that in a CA the atmosphere composition is strictly controlled during the whole storage period (in specialized rooms), whereas in an MA the changes in oxygen and carbon dioxide concentrations within the package are the result of the metabolic activity (respiration) of the produce. Therefore, CA and MA are differently applied in the fruit and vegetable supply chain, with different purposes, technical features, and overall effects on the produce (Yahia, 2009). The main goal of storing perishable horticultural commodities under CA or MA conditions is to delay ripening and senescence and maintain organoleptic quality parameters with benefits in terms of prolonging commercial and shelf-life. Other effects of CA/MA are, at least for some products, the reduction of decay incidence and severity. The decision to apply CA or MA protocols for transportation, storage, and/or packaging of a produce depends on a number of factors, the most important represented by the specific physiological and metabolic responses

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to the imposed stress that, in turn, determine the overall results in terms of produce quality and consumer acceptance (Thompson, 2010).

The observation that modifying the atmosphere composition in terms of oxygen and carbon dioxide levels is effective in extending the life of some types of food, including fruits and vegetables, dates back several centuries. Despite that, the first commercial application of CA storage for apples was established in the United Kingdom in 1929 after studies published by Kidd and West who worked at the Cambridge Low Temperature Research Station. Following this pioneering facility, the application of CA technology started to be applied in several other countries worldwide, mainly for the storage of apples and pears. If we look at the evolution of the CA technology since its early practical applications for apple storage, one trend is evident: a steady decrease in oxygen and an increase in CO_2 concentrations used in storage rooms. The standard technology, based on an oxygen concentration of about 2−3 kPa and still applied for the storage of some horticultural crops, has, in the last 15 years, markedly changed and innovations are represented by CA-based methods characterized in particular by a pronounced reduction of O_2 levels (Tonutti, 2015). It must be stressed that if, on one hand, reducing oxygen concentration and increasing carbon dioxide levels are effective in slowing down general metabolism, on the other hand, these conditions represent a stress for the commodity. Beyond certain limits of low O_2/high CO_2 concentrations and storage duration, negative effects are induced and physiological disorders may appear. In the case of oxygen, the concentration should not be lower than that corresponding to the anaerobic compensation point (ACP), when a shift from aerobic to anaerobic metabolism occurs. This dual effect (positive vs negative, depending on storage time × stress intensity interaction) must always be kept in mind when CA protocols are applied, in particular if extreme conditions (very low oxygen concentrations and/or high-carbon-dioxide levels) are used. Improvements in our understanding of stress physiology and in monitoring produce metabolic responses are accompanying the technical evolution and practical application of advanced CA technologies. One example is represented by the ultra-low oxygen (ULO) technology where O_2 is maintained near 1 kPa, and initial low O_2 stress (ILOS) in which O_2 levels are maintained as low as 0.25−0.7 kPa for short time periods after harvest. Thanks to the advances in technology that allow sensing of fruit responses to hypoxic stress conditions, a further innovative step is the dynamic CA (DCA), that represents the most advanced technology in CA-based storage protocols. With this technology (applied so far only on a few crops) fruit are kept at oxygen atmospheres of about 0.4−0.5 kPa as long as possible until the earliest stress symptoms/conditions appear. Then, this concentration is promptly adjusted to higher oxygen levels, considered "safe." Since in the DCA protocols the reduction of oxygen reaches or is very close to the lowest level tolerated by the fruit (ACP) with a high risk of severe quality losses due to the anaerobic metabolism, evaluation of the physiological and metabolic conditions of the fruit is crucial. The main parameters used to

monitor the metabolic responses and to identify the early symptoms of stress conditions are based on the measurement of ethanol production by the fruit, the chlorophyll fluorescence and/or the respiratory quotient.

As far as the CO_2 levels in CA technology are concerned, most of the protocols are set around 1.5−3.0 kPa, depending on the commodity and the oxygen level. Higher levels are detrimental for the quality of the produce due to the cellular toxicity of the gas. However, specific crops such as cherries, blueberries, raspberries, and strawberries positively respond to higher carbon dioxide levels and storage conditions based on high (>10 kPa) CO_2 atmospheres and a more limited reduction of O_2 levels is applied.

MA technique deals with the possibility of inducing changes in the atmosphere composition surrounding the produce through the use of permeable polymeric films. Due to the product's respiration and/or the gas mixtures applied together with the technical features of the film (gas permeability to O_2 and CO_2), a gas balance is established with positive effects in terms of slowing down metabolism and delaying senescence. Since the 1970s, when the earliest food packages were displayed in UK supermarkets, many implementations of MA technology have been applied, in particular concerning high-performance films and packaging material. Active packaging (the packaging interacts with the internal atmosphere and/or the food in the package) represents the new frontier of this technology.

20.2 PRACTICAL APPLICATIONS OF CONTROLLED AND MODIFIED ATMOSPHERES

CA and MA techniques have a profound effect on the physiology of harvested horticultural commodities. Under optimal conditions (that vary depending on the species/variety to be stored and the composition/developmental stage of the commodity), the marketable life of the commodity can be greatly extended and quality can be retained for a longer time. As indicated in Fig. 20.1, CA technology is effective in prolonging the storability of many horticultural commodities, when compared with regular atmosphere (RA) systems.

However, depending on the commodity, the genetic background, the physiology, the developmental stage, and the storage system, limits in applying CA technology are present and must be considered. In addition to the risk of appearance of stress-related physiological disorders resulting in produce loss and/or reduced acceptance by the consumer (see Section 20.5), the cost/benefit ratio plays a key role in the decision to apply CA systems during storage. In fact, it must be stressed that CA requires specialized facilities, expensive equipment, and skilled human resources. This, in practice, results in the use of CA facilities mostly for fruit species such as apples and European pears that are the crops for which CA technology is widely adopted. Apples and pears can be successfully stored for several months (up to 1 year for specific apple cultivars

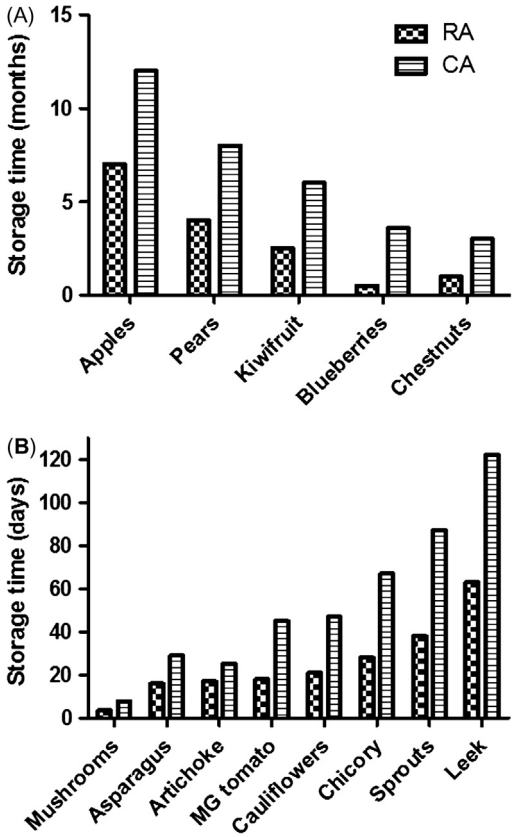

FIGURE 20.1

Storage potential of selected horticultural crops in regular atmosphere (RA) versus controlled atmosphere (CA). The graphs report some (A) fruits and (B) vegetable commodities with a beneficial effect of CA-based storage in prolonging the post-harvest life.

under optimal conditions). CA protocols are also applied to kiwifruit, persimmon, pomegranate, some cultivars of Asian pears, avocados, leeks, sweet onions, and cabbages (Kader, 2005). In some countries, where horticultural crops represent a major agricultural production (e.g., Italy), the total capacity of storage rooms equipped with CA technology nowadays exceeds that of RA facilities.

The optimization of CA technology and the development of DCA protocols in the last 10 years have resulted in the establishment of several new storage facilities, in particular in apple-growing areas. These technical advancements, coupled with the use of appropriate monitoring systems, have in general led to better and prolonged maintenance of apple fruit quality parameters and a longer marketable life. The further reduction of oxygen concentration characterizing the DCA in comparison with traditional CA protocols

results in better scores for crispness, acidity, and firmness, as observed in several apple cultivars.

