1 The Biological Basis of Fruit Quality
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Introduction

The fruits of cultivated plants may be grouped into two broad categories: dry fruits (nuts and grains), which are grown almost invariably for their seed, and fleshy fruits in which the succulent pericarp normally comprises the major nutritive tissue. Although the word fruit popularly refers to fleshy fruit, the vast majority of fruits in nature are dry when mature, as are cultivated staple grains, such as wheat (Triticum spp. L.) and barley (Hordeum vulgare L.), where botanically the “seed” is a caryopsis with the testa (seed coat) fused to the pericarp. Here, however, we are concerned only with fleshy fruit, and for practical purposes we group them into three categories:

1. Dessert fruit, for example, oranges (Citrus × sinensis [L.] Osb.), apples (Malus domestica Borkh.), and grapes (Vitis vinifera L.).
2. Salad fruit, for example, tomatoes (Solanum lycopersicum L.) and cucumbers (Cucumis sativus L.).
3. Vegetable fruit, for example, fruit that are normally cooked before consumption, such as aubergines (Solanum melongena L.) and marrows (Cucurbita spp. L.).

This classification is not exclusive, and some fruit species may belong to more than one category. For example, because of their soft structure and high perishability, berries (cranberries [Vaccinium spp. L.] and blackberries [Rubus spp. L.]) are frequently classified as “soft fruit”, whereas tomato is consumed both as a salad fruit and after cooking.

Fruit Quality

Quality is a term that when applied to fresh fruit may convey a number of interpretations. Particularly within the markets of Europe, North America, and Australia, quality refers to the external appearance of the product. Within the European Union (EU), quality standards are applied to fresh fruit and vegetables and adhere to obligatory standards within the member states (EU, 2008). Additionally, produce exported to the EU from countries outside Europe must conform to the EU standards. In general this means that according to their grade (i.e., extra, first, and second) fruit and vegetables...
are packed and graded so as to be virtually free of injury, blemishes, and disease and to be uniform in shape size, color, and maturity. Similar standards in the United States are set by the United States Department of Agriculture (USDA).

Quality, however, does not only relate to appearance, and even the most rigid application of the EU quality standards does not ensure that a particular fruit or vegetable will be tasty, rich in nutrients and vitamins, or that it will ripen to be sweet and aromatic. Moreover, the EU standards do not take into account local preferences within regional markets. Indeed, local preferences may vary widely between member states and within these states. For example, some markets prefer large (beefsteak) tomatoes; others, small tomatoes. In some markets the tomato calyx must remain attached to the fruit throughout handling and marketing; in others, the calyx is removed at harvest. The degree of acceptability of mechanical damage or shape irregularity also varies between markets.

Quality should be a prime target for plant breeders, but how universally applicable are their objectives and what markets do they aim at? Until the 1960s, fruit and vegetable seed production was in the hands of relatively small seed houses aiming largely at local markets. This meant that there was a rich biodiversity of fruit and vegetable crops and growers could select varieties or hybrids that were both suitable to their local growing conditions and that produced products that were desired within the local markets. However, since the intervention of large multinationals in the breeding and seed production industry virtually all this has changed. Economic gain forestalls traditional cultivation methods and establishes new patterns of market acceptance. Biodiversity is not seen by multinational directors as conducive to shareholders’ profits. Instead, the fewer the number of varieties of tomato and the wider their distribution worldwide, the higher the profits are likely to be. Modern tomato cultivars may be grown equally well in virtually any region of the world given the know-how. They may comply 100% with the EU or USDA quality standards, be of beautiful appearance, and as uniform as “peas in a pod”, but with indifferent texture, aroma, and flavor and low nutritional value. Therefore, in considering the biological basis of quality, we shall concentrate primarily on organoleptic and nutritional quality traits, particularly the constituents of fruits that are important for a healthy, human diet, and the biological processes involved in their metabolism. The way in which breeding contributes to quality forms the subject of the subsequent chapters.

Fruit Constituents and Their Contribution to the Human Diet

Some fruits (e.g., tomatoes, bananas [Musa spp. L.], oranges, and apples) are consumed widely throughout the whole world, whereas others are more localized in demand (e.g., olives (Olea europaea L.), berries). Moreover, the increase in global travel and communication has raised consumer awareness of fruits, which until just a few decades ago were virtually unknown. However, although the consumption of fresh fruits within the western world has tended to increase over recent decades, per capita consumption is frequently lower than that recommended for a healthy diet and varies with consumer habits and the availability of supply (Lock et al., 2004).

Fruits are an important natural source of essential vitamins, in addition they contain water, organic acids, fats, carbohydrates, proteins, fiber, antioxidants, and inorganic minerals (Lock et al., 2004). The concentrations of these substances vary among species and cultivars and can be influenced by environmental factors, cultivation practices, and postharvest handling (Kays, 1991). Recently, emphasis has been placed on the occurrence of antioxidants because these play a crucial role in removing reactive oxygen species (free radicals), such as singlet oxygen (O·), hydrogen peroxide (H₂O₂), superoxide (O₂⁻), or hydroxyls (OH⁻) (Asada, 1999), which may cause oxidative damage to
cells and are implicated in chronic illnesses, such as cancer and cardiac disease (Mittler, 2002). Apart from vitamins A and C, a number of other fruit constituents (e.g., flavonoids and phenolics) have significant antioxidant properties and are considered to be of particular value to human health.

**Vitamins**

Vitamins are organic molecules that are essential in trace amounts for human metabolism. They may be grouped into six categories: A, B complex, C, D, E, and K. Fresh fruits contain significant amounts of vitamins A, B, C, and E.

Vitamin A is essential for the functioning of the retina of the eye and is crucial for normal vision. Deficiency leads to impaired vision and even blindness (Rice et al., 2004). In developing regions of the world, vitamin A deficiency is estimated to cause blindness in 250,000 to 500,000 children each year. Additionally, it plays an important role in gene transcription, cell division and differentiation, reproduction, and the maintenance of normal skin health, as well as being a powerful antioxidant (Rice et al., 2004). Vitamin A can be of animal or plant origin. In plants, the carotenoids (i.e., α-carotene, β-carotene, γ-carotene, and the xanthophyll, β-cryptoxanthin) function as precursors of vitamin A. The human organism requires about 700 (female adults) to 900 μg (male adults) vitamin A per day. Fruits that are particularly good sources of provitamin A include cantaloupe melon (Cucumis melo var. cantalupensis Naud.), apricot (Prunus armeniaca L.), papaya (Carica papaya L.), and mango (Mangifera indica L., 40–170 μg 100 g⁻¹ fresh weight) (Kays, 1991).

The B vitamins (i.e., B₁, B₂, B₃, B₅, B₆, B₇, B₉, and B₁₂) are a group of water-soluble compounds that contribute to human health by supporting cell growth and metabolism, skin and muscle tone, the function of the immune system, erythrocyte metabolism, and the prevention of anemia. With the exception of vitamin B₁₂, all the other B complex vitamins are available from plant sources; for example, avocado (Persea americana Mill.) contains vitamins B₂, B₃, B₅, B₆, and B₉; chili pepper (Capsicum spp. L.) contains vitamins B₂ and B₆; okra (Abelmoschus esculentus [L.] Moench.) contains vitamins B₁ and B₉; and banana contains vitamins B₃, B₅, and B₆. A regular intake of the B vitamins is required because any excess is excreted in the urine. A lack of B vitamins is associated with various skin disorders and dermatitis, as well as diseases such as beriberi (B₁ [thiamine]), hyperemia (B₂ [riboflavin]), and anemia (B₆ [pyridoxine], B₉ [folic acid], and B₁₂ [cobalamin]).