20.3 LOW OXYGEN STRESS PHYSIOLOGY IN PLANTS

Oxygen deficiency (hypoxia) or its complete absence (anoxia) in the environment surrounding the plant causes unavoidable abiotic stress, named anaerobic stress. Considering plants in natural conditions, anaerobic stress can occur within the roots during flooding (waterlogging) or microbial activity in the soil, and within the whole plant during submersion due to flooding. Apart from environmentally created anaerobic stress, hypoxia and steep oxygen gradients are known to occur inside fruit tissues, especially in large bulky fruit (such as pears and apples), even in the normally oxygenized environment (normoxia). Formation of the oxygen gradient can be explained due to the absence of an active oxygen transport mechanism, existence of diffusion barriers (e.g., fruit peel), low tissue diffusivity, and high oxygen consumption by respiration. Hypoxia is also known to occur within germinating seeds and within tissues with high metabolic rates. In general, we can distinguish plant tissue hypoxia caused by environmental factors that decrease the oxygen availability or due to anatomical, morphological, physical, or biochemical components that can gradually decrease the oxygen level inside the tissue. Understanding the physiology of anaerobic stress in model species is of great interest with regard to low-oxygen-based storage of fruits and vegetables.

In order to cope with abiotic stress, plants have developed different strategies, which allow them to survive under unfavorable environmental conditions (Van Dongen and Licausi, 2014). Many wetland and aquatic plants have developed specialized tissue that forms spaces or air channels (aerenchyma) to withstand emerged, submerged, or floating conditions. As part of aerenchyma formation, some plants (e.g., rice) during flooding can induce rapid stem elongation in order to reach the water surface. Both strategies are based on morphological changes in order to overcome the limiting (for normal growth processes) stress conditions.

On the other hand, adaptation to anaerobic stress can induce a series of metabolic changes at the cellular level. Namely, oxygen deficiency has a profound impact on many different metabolic pathways. Plants are aerobic organisms, meaning that the majority of needed energy (in the form of ATP) is obtained via the oxygen-dependent process of aerobic cellular respiration. Therefore, it comes as no surprise that rearrangement of primary metabolism represents a common adaptive strategy to low oxygen stress amongst plant species. One of the most studied adaptive responses to low oxygen is the rearrangement of cellular respiration from aerobic to anaerobic. When molecular oxygen is present, plants rely on energy production via aerobic respiration, which is a highly efficient process. Aerobic respiration represents a set of metabolic processes including glycolysis, pyruvate oxidation, the Krebs cycle (also known as citric acid cycle or tricarboxylic acid, TCA), and oxidative phosphorylation. Firstly,

in glycolysis, glucose is converted into two molecules of pyruvate creating ATP and converting NAD^+ to NADH. Secondly, pyruvate, in the presence of oxygen, is converted to acetyl CoA, with the release of carbon dioxide and the generation of NADH. Acetyl CoA goes through the Krebs cycle in the mitochondria, producing ATP, NADH, $FADH_2$, and carbon dioxide. Finally, NADH and $FADH_2$ produced in other steps deposit their electrons on O_2 by a series of electron carriers, and releasing most of the ATP molecules. During the anaerobic stress, the Krebs cycle and oxidative phosphorylation are inhibited, thus, in order to support the energy requirements, glycolysis is enhanced with a higher accumulation of pyruvate. In order to decrease its concentration and further produce ATP, pyruvate is redirected toward the fermentation pathways producing ethyl alcohol (ethanol) which represents one of the compounds indicating the metabolic shift from aerobic to anaerobic. Indeed, under low oxygen condition an upregulation of *alcohol dehydrogenase* (*ADH*) and *pyruvate decarboxylase* (*PDC*) genes, coding for the enzymes which are involved in pyruvate fermentation, is observed in many plant systems.

However, since ethanol diffuses out of the cells its production depletes the plant's carbon reserves. Therefore, the additional/alternative metabolism of pyruvate to alanine is observed in many plant species. In general, the adaptive response to low oxygen is known as the Pasteur effect, whereby glycolysis is promoted and the TCA and mitochondrial respiration are repressed. Both processes of cellular respiration generate energy, but anaerobic respiration provides far less energy than aerobic respiration. In order to compensate for the lower energy production via anaerobic respiration, many biosynthetic activities and growth are inhibited in order to save ATP and to allow oxygen consumption to be decreased. Additionally, low oxygen triggers a switch to metabolic pathways that consume less ATP and utilizes oxygen more efficiently. One example is the alternative biochemical pathway for sucrose degradation to hexose phosphates.

Moreover, low oxygen conditions are also known to cause accumulation of toxic end products from anaerobic respiration and reactive oxygen species (ROS). Also, hydrogen peroxide is generated during hypoxia and has been suggested to act as a signal component that triggers downstream responses in hypoxia signaling.

At the molecular level, low oxygen has a profound impact on the gene expression patterns. Hypoxia is known to modulate expression of numerous different genes, many of which have a cell type-, tissue type-, or species-specific gene expression modulation. However, a set of around 50 different genes (composed of TF, signaling protein, anaerobic metabolism enzyme, and uncharacterized protein-coding genes) were identified as common (core) hypoxia-responsive genes among different cell types and species in plants (Mustroph et al., 2010). Considering the regulatory mechanisms involved, in *Arabidopsis* the transcription of around 80% of hypoxia-responsive genes is putatively modulated by a group of transcriptional factors named ethylene

response factors (ERFs) subgroup VII. These transcriptional factors are known as direct oxygen sensors and are responsible for triggering the expression of the majority of hypoxia-responsive genes, which in turns lead to an adaptive response to hypoxia. Oxygen sensing and signaling are based on the oxygen-dependent posttranslational modification of ERFs VII under aerobic, but not during anaerobic, conditions. In fact, during hypoxia ERF VII TFs are stabilized and relocated into the nucleus, where they can trigger gene expression of hypoxia-responsive genes, while under normoxia they are continuously degraded through the N-end rule protein degradation pathway (NERP) (Gibbs et al., 2011; Licausi et al., 2011). In addition to NERP, indirect oxygen-sensing mechanisms could be based on low energy status, variations in carbohydrate availability, ROS signals, nitric oxide (NO)-related responses, NAD/NADH ratio, calcium fluxes, and homeostatic reactions to intracellular pH changes (Bailey-Serres et al., 2012).

20.4 PHYSIOLOGICAL AND MOLECULAR RESPONSES OF FRUITS AND VEGETABLES TO CONTROLLED AND MODIFIED ATMOSPHERE CONDITIONS

As described in Sections 20.1 and 20.2, low-oxygen-based storage techniques (CA, MA) are a means to preserve quality parameters and the shelf-life of specific agricultural commodities, in particular some perishable fruits and vegetables. The effects of these storage protocols on the physiology of horticultural crops are many, involving changes in primary and secondary metabolism and depending on several factors (Lurie and Tonutti, 2014). First of all, it must be kept in mind that the metabolic activity in fruits and vegetables continues after harvest and, by controlling the atmosphere composition (in particular regarding the oxygen concentration) in the storage room, several postharvest changes such as respiration rate, ethylene production, ripening, and senescence processes can be controlled. Secondly, when applying MA/CA, the susceptibility to microorganism infections, the stage of development or maturity at harvest, the conditions of growth, and the type of commodity (e.g., climacteric vs nonclimacteric fruits) need to be taken into consideration to obtain the best benefits from the application of such technologies.

As reported earlier and as a general indication, the effects of low-oxygen-based storage protocols depend on the intensity and duration of the stress, the physiological and developmental stage of the commodity, the growing conditions, and the type and physiology of the produce (plant organ, climacteric vs nonclimacteric fruits, etc.). In fact, although some basic responses are in common, some metabolic changes may depend on the commodity and the genetic background, as demonstrated in apples where different cultivars show different responses to the same storage conditions (ULO, DCA) (Brizzolara et al., 2017).

Nevertheless, several general considerations on the effects of MA/CA on fruits and vegetables can be described starting from the changes in gene expression profiling. The research focused on the understanding of the molecular changes of fruits during CA storage started in the late-1980s, when some primary metabolism-related genes (*ADH*, *PDC*, *lactate dehydrogenase*, and *pyruvate dehydrogenase*) were identified as oxygen-responsive. These genes are coding for the enzymes which are involved in pyruvate fermentation, therefore their upregulation in turns leads to a switch from aerobic to anaerobic respiration and the accumulation of anaerobic metabolites such as acetaldehyde and ethanol. Other hypoxia-responsive genes have been identified in fruit tissues, such as *alanine aminotransferase* (*AlaAT*), which codes for an enzyme involved in the reversible catalysis of pyruvate and glutamate to alanine and α-oxoglutarate, and *sucrose synthase* (*SuSy*), which catalyzes the formation of UDP-glucose and fructose from sucrose, are induced to low oxygen conditions. The hormonal metabolism is also deeply affected in fruit stored under CA, and this is particularly evident for ethylene, the climacteric fruit-ripening hormone. The reduction of ethylene biosynthesis in CA-stored climacteric fruit is indeed associated with the downregulation of *ACC-synthase* and *ACC oxidase* genes, which are involved in the last steps of ethylene biosynthesis. Early molecular studies also pointed out that CA conditions resulted in the downregulation of genes with key roles in processes characterizing the ripening syndrome. This is the case for *cellulase* (*endo-beta-1,4 glucanase, EG*) and *polygalacturonase* (*PG*) genes, involved in cell-wall metabolism and, consequently, in the loss of flesh firmness that is reduced in fruits stored under CA conditions.