Vitamin C (L-ascorbic acid) is a water-soluble sugar-lactone and a strong antioxidant. In humans it acts as an enzyme cofactor for biosynthetic reactions, a substrate for ascorbate peroxidase, and an electron donor for certain enzymes (Hancock & Viola, 2005). Vitamin C (in the form of lime juice) was used to prevent scurvy among seamen long before its isolation in 1932. Moreover, patients suffering from oxidative stress, such as that related to cardiovascular disease, hypertension, chronic inflammatory disease, and diabetes, exhibit a lower plasma ascorbate concentration (45 μmol l⁻¹) than that of healthy individuals (61.4–80 μmol l⁻¹) (Schorah et al., 1996). Fruits that are particularly rich in vitamin C include blackcurrant (Ribes nigrum L., 155–215 mg 100 g⁻¹ fresh weight), pepper (Capsicum annuum L., 134–155 mg 100 g⁻¹ fresh weight), kiwi (Actinidia deliciosa A. Chev., 65–100 mg 100 g⁻¹ fresh weight), and citrus (Citrus spp. L., 65–85 mg 100 g⁻¹ fresh weight) (Kays, 1991).

Vitamin E is a generic name for tocopherols (i.e., α- , β- , γ- , and δ-tocopherols) and tocotrienols, which are lipophilic antioxidants considered to be important for the removal of reactive oxygen species created during lipid oxidation and for the protection of cell membranes and the reduction of blood cholesterol levels. The recommended daily intake of vitamin E (α-tocopherol) by adult males
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or females is 15 mg (22.4 IU). Although the most valuable natural sources of vitamin E for the human diet are wheat germ, nuts, and vegetable oils (Kays, 1991), significant amounts can be derived from a number of fresh fruits, including olives and avocados.

Vitamin K (K for Danish, koagulation) denotes a group of lipophilic, hydrophobic nutritional factors required for blood clotting and other metabolic processes relating to vascular biology and bone metabolism of which vitamin K₁ (also named phylloquinone, phytomenadione, or phytotridione) is found in a number of green plants and fruits. The recommended daily intake of vitamin K is 90 μg for adult females and 120 μg for adult males. As in the case of other lipophilic vitamins (i.e., A, D, and E), vitamin K is stored in the fat tissue of the body. Fruits with high vitamin K levels are kiwi (34–50 mg 100 g⁻¹ fresh weight), blueberry, blackberry (Vaccinium spp. L., 15–27 mg 100 g⁻¹ fresh weight) and grape (Vitis vinifera L., 14–18 mg 100 g⁻¹ fresh weight) (MacKenzie et al., 2003).

Fiber

Fibers formed from macromolecules, such as cellulose, hemicelluloses, pectins, lignin, resistant starch, and nondigestible oligosaccharides, are important for the proper function of the peptic system. Fibers are not digested within the human gut, but by adding bulk, they shorten the transit time through the intestinal tract and regulate bowel function (Anderson & Chen, 1979). Soluble fibers may absorb water to become gelatinous and fermentable by bacteria. They can also bind bile acids, thus restricting their entry into the body and reducing cholesterol levels, as well as regulating blood sugar levels and balancing intestinal pH. Lignin is also believed to have antioxidant properties. The human fiber requirement is estimated to be about 20 to 35 g per day. Although this may be provided mainly by the ingestion of vegetables, fruits also contribute about 1 to 3% of their fresh mass, with 30 to 35% in the form of cellulose, 25 to 35% hemicelluloses, and 20 to 35% pectins (Marlett, 1992).

Phenolic Compounds

Phenolics are substances (nearly 10,000) with aromatic rings and variable degrees of hydroxylation (Taiz & Zeiger, 2002; Mattila et al., 2006). Some phenolic compounds are carboxylic acids soluble in water; some are soluble only in organic solvents, whereas others are insoluble polymers (Taiz & Zeiger, 2002). Phenolic compounds are invariably present in small amounts in fruits, but in strawberries can constitute as much as 0.1% of their fresh mass. In general, phenolic compounds are located more in the peel than in the pulp. They may be grouped into two main categories: phenolic acids and flavonoids, as well as other substances such as lignans, stilbenes, tannins, and coumarines.

Phenolic acids include the products of benzoic and cinnamic acid, such as p-hydroxybenzoic, vanillic, syringic, gallic acid, and p-coumaric, caffeic, ferulic, and sinapic acid, respectively. The antioxidant properties of phenolic acids vary and depend on the structure of their molecule. Some fruits, especially berries, are rich in caffeic acid (Mattila et al., 2006).