From the early 2000s, postgenomic tools have contributed to further understanding processes involved in low oxygen responses at the molecular level, in particular in apple fruit. Transcriptomics and metabolomics studies have been addressed to identify and describe processes related to the onset and development of hypoxic-induced physiological disorders (e.g., flesh browning, see later) and to discriminate the effects and impacts of different CA conditions and protocols (e.g., ULO vs DCA). Genes involved in energy (linked to the tricarboxylic acid cycle and the electron transport chain), lipids (related to membrane alterations and fatty acid oxidation), secondary metabolisms, as well as in the redox state, are differently affected in CA-stored apple fruit. As reported in Section 20.1, different CA protocols are applied to prolong the commercial life of specific fruit crops. These protocols slightly differ in terms of oxygen levels (e.g., 0.3−0.4 vs 0.8−1.0 kPa), nevertheless they are highly effective in inducing different molecular responses. In apples, genes involved in the cell wall, minor and major carbohydrates, amino acids, secondary metabolisms, fermentation and glycolysis, as well as genes involved in transport, defense responses, and oxidation−reduction, are indeed selectively affected by different oxygen levels. This might be the result of sensing mechanisms active in fruit tissues. The regulation of the expression of fruit-specific ERFs and the different accumulation of the specific proteins under hypoxic conditions indicate that also in apple fruit the oxygen-sensing mechanism detected in

Arabidopsis (NERP, see Section 20.3) is present and appears to be involved in the selective molecular responses observed in relation to different oxygen levels (Cukrov et al., 2016). ERFs seem to play a key role in regulating gene expression also in relation to treatments with high-carbon-dioxide atmospheres. In addition to ERFs, other transcription factor families (e.g., AUX/IAA, WRKY, HB, Zinc-finger MADS box) have been recognized as being involved in modulating the low-oxygen molecular responses in apple fruit.

20.4.1 Effects of Low Oxygen Levels on Fruit Metabolism and Composition

As observed in model plant species, a lack of oxygen causes a reduction in respiratory efficiency and, as a consequence, in energy production. Therefore, under hypoxia/anoxia, ATP synthesis is mostly provided by glycolysis coupled with NAD^+ regenerative pathways, including ethanolic fermentation and alanine production. Indeed, ethanol and alanine have been detected as major metabolites accumulating during CA storage of apples, the most studied fruit crop in terms of metabolic responses to CA storage. As a general trend, the production of these compounds is related to oxygen pressure, with increasing accumulation detected in relation to decreasing oxygen supply. Low concentrations of ethanol in the fruit tissue (variable in relation to species and varieties) are not only acceptable but also may induce positive effects (e.g., a reduction of the physiological disorder superficial scald, better taste), however, above a certain concentration ethanol has a negative impact on general metabolism and organoleptic traits (Pesis, 2005). In general, too long CA storage periods and/or extreme low oxygen (or high carbon dioxide) concentrations lead to the accumulation of metabolites (such as ethanol) associated with off-flavors and the onset of physiological disorders (see Section 20.5). In order to reduce this risk, strict control of the physiological and metabolic responses of fruit during CA storage is applied.

In addition to ethanol, other classes of compounds and related metabolic processes appear to be highly sensitive to the oxygen level. Among them, amino acid metabolism is one of the most affected by low-oxygen stress. The concentration of many different molecules belonging to this class, such as alanine, aspartate, γ-aminobutyric acid (GABA), proline, serine, and threonine, has been often found to be modulated by oxygen level during fruit CA storage.

Alanine has been identified as a hypoxia-related metabolite in both model species and fruit tissues. This amino acid derives from pyruvate transamination (AlaAT) coupled to two different possible reactions: the conversion of glutamate to 2-oxoglutarate (2-OG) or the conversion of GABA into succinic semialdehyde (SSA). From an energetic point of view, the production of SSA via GABA shunt is less efficient than producing 2-OG, and this could imply that under low oxygen levels alanine is, therefore, formed by glutamate and plants can use it as a storage form of pyruvate.

Also, GABA is considered to play an important role under stress conditions, including hypoxia, given its potential role both as a signaling molecule employed by plants to modulate stress responses and as a protectant molecule itself. Information on GABA pattern of accumulation under fruit CA storage appears to be controversial, with evidence reporting both increases and decreases following low-oxygen stress. A solid evidence concerning GABA is that this compound seems to have an important role in energy metabolism and defense against different abiotic stresses. GABA synthesis is promoted when the cytosolic pH decreases and so its production could be related to the fate of other organic acids in the cell, consequently being a tissue- or treatment-specific event, in a way relatively independent from the oxygen level itself. Alanine and GABA are the major amino acids produced under hypoxic conditions, accounting for a large portion of the free amino acid pool under low-oxygen stress. Generally, amino acid accumulation is a kind of defensive mechanism protecting from stresses of a different nature: alanine and GABA production are thought to be an important adaptive process aimed at carbon and nitrogen storage, as well as to maintain the osmotic potential in stressed tissues, allowing cells to balance the fast decrease in carbohydrate levels.

Amino acids interconversion represents a plastic tool to readapt a cell's metabolism to different conditions. As a consequence, the accumulation of other amino acids is also affected by hypoxia. Proline, as well as isoleucine, threonine, and serine production/accumulation are increased in fruit stored under low-oxygen concentrations. Aspartate, asparagine, and glutamate present low levels during hypoxia, consistent with the role of these amino acids as precursors of alanine and GABA synthesis. In general, when the level of energy decreases asparagine is used in order to supply the TCA cycle with carbon skeletons: consequently, alanine is accumulated as the major amino acid in hypoxic condition.

The concentration of several organic acids is also affected by CA conditions. As an example, an increase in succinic acid is often recorded during fruit CA storage. Other important organic acids have been found to be significantly affected by fruit CA storage, such as quinic acid that tends to increase under CA and MA conditions. Quinate contributes to the production of some important phenolic compounds, such as esters of caffeic and *p*-coumaric acids with quinic acid, flavanol monomers, di- and oligomers, quercetin glycosides, and dihydrochalcones. Their role under hypoxic conditions still needs to be clarified, but it can be hypothesized that they can help cells to cope with low-oxygen stress and ROS. Quinate is also known to impact astringency in fruit, also playing an important role in the perception of the sweet taste, as well as in the biosynthesis of aromatic compounds. Malate, one of the most important organic acids in fruits such as apples and pears, is also affected by low-oxygen conditions and, in general, the lower the oxygen level, the higher the malic acid concentration, as is observed in apples stored under DCA. This is mainly due to the effects that hypoxia induces on the aerobic respiration and the TCA cycle enzymes.

Sugar metabolism is modulated under CA conditions. Glucose, galactose, and melibiose concentrations show increasing trends when hypoxic conditions are applied. Galactose in fruit tissue is mainly bound to the side chains of cell-wall polysaccharides and its level increases as a result of cell wall breakdown (which happens also under CA/MA storage despite softening inhibition). Other sugars, such as arabinose, melibiose, raffinose, and sucrose, as well as many sugar alcohols, are known as osmoprotectants and stress markers in plants. As an example, melibiose is a member of the raffinose oligosaccharide family, known to be implicated in stress responses and accumulated in stress-tolerant species/varieties. Several disaccharide species are accumulated by plants during stress events and they are protective osmolytes which contribute to membrane and protein stabilization, as well as to maintaining proper cell hydration levels. Moreover, their increase under stress conditions serves also as a carbohydrate reserve of disaccharides, which could be important in protecting tissues from prolonged stress periods and energy losses.

One of the main effects of CA/MA on fruit quality parameters is the retention of flesh firmness. Firmness loss mainly relates to disassemble the middle lamella and cell wall by specific enzymes, such as PG, β-galactosidase, β-xylosidase, α-arabinofuranosidase, pectate lyase, endoglucanase (EGase), or pectinmethylesterase. MA/CA conditions downregulate the expression of some genes coding for such enzymes, allowing a good maintenance of firmness levels, with the best effects obtained under the lowest oxygen concentrations tolerated. Also, high-carbon-dioxide levels could potentially have a similar effect, but very high concentrations may cause the development of physiological disorders. Although a direct effect of hypoxic conditions on the modulation of the cell wall-related gene expression is hypothesized, it cannot be ruled out that the downregulation of specific genes, at least those that are ethylene-dependent, is due to the inhibitory effects of low oxygen concentrations on ethylene biosynthesis and action.

20.4.2 Influence of Oxygen Level on Fruit Aroma Profile

CA and MA affect the accumulation pattern of volatile organic compounds (VOCs), which have a great impact on fruit aroma and flavor, and are considered one of the most important quality parameters influencing consumer acceptance. Fruit at ripening produces a great number of volatiles, the most important belonging to the chemical classes of aldehydes, alcohols, esters, terpenes, ketones, carbonyl, lactones, and apocarotenoids. In addition to the genetic background, the contribution of each volatile compound depends also on its odor threshold (OTH) and relative concentration, which in turn depends on the activity level of the related enzymes and on the substrate availability. The fruit overall aroma is the result of a balance between all compounds, also considering the role played by the interactions between volatiles in terms of perceived aroma. Genetically, this complex quality trait appears to be regulated by several genes/enzymes involved in different pathways. One of the most important is the lipoxygenase (LOX) pathway responsible for the

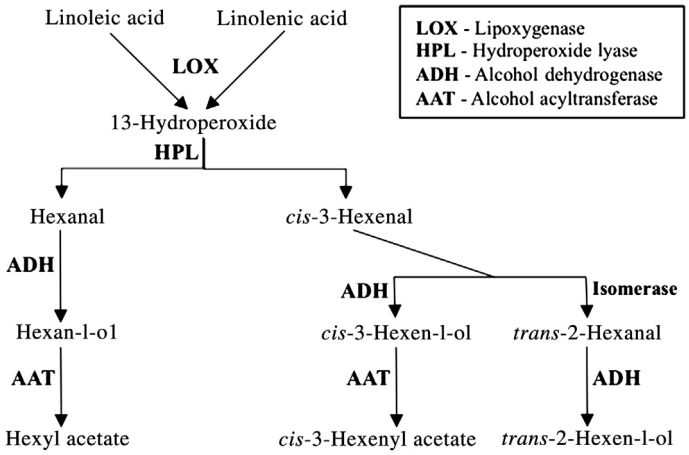

FIGURE 20.2

Schematic biosynthetic pathway of C6 volatile aliphatic compound (LOX pathway).

production of aliphatic volatile compounds. Enzymes such as LOX, hydroperoxide lyase, ADH, and alcohol-acyl-transferase (AAT), are involved in the production of C6 compounds starting from fatty acids (linoleic and linolenic acids) (Fig. 20.2). The C6 compounds (aldehydes, alcohols, and esters) produced in this pathway have a profound impact on the volatile profile in many fruit species. These enzymes are regulated by a number of factors and the same enzyme may be involved in the production of different compounds depending on the tissue specificity, the availability of different substrates, and the environmental conditions (e.g., atmosphere composition).

An example of the complexity of these biosynthetic reactions is esterification, which combines alcohols with CoA derivatives of carboxylic acids derived from fatty acids and amino acid metabolism and is an oxygen-dependent reaction catalyzed by AAT. The type of esters produced depends on the tissue specificity of AAT but also on the availability of the different pool of substrates. Comparing different CA protocols (regular CA vs DCA), it has been found that AAT enzyme combines acetic acid with different alcohol types (1-butanol, 2-methyl-1-butanol, and 1-hexanol) to originate esters under regular CA conditions, whereas in fruit stored under DCA this enzyme tends to combine different acids (butanoic acid, 2-methylbutanoic acid, hexanoic acid, and heptanoic acid) with ethanol. The high dynamic activity of AAT enzyme can explain the different results and variability according to the tissue, cultivar, precursor, fruit-ripening stage, and storage conditions. In some species, such as apple, acetyl CoA is the principal CoA derivative present, hence most of the esters produced are acetate esters. AAT enzymes have higher affinity for 2-methylbutanol than for hexanol and butanol when low concentrations of alcohol substrates are present, but the same enzyme had the opposite behavior at higher concentration of alcohol substrates.

As has already been mentioned, higher levels of VOCs might be produced in CA and MA stored fruit. It is worth noting that the production/accumulation of fermentation-related volatile compounds takes place also in fruit stored in regular air, and typically accompanies over-ripening, general senescence, tissue breakdown, and development of off-flavors. The way in which fruits are exposed to air after CA storage may have a marked effect on the final flavor profile. Ethanol, acetaldehyde, and ethyl acetate have also been considered as negative traits of fruit quality, contributing to off-flavor development when present in amounts greater than their OTH. High concentrations of these compounds can persist throughout fruit storage and shelf-life but, generally, the presence of low levels of these molecules could even improve fruit aroma and flavor. Moreover, these potential off-flavors disappear after some time when fruit are kept under normoxia after storage. Furthermore, it must be considered that off-flavor perception is subjective.

The increased activity of the fermentative pathway under hypoxia provides substrate (ethanol) for ethyl esters production, which is enhanced under CA and MA and, usually, flanked by a decreased production of nonethyl esters that may depend on competitive inhibition. Ethyl esters production is reported to be enhanced under low-oxygen storage in different fruit species and an indirect relation has been observed with oxygen concentration, with CA-stored fruit accumulated more fermentative products with decreasing oxygen levels. The recorded increase in ethyl esters production/accumulation could be due to the competitive inhibition exerted by ethanol on esters biosynthesis from other alcohols, and/or to the modulation of the activity of enzymes involved in the biosynthesis of VOCs.

Considering the effect of CA on consumer acceptance, it needs to be pointed out that ethyl esters are also known as potent odorants being characterized by an apple-like odor, often owing to their low OTH values. These compounds contribute to the flavor and aroma of several fruit species, such as apple, pear, pineapple, kiwi, and star fruit. Generally, alcohols and some esters, such as ethyl acetate, are generally emitted at high levels but, since they have high OTH, they can be perceived by consumers only when their amount is very high. As a consequence, a slight increase or decrease in their level does not really influence the final aroma. Indeed, they generally have a reduced impact on fruit flavor and are only rarely reported as main compounds but, typically, as molecules conferring background notes to the characteristic flavor of each fruit. On the other hand, compounds with a very low OTH can strongly affect fruit aroma (also negatively depending on their nature).

Fermentation-related compounds and ethyl esters are the most typical VOCs associated with CA storage, but other esters (e.g., butyl, 2-methylbutyl, pentyl, and hexyl esters) are also affected by the application of these storage conditions. The concentration of nonethyl esters tends to decline and the reduced metabolism of isoleucine (which is known to be the precursor for several volatile molecules) has been considered the primary cause for the observed general

decline in ester synthesis in CA-stored fruit. In addition to esters, other chemical classes of aroma volatiles are produced during normal ripening of climacteric and nonclimacteric fruits, such as aldehydes, alcohols, lactones, terpenoids, ketones, and volatile acids, and they can also be modulated by the applied storage conditions, characterizing altogether the stored fruit aroma. Based on the abundant scientific literature published on the effects of CA/MA storage on volatile compounds, it is evident that there is not a general common effect on VOC metabolism. This is because too many factors (genetic background, developmental stage, type of tissue, applied temperature, etc.) interact among them, with the result that different CA systems (e.g., regular CA, ULO, ILOS, DCA) have different or even opposite effects on fruit VOC profiles.

20.5 PHYSIOLOGICAL DISORDERS ASSOCIATED WITH CONTROLLED AND MODIFIED ATMOSPHERES STORAGE

As reported earlier, low O_2/high CO_2 concentrations are applied in order to prolong the commercial life and maintain the quality of specific fruits and vegetables. However, these conditions are stressful and, under certain conditions, may result in the onset of physiological disorders and the appearance of defects. Abnormal ripening, tissue browning and breakdown, and toxic accumulation of fermentative products are some of the problems that occur in fruits during or after prolonged CA storage or use of inadequate atmospheres. In addition to the storage duration, the onset and incidence of physiological disorders depends on a number of factors, including the sensitivity of the specific cultivars or variety, the anatomical structure (mainly intercellular space capacity and tissues' rate of gas diffusion), growing conditions, developmental stage, and harvest time.