Flavanoids are low-molecular-weight polyphenolic compounds that may be grouped as flavones and flavonols, flavanones and flavanols, isoflavones, proanthcyanidins, and anthocyanidins (Le Marchand, 2002). Flavones (e.g., rutin, luteolin, and apigenin) and flavonols (e.g., quercetin and kaempferol) are present in high amounts in blueberries and citrus, especially in the peel. Flavanones
are present in citrus (e.g., hesperidin, which is a glycoside form) (Tripoli et al., 2007), whereas flavanols, such as catechin and epicatechin, are present in grapes (Rice-Evans et al., 1997). Isoflavones, such as genistein, glycitein, and daidzein, are present mainly in legumes, whereas proanthocyanidins (derived from catechin and epicatechin) are present in grapes, apples, and blueberries (Gu et al., 2004). Anthocyanidins (e.g., pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin) are always present in the glycoside form, which has fewer antioxidant properties than the nonglycoside form. The concentration of flavonoids is invariably higher in mature fruits, and they may be present only in the peel or in the flesh of the fruit as well, depending on the plant species and variety. For example, red-flesh varieties have a higher flavonoid content than white-flesh ones.

Lignans are usually present in small amounts in fruits, with the exception of legume fruits. Resveratrol is a stilbene that is present in grapes, especially when they are produced under stress conditions (Langcake & Pryce, 1976) and in blueberries.

**Organic Acids**

Organic acids in fruits may be: (a) monocarboxylic acids (i.e., formic, acetic, and butyric acids), monocarboxylic acids with alcohols (i.e., glycolic, lactic, glyceric, and mevalonic acids), ketones (i.e., pyruvic acid), or aldehydes (i.e., glyoxylic acid); (b) di- or tricarboxylic acids (i.e., oxalic, succinic, fumaric, malic, tartaric, citric, and isocitric acids); (c) acids derived from sugars (i.e., saccharic, galacturonic, and glucuronic acids); or (d) cyclic monocarboxylic acids, such as aromatic acids (i.e., benzoic, salicylic, and caffeic acids) or alyclic acids (i.e., quinic and shikimic acids). They possess acidic properties due to the presence of their carboxyl (COOH) group(s), exist either as free acids or anions or in the form of esters and glycosides, and are located in active pools within the cytoplasm that contribute to cellular metabolism or are stored within the cell vacuole. Additionally, some acids may exist in the form of insoluble salts (e.g., oxalates) (Kays, 1991).

Apart from their role in cell metabolism (i.e., as components of the tricarboxylic acid cycle or in photosynthesis), organic acids significantly contribute to the flavor and aroma of fresh fruit. Although most organic acids within fruits are present only in trace amounts, some occur in much larger concentrations. For example, citrus fruit (e.g., oranges, lemons [Citrus × limon L.]) contain particularly large amounts of citric acid; apples, pears (Pyrus communis L.), and peaches (Prunus persica [L.] Batsch.) contain mainly citric and malic acids, whereas in grapes tartaric and malic acids predominate. In other fruits (e.g., bananas, cranberries), quinic acid and benzoic acid, respectively, are important aromatic constituents (Kays, 1991; Vicente et al., 2009).

**Proteins**

Although the protein and amino acid content of fresh fruit is rather low (typically <1% fresh weight), these components play a significant role in fruit maturation and ripening. In leguminous species (e.g., beans [Phaseolus vulgaris L.]) the protein content of the pods may be as high as 30%, due mainly to protein accumulation in the seed (Vicente et al., 2009), whereas in climacteric fruit, such as apple and avocado, the protein concentration increases during the early stages of ripening. The newly synthesized proteins play a direct role in the ripening process because inhibition of protein synthesis also inhibits ripening.
Lipids and Fatty Acids

Lipids are composed of long-chain fatty acids that may be saturated (i.e., lauric, myristic, palmitic, stearic, behenic, and lignoceric acids), monounsaturated (i.e., palmitoleic, cis-vaccenic, and oleic acids), or polyunsaturated molecules (i.e., arachidonic, linoleic, or α-linolenic acids) (Kays, 1991).