In apples and pears, the most frequent external symptoms due to low-oxygen injury are the appearance of brownish areas on fruit peel, ranging from small patches to large areas covering almost the whole fruit. The intensity of the brown coloration varies based on the background color of the fruit peel. Considering, on the other hand, symptoms related to fruit internal injury, they are characterized by brownish corky sections with occasional cavities, which often are contiguous with the external damages. In pome fruits, high-carbon-dioxide levels induce the accumulation at toxic levels of succinic acid and possible negative effects of high carbon dioxide are represented by the development of peel injuries, core browning, and internal cavities. As it occurs under low oxygen levels, high carbon dioxide can also induce the accumulation of fermentation-related compounds at toxic levels.

The negative effects exerted by these compounds mainly depend on their amount and, despite their accumulation, seems to be the result more than the cause of tissue disorganization, their increase at high levels may induce cell

collapse and tissue browning. Browning has been associated with higher polyphenoloxidase activity, which catalyzes the oxidation of phenolic compounds to brown pigments and causes core browning. In sound tissues, the oxidase enzyme and the phenols, which are the enzymatic substrates, are situated in separate subcellular compartments. The alteration of cellular respiration under CA and MA storage reduces energy availability for the proper maintenance of membrane integrity and, as a consequence, cellular compartmentalization may be corrupted, allowing polyphenol oxidases to come into contact with their phenolic substrates, determining the browning of the tissue. Browning disorder has also been related to a reduction of ascorbic acid (probably the most important reducing substrate for hydroperoxide detoxification in plant cells) at a cellular level.

The onset of physiological disorders has been associated with specific metabolic alterations. An increase in cellobiose, galactose, mannose, and xylose, which indicates a corrupted cell-wall architecture, has been observed in brown tissues. Moreover, an increase in sugar alcohols such as mannitol, ribitol, and sorbitol, has been identified in altered tissue and associated with stress defense mechanisms induced by storage conditions, whereas an increase in succinate and a decrease in aspartate have been linked to an altered TCA cycle. If appropriately managed, DCA maintains fruit quality in terms of aroma, firmness, acidity, and also prevents the onset of the most important disorders better than other static CA protocols. Tissue browning, internal breakdown, and also mealiness incidence are reduced in DCA-stored apples.

Also, other fruit commodities can develop disorders after CA or MA storage. Among them, peaches and nectarine can display external (skin browning) and internal (flesh discoloration) disorders. Strawberries can develop dark or purple discoloration of the outer tissue of the berries, which may be due to the migration of anthocyanins from inner to outer tissues, or to changes in the type of anthocyanins as a result of pH changes induced by high-carbon-dioxide content. In cherries, stem discoloration, fruit darkening (inhibition of red pigment synthesis), fruit exudate, and surface browning (droplets of exudate on the skin surface and subsequent development of browning), as well as the production of off-flavors, are the most important disorders associated with CA and MA storage. Honeydew melon stored under CA or MA display, in particular during shelf-life, water-soaked and cracked epiderm disorders. Kiwifruit can develop hard core disorder (in which the fruit core fails to ripen), as well as pericarp translucency, pericarp granulation, and white core inclusion disorders (probably due to a synergistic interaction between carbon dioxide and ethylene). After nonoptimal, or too long, CA and MA storage, vegetables can also show several disorder symptoms. As an example, artichokes may develop blackening of the bracts, as well as discoloration of tissue within the receptacle, with symptoms that appear to be more severe in more mature buds. Bract blackening could be the result of low-oxygen injury, high-carbon-dioxide injury, or chilling injury aggravated by MA/CA. Another example is the discoloration of heart

leaves in Brussels sprouts. Typically, in this latter commodity a reddish discoloration of heart leaves is associated with low-oxygen injury, but also bitterness can occur without internal discoloration. Moreover, discoloration of the stem is often found in Brussels sprouts after CA storage under high-carbon-dioxide content, typically happening close to the vascular ring at the stem end.

Also, cabbage can take advantage of CA and MA conditions, but browning of the meristem tissue can be the result of nonoptimal storage. The young meristem tissue is much more sensitive to low-oxygen injury because it is actively respiring, and it can turn brown and desiccated after storage. Considering cabbage, a general leaf browning is also often observed: carbon-dioxide injury appears as browning of the tissue around the apex, with noticeable off-flavor production. Low oxygen levels can also induce the discoloration of cauliflower florets, sometimes flanked by soft rot. Moreover, the discoloration of the tissues of this commodity can also appear once it has been cooked. Lastly, lettuce may also show storage-related disorders, such as water-soaked leaves, heart leaf injury (reddish brown discoloration of the leaf margins or the entire leaf in the heart leaves), and brown stain, which are the most important disorders linked to CA and MA.

20.6 CONCLUSIONS

The successful application of CA/MA for prolonging commercial life and reducing postharvest losses of horticultural products relies on the better knowledge of physiological responses to low-oxygen/high-carbon-dioxide atmospheres. Integrated and innovative research approaches will allow better identification and description of the metabolic changes and reset that is occurring in fruits and vegetables under these imposed postharvest stresses. This will result in the identification of biological markers and the implementation of tools more effective in monitoring and reporting the physiological conditions of the produce during storage, packaging, and transportation under CA/MA. Coupled with the technical implementations (and reduced costs) of equipment/facilities, this will allow to: (1) optimize the CA/MA systems in relation to the species/cultivar response specificity with more limited risks of physiological disorders and increased quality parameters and (2) widen the application of these technologies to different horticultural crops.

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CHAPTER 21

Molecular Biology and Biotechnology of Horticultural Crops

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21.1 A LOOK INTO THE PAST, PRESENT, AND FUTURE OF PLANT FOOD PRODUCTION

All through human history, we have faced food shortages which have led to the transition from nomads (hunter-gatherers) to sedentary (farmers), initiating plant-breeding programs of wild plant species through domestication, to obtain human food. This process has been carried out for ~10,000 years and consisted of selection to adapt plants to human consumption and cross-breeding for continued propagation based on desired characteristics, such as plant vigor, size, flavor, color, resistance to abiotic stress, to facilitate harvesting and nutritional improvement (Gepts, 2002). These selections were carried out without knowledge of the genetic architecture of the selected characteristics. However, the elucidation of the genetic basis of traits of a desirable horticultural crop has the potential to facilitate ongoing plant-breeding efforts (Burke et al., 2007).

Several important horticultural crops have been developed in the Americas, such as maize (*Zea mays*), potato (*Solanum tuberosum*), and common bean (*Phaseolus vulgaris*) (Pickersgill, 2007). The major example of plant domestication in America is maize (*Z. mays*) from its ancestor, the Mexican teosinte (*Z. mays* ssp. *parviglumis*), in the Mesoamerica regions ~7500 years ago, where

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there is evidence for both morphological and genetic changes during this process. Nevertheless, domestication has resulted in the plant being dependent on humans because it cannot be propagated without human intervention.

However, the basis of knowledge regarding the genetic control of features from domesticated plants remained unraveled until the Mendel experiments hybridizing different varieties of peas to demonstrate the process of heredity in the 1860s, based on experimental evidence and mathematical analysis, which led to the establishment of the basis for genetics science (Kampourakis, 2017). Furthermore, these experiments allowed modern agriculture to be able to predict or control genetic characteristics through controlled pollination crosses.

Later, several technologies were developed to investigate crop origins, such as chromosome homology and allelic variants of enzymes. However, the development of DNA-based molecular markers was the panacea to domestication studies, e.g., for the identification of progenitors (Burger et al., 2008). Maize contributed to plant breeding and genetic science, leading to important discoveries about chromosomes and mobile DNA (Williams, 2011).

The ongoing technologies, such as quantitative trait locus for localizing domestication- and improvement-related genes, can provide great insights into the genetic architecture; however, they are both labor-intensive and time-consuming (Burger et al., 2008).

Recently, the advent of the "omics" era and the new sequencing technologies have facilitated the knowledge of how plants function and the identification of genes underlying crop-related traits to perform a large-scale genomic scan in a crop species and its wild progenitor (Burke et al., 2007). However, the new challenges due to increasing population and changing world food habits are demanding the production of more food in less time, with new plant varieties and cultivation methods. In this regard, the advent of molecular genetics and new technologies could accelerate the improvement of horticultural crops in a sustainable way.