Lipids function as storage reserves (e.g., fatty acids and triacylglycerols or triglycerides) or as structural components of biological membranes (e.g., glycerol-phospholipids, glyceroglycolipids, sphingolipids, and sterols) (Taiz & Zeiger, 2002). Esterified fatty acids coupled with one of the three hydroxyl groups of the glycerol molecule can form natural triglycerides (Mazliak, 1970), resulting in oil inclusions in the cell. Additionally, the cuticle, which protects the outer tissue layers of fruit and other plant organs, contains waxes (typically high molecular weight esters of fatty acids and higher alcohols) the composition of which is important for the restriction of water loss and protection against mechanical damage and pathogens (Mazliak, 1970).

Lipids in fresh fruits usually account for less than 1% fresh weight, with the exception of avocado (4–20% fresh weight) and olive (15–40% fresh pulp weight), but with significant variation between cultivars (Mazliak, 1970). The monounsaturated fatty acids are considered to be of great importance for decreasing the level of low-density lipoprotein (LDL) cholesterol (the so called “bad” cholesterol) in the blood. Generally, fatty acids affect arterial blood pressure and the response of the organism to inflammation. The occurrence of linoleic acid and α-linolenic acid is important because these acids cannot be synthesized in the human body. Moreover, these polyunsaturated fatty acids are enlisted as omega fatty acids, which are considered to be important for human health.

Carbohydrates

Carbohydrates are the principal storage components of most fruit and may constitute between 3 and 20% fresh weight (e.g., cucumber 3.5%, watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai] 10–14%). In addition to acting as important energy reserves and substrates for respiration, carbohydrates constitute important structural components of the cells. During fruit maturation and ripening, significant changes in the carbohydrate composition (e.g., starch breakdown into sugars, hydrolysis of pectins) lead to fruit softening and sweetening, which are essential for fruit quality. Carbohydrates are present in the form of polysaccharides, oligosaccharides, and monosaccharides. Glucose, fructose, and sucrose are the most abundant water-soluble carbohydrates in fruits and are the principal sources of sweetness (Taiz & Zeiger, 2002). However, the relative concentrations of individual sugars vary greatly between species and cultivars, as well as with the stage of maturation and ripening. For example, in apple, pear, strawberry (Fragaria × ananassa Duch.), and grape, the concentrations of glucose and fructose are higher than that of sucrose, whereas in banana, pineapple (Ananas comosus [L.] Merr.), peach, and melon (Cucumis melo L.), the major soluble sugar at ripeness is sucrose. During fruit growth and maturation, starch may accumulate (e.g., in tomato), but at full ripeness the starch content is relatively low.

Minerals

Fresh fruits are a good source of minerals, many of which are considered to play an important role in human health.
Nitrogen, which is present in all fresh fruits, is essential for protein and amino acid synthesis. Nitrogen intake by humans may be from water, animals, or plants. However, the occurrence of high nitrate concentrations within some plant products (particularly leafy vegetables, but also some fruit) has been implicated in the occurrence of methemoglobinemia in infants and the formation of potentially dangerous carcinogens within the digestive tract (Sanchez-Echaniz et al., 2001; Weyer et al., 2001). On the other hand, there may also be a beneficial role for nitrates and nitrites in human health, where according to Dietary Approach to Stop Hypertension (DASH), diets rich in vegetables and even exceeding the recommended daily intake of nitrates cause vasodilation, decrease blood pressure, and support cardiovascular function (Hord et al., 2009).

Phosphorus participates in vital cellular functions (e.g., DNA and RNA synthesis) and is considered to be an essential mineral for human growth. Although human phosphorus intake occurs mainly from nonplant sources, fresh fruits may also contribute (e.g., avocado, kiwi, raspberry [Rubus idaeus L.], strawberry, and apricot).