21.2 PLANT GENETIC ENGINEERING

The technology to manipulate DNA in vitro was developed with the discovery, isolation, and production of enzymes with activity over nucleic acids, such as restriction enzymes, exonucleases, ligases, phosphotransferases, DNA polymerases, reverse transcriptases, RNAses, and DNAses (Sambrook et al., 1989). Furthermore, plant organogenesis, defined as the protocols to induce a complete plant from plant tissue, were well developed by the 1960s (Sussex, 2008).

With all the knowledge generated, the field of genetic engineering started with the creation of the first transgenic plant more than 30 years ago (Herrera-Estrella et al., 1983).

Genetic engineering is a discipline which combines different technological tools, including DNA recombinant technology, the manipulation in vitro of plant tissues, also known as tissue culture and analysis in silico of DNA sequences, also known as bioinformatics.

With the tools to sequence DNA, it was possible to know the sequence and structure of genes. It was discovered that genes are mainly made of two regions, the coding regions which are transcribed and translated to synthesize the protein and the promoter region. There are other types of genes that are only transcribed but not translated. The promoter region of a gene controls which tissue, in which type of cell, at what specific stage of plant development, and under what kind of stress the gene will be transcribed and the specific protein that will be synthesized. Knowledge about the function of proteins encoded by different genes has grown exponentially. In this way, genes playing a function in plant defense in response to a pathogen attack (infection of plant with bacteria, nematodes, virus, or fungi), in response to different types of stress such as hydric stress (low water availability), heat stress (high temperature), cold stress (low temperature exposition), low oxygen stress (tissues in atmospheres with a low oxygen concentration), salt stress (the presence of a high concentration of salt in the soil), among others, were identified and isolated. Today, it is possible to predict without experimentation, the putative physiological function of a gene by in silico analysis of its regulatory region. For instance, using this approach, it was found that the rhamnogalacturonan lyase enzyme plays a role in pollen tube development, plant growth, fruit development and ripening, and the stress response (Berumen-Varela et al., 2017).

Genetic engineering has been utilized to create plants, fruits, and vegetables with new phenotypic characteristics for several objectives, as described next. The nutritional quality has been increased to induce positive effects on human health and elimination of natural toxic compounds to reduce negative effects on human health. The possible utilization of fruits to deliver vaccines by eating the fruit has also been tried. In this regard, transgenic potatoes to enable oral immunization against hepatitis B virus were created. Also, leaves of a spinach (*Spinacia oleracea*) transgenic plant were designed to deliver a rabies vaccine. With the goal of reducing postharvest fruit losses, tomato fruits with a phenotype of fungi resistance and fruits with a prolonged postharvest shelf-life as compared with normal fruits were created. On the other side, plants resistant to herbicides were developed to eliminate weeds growing in the field without negative effects on crop production. Also, plants have been developed with a higher resistance to abiotic stresses, such as heat, drought, as well as a high concentration of salts and aluminum in the soil. Furthermore, plants have been designed to eliminate toxic compounds from the soil in what is known as phytoremediation. In addition, there are plants designed to produce important substances for industry by an approach known as molecular pharming. Further, plants have been designed to produce vaccines against the *Escherichia coli* toxin inducing diarrhea in humans, hepatitis B, rabies, and Norwalk virus, and also to produce antibodies to treat non-Hodgkin lymphoma, colorectal cancer, and dental caries.

21.3 PERSPECTIVES OF GENETICALLY MODIFIED ORGANISMS

There are reasons to be afraid of a new technology and, as an example, it will be illustrative to mention X-ray technology. At the beginning, this technology was thought to be a panacea. Unfortunately, it was discovered very soon after its use that this radiation was harmful to human health as described in Sansare et al. (2011). Because of this, radiation safety procedures were developed to reduce the negative effects of X-rays and other ionizing radiations on human health. In the case of transgenic organisms, there are many possible risks that have been mentioned. Among those, it is believed that the selectable markers, usually genes coding for antibiotic resistance, that are used to discriminate between transformed and nontransformed tissue during the tissue culture procedure, can be transferred to normal bacteria present in human intestines and in this way, this bacterium will become resistant to the antibiotic. This phenomenon is known as horizontal gene transfer and it has been suggested that the probability of this event taking place is extremely low (Altincicek et al., 2012). It does make sense that this event does not happen frequently, taking into account that humans have been eating foods like meat, fruits, and vegetables, which include DNA, and no transfer of DNA between these organisms and humans has been recorded.

Due to the rather random insertion of the transgene into the plant genome which can include, by chance, the gene-coding region, the creation of chimeric proteins with a possible toxic activity or ability to induce allergic symptoms is another issue related to plant transformation.

It has also been suggested that the transgene can escape from transgenic plants through pollen, which can fertilize wild-type relatives. In this way, the wild plant can acquire resistance to herbicides, resistance to insect attack, fungi attack, and higher resistance to abiotic stress, which will give the wild-type plants an advantage in competing and surviving.

Based on all the issues related to transgenic plants, laws for management of the risk related to transgenic plants have been developed in several countries, based mainly on the precautionary principle which states that it is better to take measures now than to face the negative effects on human health in the future. For example, the Cartagena Protocol on Biosafety to the Convention on Biological Diversity has been signed by 171 countries (Rivera-Domínguez and Tiznado Hernández, 2013).

We suggest that the most important risk related to transgenic plants is the effects of their release into the biosphere. Every species has natural access to a limited number of genes because there are physical as well as molecular barriers which negate the uncontrolled exchange of genes among the different species. It is not known why these barriers are there and therefore, it is unknown what would happen if species were allowed to access genes which are not naturally available to them. This situation could bring unexpected and difficult to predict effects on the species growing in the biosphere.

Despite the debate about the possible negative effects of transgenic plants in the biosphere, DNA recombinant technology is the only tool available to face problems that human kind already has, like the reduction in agricultural production due to the increase of saline soils, contamination with heavy metals, or contamination with mercury. There is a need to increase food production from both animals and plants, to produce low-cost vaccines, low-cost medicines, more effective antibiotics, better medicines to control virus infections, increase the nutritional quality of the food, etc. As an important example, insulin to treat diabetes is very cheap thanks to the utilization of DNA recombinant technology to produce it.

21.4 DEVELOPMENT OF NEW TECHNIQUES TO MODIFY THE PLANT GENOME

Mainly due to the large debate about the negative effects of the first protocols designed to create transgenic plants, new experimental approaches to change the plant genome sequence were created. We classified these new and most important technologies into two groups. The first group are referred to as technologies to edit the genome, also known as genome editing with engineered nuclease protocols. In this group, the following will be included: oligonucleotide-directed mutagenesis, zinc finger nuclease, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats associated with the Cas9 protein. In the second group, there are techniques known as new plant breeding, including seed production technology, RNA-dependent DNA methylation, reverse breeding, cisgenesis and intragenesis, and genetically modified rootstock grafting. The gene edition protocols basically are designed to find specific sequences in the plant genome. Once the DNA region is located, the enzymes will eliminate some bases, which will eliminate the activity of the specific gene. In this way, the genome edition protocols do not change the plant genome through the introduction of a new DNA region like in the older technologies to create transgenic plants.

The technique of transcription activator-like effector nucleases was successfully utilized to change the sequence of the acetolactate synthase in tobacco. The protocol of zinc finger nuclease was used to induce mutations in a tobacco gene to make the plant herbicide resistant. Further, with the technique of oligonucleotide-directed mutagenesis, it was possible to mutate a gene encoding for blue fluorescence protein and turn it into a green fluorescence protein in *Arabidopsis*.

Probably one of the most important gene-editing protocols available today is the Clustered Regularly Interspaced Short Palindromic Repeats associated with the Cas9 protein (Crisp/Cas9) protocol. This system is present in some bacteria and all organisms belonging to the *Archaea Phylum* which groups organisms that are different from bacteria and eukaryotes. This system was engineered to find and cut both strands of the DNA. The double-stranded DNA break will be corrected and the gene will lack part of the sequence and therefore it will

not be active. It had been suggested that the new protocols of genome edition would be important to ensure global food security (Ma et al., 2018). For the interested reader, there is an excellent review describing the gene edition protocols (Germini et al., 2018).

After editing the plant genome sequence, it is important to be able to carry out the crossing of the new plant created with another plant, which shows important characteristics with the goal of transferring the new genes into another genomic background. This is usually a time-consuming procedure and with a low probability of success, and that is why the new plant-breeding protocols are important because it is possible to do the crossing in a shorter period of time and with much better chances of obtaining a plant with the desirable characteristics. For instance, with the cisgenesis protocol, it is possible to obtain a plant with a good phenotype and homozygous for all the locus in a relatively short period of time. In addition, with the root grafting protocol, it is possible to utilize a root from a transgenic plant resistant to salinity stress grafted with a plant susceptible to the saline stress but commercially important due to the phenotypic characteristics of the fruit it produces.

21.5 DEVELOPMENT OF THE NEXT-GENERATION DNA SEQUENCING TECHNOLOGIES

The first generation of DNA sequencing protocols is the Sanger Dideoxy, which is still in use because it is fairly useful to sequence DNA fragments of an average length of 700 pair bases. However, the sequences of a transcriptome or a genome with this technique are difficult, expensive, and time-consuming.

The next generation of DNA sequencing technologies was developed for sequencing of large genomes or transcriptomes made of thousands of different genes. Now, it is possible to sequence completely the large human genome with a size of 3234.8 Mb and plant genomes with variable sizes, such as 371.64 Mb for papaya, 439 Mb for mango, 537.9 for pineapple, and 1202.94 Mb for sugar apple fruit (Bennett and Leitch, 2012).

In the case of the next-generation sequencing technologies, there is already the second generation, second-third generation, third generation, and even a fourth generation. Basically, with the exception of the fourth technology, the new-generation sequencing technologies are based on the technique developed in the first-generation sequencing technology, with specific differences, including that these are able to carry out simultaneously many thousands of sequences of DNA short regions and reads of bigger length. In the end, the machine will produce millions of short reads that need to be assembled by using bioinformatics tools. However, this step is crucial to be able to have the whole sequences of the different gene-coding regions of the library or the different chromosome sequences in the case of a genome. In the second generation, the following can be mentioned Illumina, Solid, Ion Torrent, and Roche 454.

These protocols utilize the DNA polymerase enzyme to incorporate the different bases based on a template which is the DNA fragment being sequenced. This approach is known as sequencing by synthesis. The main difference between these is the way in which the incorporation of the base is detected and registered. In the case of Illumina, the different bases fluoresce with a different wavelength. In the case of Roche 454, the pyrophosphate released during the incorporation of a nucleotide is utilized by the enzyme ATP sulfurylase adenosine to produce ATP, which in turn is utilized by the enzyme luciferase to produce light. In the case of Ion Torrent, the detection is carried out by measuring the change in pH due to the proton release when a nucleotide is incorporated into the growing DNA chain.

Besides this, another difference is the size and number of fragments obtained with the different technologies. The Roche 454 can produce up to 700 Mb of 700 bp reads. Ion proton can produce up to 10 Gb of 200 bp reads. With the powerful Illumina Novaseq equipment, it is possible to sequence up to 6000 Gb of 2×150 bp fragments.

The single molecule real-time sequencing technology (also known as PacBio sequencing) corresponds to the third generation of sequencing protocols. In this technology, a DNA polymerase is immobilized in the bottom of a cell known as zero move wideguide. After that, a DNA fragment is circularized by using special adapters known as hairpins along with the four-nucleotide labeled with fluorescent dyes of different colors. The DNA polymerase will begin the incorporation of the different bases following the sequence of the DNA fragment and the colors released due to the incorporation of different nucleotides will be recorded in a movie. Due to the fact that the DNA fragment is circular, the DNA polymerase can carry out more than one round of sequencing and, in this way, it is possible to sequence both DNA strands and even each DNA strand can be sequenced more than once. This technology offers the advantage of sequencing up to 1 Gb of fragments with 60 kb maximum length with half of the reads having a size between 15 and 20 kb.

The nanopore technology belongs to the fourth generation of DNA sequencing technology and it does not rely on the utilization of DNA polymerase, unlike the other technologies described above. In this technology, a nanopore is created using proteins inserted in a semiconducting material. This pore is between two solutions containing charged ions. An electric potential or voltage is used to induce an electric current between the two solutions, which is monitored. The ions can only pass through the pores created with the protein. Once the system is in place, the DNA is cut into large pieces and the two DNA strands are connected through a hairpin adapter in such a way as to make one long DNA strand. Also, in one of the sides of the DNA, a protein known as a motor protein is ligated. This protein carrying the DNA strand will interact with the protein of the nanopore and will move the DNA strand through the pore. The movement of each base pair will affect the voltage and, in this way,

it is possible to know the base which is altering the voltage and as a consequence the DNA sequence. With this technology, it is possible to sequence up to 5 Gb with a fragment size of up to 900 kb.

21.6 BIOINFORMATICS AS A KEY TOOL IN GENOMICS AND TRANSCRIPTOMICS ANALYSES

Bioinformatics is an emerging multidisciplinary area that fuses computational science, statistics, mathematics, and biology to organize and analyze biological data with the aim of answering biological questions. It is based on the use of a list of instructions to solve a calculation (algorithms), which commonly integrate mathematics and statistics calculations. Bioinformatics has played a very important role in the scientific progress obtained to date, including advances in medicine through the development of algorithms (computational tools based on mathematical formulas) to identify changes (mutations) in the DNA sequence, as is the case of punctual mutations (single nucleotide polymorphisms, SNPs) related to diseases. In agriculture, the recent bioinformatics tools have allowed knowing the complete DNA sequence (genome), the sets of transcripts (transcriptome), and proteins (proteome) of several plants.

The history of bioinformatics is closely related to molecular biology. Since the discovery of the DNA structure in 1953, some of the major conceptual problems of molecular biology have been solved with the support of algorithms development. The 1960s and 1970s have been considered as the birth of computational biology, because several algorithms were created, which are still being used or were the foundation for the development of more robust ones. The decades of the 1980s and 1990s demonstrated that bioinformatics is an independent discipline and that it is essential for a better understanding of biology. In 1990, the most famous algorithm was published, known as the Basic Local Alignment Search Tool (BLAST), which allows us to search for similarities and differences in the nucleotide and amino acid sequences of molecules from different organisms. This tool allows us to analyze the evolutionary origin and functions of genes and proteins, among other applications (Ouzounis and Valencia, 2003).

Bioinformatics use in the biological sciences has been extensive, however, in 2001, the publication of the human genome sequence was a breakthrough for molecular biology, leading to great advances in science and the beginning of the "omics" era of sciences. These technological developments have allowed analysis of the complete DNA sequence (genome), and the expression of sets of transcripts (transcriptome) and proteins (proteome) of an organism. Due to this, the number of sequences has been increased exponentially, generating a large number which needs to be analyzed. By December 2017, $2.47E^{12}$ bases and $5.51E^{8}$ sequences of Whole Genome Shotgun had been processed at GenBank (https://www.ncbi.nlm.nih.gov/genbank/statistics/). This huge amount of data represents a major challenge for bioinformatics. Because of this, it had to evolve to adapt and to be able to develop tools that permit us

to analyze and integrate the large amounts of information generated. Some people called this stage "next-generation bioinformatics," where computational tools have been created to integrate, evaluate the quality, filter and analyze, and, above all, to facilitate access to the information generated from projects involving "omics" analysis (Diniz and Canduri, 2017; Kumar and Dudley, 2007). Additionally, at this stage, the GNU/Linux operating system (first version published in 1991 under the GPL license, which indicates that it does not require a payment for its use) was positioned as the best choice for bioinformatics analysis, offering better performance in comparison with others. Besides this, the ability to store data increased a lot, helped by improved computer processing power.

For a better understanding of how bioinformatics tools help us to analyze the huge amount of DNA sequences generated to be able to answer complex biological questions, we will describe a summary of computational tools that have been developed to determine the transcripts sequences and how they are expressed under a given condition (transcriptomics analysis). The first step is to sequence the RNA samples using the next-generation sequencing technologies such as Illumina, 454 Roche, IonProton, etc.; this process generates millions of reads (short sequences of 75−600 nt of length, depending upon the technology utilized). Subsequently, in order to check the quality of the reads, we can use the FastQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), which allows us to visualize each base sequence quality obtained in Phred scale, represented by the Q-value (Q-value indicates the probability of error in the sequencing process; in this way, a Q30 means that we expect one error for each 1000 bp sequenced); then, to filter poor-quality reads we can use tools like Fastx Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and Trimmomatic (http://www.usadellab.org/cms/?page = trimmomatic), which remove the low-quality reads, with the Q-value lowered to 25 or other quality value. The good-quality short sequences are used to assemble the transcriptome, which is like putting together a puzzle but with more than 100 million pieces, where the pieces are the filtered reads. That sounds very difficult, however, to solve the puzzle (assemble the transcriptome), several bioinformatics tools have been developed, the most commonly used when we work with nonmodel organisms is the Trinity tool (https://github.com/trinityrnaseq/trinityrnaseq/wiki). After the transcripts are assembled, it is necessary to determine how these are expressed. In order to achieve this, we need to map the reads filtered against the transcriptome assembled and quantify the number of reads that anneal to each transcript. For this, the most commonly used tools are bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml), RSEM (https://deweylab.github.io/RSEM/), and HTSeq (https://htseq.readthedocs.io/en/release_0.9.1/). Afterward, we need to identify the set of differentially expressed transcripts in a particular condition with respect to a reference sample (control). To do this, the most commonly used tools are DESeq (https://bioconductor.org/packages/release/bioc/html/DESeq.html) and edgeR (http://bioconductor.org/packages/release/bioc/html/edgeR.html), which utilize

statistical calculations to determine whether the difference in expression levels of transcripts between two different samples is statistically significant. Finally, to determine the possible biological functions of the transcripts expressed under any given condition, we can use the software BLAST2GO, which integrates several algorithms that allow us to assign a function based on the similarities and differences with sequences from other organisms deposited in public databases like GenBank of the National Center Biotechnology Information (Kumar and Dudley, 2007; Oshlack et al., 2010). It should be mentioned that all tools previously mentioned work in the Linux operating system and they are published under the GPL license, except for the software BLAST2GO, which requires paying for a license to get full access.

As described earlier, bioinformatics has become an independent discipline and played an essential role in the history of biology research. The new technologies are building scenarios that will allow us to answer much more complex biological questions and without a doubt, bioinformatics will play an essential role in performing it.

21.7 THE PLANT GENOMICS ERA: THE CURRENT STATUS OF GENOMICS AND TRANSCRIPTOMICS IN HORTICULTURAL PRODUCTS

Since 1977, the Sanger sequencing method publication gave us the possibility of knowing the gene sequence. Subsequently, in the 1990s, the Sanger method automation allowed to obtain gene sequences faster and cheaper, giving rise to the beginning of "omics" era, which was consolidated with the human genome sequencing project and the next-generation sequencing technologies. The omics era changed the way to study the organism's biology, causing the establishment of new fields of science, such as genomics and transcriptomics. This allowed studying of the structure, function, and evolution of all gene sets of an organism (genome) through genomics. On the other hand, transcriptomics is used to study how, when, and where the set of RNA transcripts of an organism (transcriptome) are expressed under a given environmental or development condition. These interdisciplinary new fields of science implicate the involvement of several disciplines, such as mathematics, statistics, chemistry, and especially bioinformatics; allowing us to answer ever more complex biological questions.

In the last 15 years, genomics and transcriptomics have had a huge impact in the plant sciences, agronomy, plant pathology, and horticulture, among others. In 2000, the *Arabidopsis* genome sequence, considered as the quality standard for plant genomes, set up the beginning of plant genomics. Since this event, the number of plant genomes sequenced has increased considerably. To date, about 367 terrestrial plant genome projects have been registered at GenBank-NCBI (https://www.ncbi.nlm.nih.gov/genome/browse/). These include sequences of either complete or partial genomes of several plants of agronomic interest, such

as grapevine, papaya, cucumber, sugar beet, woodland strawberry, apple, potato, tomato, chickpea, banana, Chinese cabbage, orange, watermelon, mango, peach, and chili (Bolger et al., 2014; Hamilton and Robin Buell, 2012). On the other hand, transcriptomics has generated information about the gene expression of an organism's different organs, tissues, and/or cells under many different conditions. At the beginning of transcriptomics, microarray technology (chips that contain short gene sequences of an organism) was widely used to determine the gene expression levels in some crops, helping in the search for the biological functions of genes in apple, banana, grapevine, papaya, and strawberry. The advances in sequencing technologies and bioinformatics allowed the establishment of the RNA-seq technology (high-throughput RNA sequencing), which has displaced the microarrays in transcriptomics analyses. One of the many uses of RNA-seq is that we can obtain the sequence of the plant transcriptome without knowledge of the genome sequence (de novo transcriptome assembly). This has led to massive sequencing of transcriptomes of plants under different conditions, adding to the knowledge of plant biology (Rai and Shekhawat, 2015; Simsek et al., 2017).

The integration of an immense amount of information generated by genomics and transcriptomics has accelerated plant biology knowledge in several areas, including the domestication process, evolutionary history, the development and maturation of fruits, growth, response to different stress types, and the fruit postharvest life of several plants of agronomic interest. In this regard, genomics has allowed knowing the changes in the DNA sequence (natural mutations, SNPs, DNA regions deletion and duplication) that have occurred during the domestication process, which has led to improving fruit size, color, flavor, and shape, flowering time, plant height, and resistance to abiotic and biotic stresses in some of the most important crops (Kantar et al., 2017; Rai and Shekhawat, 2015).

On the other hand, transcriptomics has contributed significantly to determining how genes are expressed in some horticultural products, obtaining important advances in the understanding of their biology and how they respond to several stress types. These advances, including the elucidation of physiological and molecular mechanisms that control fruit maturation, plant life cycle, cuticle biosynthesis, response to biotic and abiotic stresses in important horticultural crops, such as tomato, potato, cherry, apple, mango, and citrus, among others (Rai and Shekhawat, 2015; Simsek et al., 2017; Tafolla-Arellano et al., 2017). This knowledge has provided data to support the biotechnological applications for the development of crops with more tolerance to abiotic and biotic stresses, fruits with better organoleptic and nutritional characteristics, and plants with higher production, among others (Imadi et al., 2015; Simsek et al., 2017). Interestingly, the omics era has been characterized by deepening the biological analysis of organisms, in this sense, these analyses have helped to find functions for DNA which were considered to be "junk." Previously, it was thought that only that fraction of the genome sequence encoded for genes, those that in turn code for proteins, was important, whereas the rest

was "junk" DNA without a function assigned. Currently, genomics and transcriptomics have helped to uncover that DNA "trash" includes sequences of genes that do not encode for proteins (noncoding RNA), such as the microRNAs and long noncoding RNAs, which have a very important role in the regulation of essential biological processes, such as fruit development, fruit ripening, flowering control, resistance to biotic and abiotic stresses (Rai and Shekhawat, 2015; Simsek et al., 2017; Zhu et al., 2015). This opens a new field to study the roles of these noncoding genes in the biology of horticultural products.

As described earlier, the omics era has increased the knowledge of plant biology. Now we have a better understanding of how plants develop, grow, and reproduce, and how they adapt to their environment; however, several questions still need to be answered to elucidate all the information encoded in the DNA sequence. In order to answer these questions, a new omics era has emerged that scientists have called "the postgenomic era," whose fundamental objective is to integrate the genomic, transcriptomic, proteomic (study of the proteins of an organism), and experimental information to identify the biological functions of each gene and protein, through functional and comparative genomics. This will provoke a new paradigm in the method of performing research, particularly in plant genomics. However, the future is hopeful and promises an exponential improvement in the understanding of plant biology and in consequence in its application to face the food demand of the world's population.

21.8 CONCLUDING REMARKS

We have witnessed a large change in the evolution of technology to manipulate DNA since the elucidation of the three-dimensional double helix back in 1953. The growing knowledge about molecular and biochemistry of DNA along with the technological advances in DNA sequencing, software development, computer processing, and read-only memory storage capacity are the platform that has made possible these developments.

In the near future, it is envisioned that there will be the design of horticultural products with better organoleptic characteristics, improved nutritional qualities, with wide positive effects on human health and optimal postharvest shelf-life behavior.

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Postharvest Physiology and Biochemistry of Fruits and Vegetables

Editor: Elhadi M. Yahia
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- Deals with the developmental aspects of the lifecycle in whole fruits
- Describes issues, including the morphology and anatomy of fruits, beginning with the structural organization of the whole plant and explaining the fruit structure and its botanical classification
- Addresses biotechnological concepts that control diverse quality components such as firmness and the nutritional value of fruits

Postharvest Physiology and Biochemistry of Fruits and Vegetables presents an updated, integrated, and sequenced view of the many characteristics of fruits and vegetables. These include their contribution to human health, plant metabolism, physical and chemical or compositional changes during their development, several important physiological disorders, biochemical effects of modified and controlled atmospheres, and the use of biotechnology to improve their quality and prolong postharvest life. The book is written specifically for those interested in pre- and postharvest crop science and the impact of physiological and biochemical changes on their roles as functional foods, and the important biological, physiological, and biochemical concepts for the preservation of these food commodities. The book is an essential resource for students, researchers, and academics in fields related to horticulture, agronomy, plant biology, food science and technology, and human nutrition.

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