Potassium is an important electrolyte concerned with the regulation of blood pressure and heartbeat (McCarron & Reuser, 2001), the maintenance of bone mass during aging (McDonald, 2007), and the release of energy from carbohydrates, proteins, or fats (Ignarro et al., 2007). Although it is present in all plant organs, fresh fruits such as avocado, apricot, banana, kiwi, fig (Ficus carica L.), pomegranate (Punica granatum L.), orange, and melon are considered to be among the richest sources.

Calcium is essential for bone and tooth formation, and calcium deficiency increases the risk of osteoporosis (McCarron & Reuser, 2001). Human calcium intake occurs largely from dairy products, but it is present in relatively high amounts in fresh fruits such as orange, fig, kiwi, lemon, and blackberry.

Magnesium participates in protein synthesis, enzyme activation, body temperature regulation, and bone formation (together with other minerals). Raspberry, avocado, banana, and blackberry are good sources of magnesium intake.

Plant micronutrients (i.e., iron, copper, manganese, zinc, sulfur, selenium, cobalt, sodium, chlorine iodine, and fluorine), although present only in trace amounts, are also important for the human diet.

Iron is an essential component of hemoglobin and also takes part in human body functions, such as the immune response, bone structure, and protein and enzyme structure (Arredondo & Núñez, 2005). Avocado, berries, lemon, cherry (Prunus cerasus L.), fig, and grape are good sources of iron.

Copper is important for some protein functions and for the formation of hemoglobin. It affects the progression of cardiovascular disease and diabetes, and in the case of deficiency during pregnancy, it can lead to structural malformations in the fetus and persistent neurological and immunological abnormalities in the offspring (Uriu-Adams & Keen, 2005). Avocado, blackberry, kiwi, grape, and fig are valuable sources of copper for the consumer.

Manganese participates in bone structure, brain function, blood sugar regulation, and is a cofactor for certain antioxidant enzymes. It occurs in relatively high amounts in avocado, banana, various berries, and pineapple.

Zinc is considered to have antioxidant properties and may affect the immune response. It also affects the structure and activity of certain enzymes. Avocado, blackberry, raspberry, and fig are relatively good sources of zinc.

Sulfur and sodium, both essential for human metabolism, are supplied primarily by water and the environment, although melon (cantaloupe) and avocado have relatively high concentrations of sodium.
Fruit Metabolism during Fruit Development, Maturation, and Ripening

Ripening is an essential process for the development of fruit quality, but in view of the wide range of fruit types, it is hardly surprising that differences are observed with respect to ripening metabolism even though the central biochemical pathways are common.

Fruit development and growth are dependent on photosynthetic CO₂ fixation in leaves and the translocation of sugars (i.e., sucrose, in particular, stachyose, raffinose, and sorbitol), amino acids, and organic acids to the fruit cells (Ho, 1988). During the early phase of development, most fruits, like meristems, can be regarded as utilization sinks because of their high metabolic activity and rapid cell division. During the later phase of development, which is characterized by cell expansion, seed development and maturation, most fruits accumulate high levels of carbohydrates in the form of starch or sugars and are thus more typical of storage sinks (Ho, 1988).

Our perception of flavor relies on two senses, taste and smell, and taste sensations may be characterized as sweet, sour, or bitter (salty not contributing to the flavor of fresh fruit). Fruit flavor, however, is also affected by our sense of smell and the presence of specific flavor volatiles. Hence, the flavor and aroma profile of an individual fruit derives from a complex interaction of sugars, organic acids, phenolics, and more specialized flavor compounds, including a wide range of volatiles (Tucker, 1993).

Carbohydrate Metabolism

Although most fruit cells have functional chloroplasts during their development, they do not appear to contribute significant amounts of photo-assimilates to fruit growth (Gillaspy et al., 1993), and so fruit sugars and organic acids mainly originate from leaf photosynthetic assimilates. Most fruit accumulate the bulk of their carbohydrate before the onset of ripening. Growing tomato and banana fruits tend to accumulate high amounts of starch (up to 20% of dry weight in young tomato fruit), which is degraded at the later stages of ripening to produce sucrose and hexoses (Seymour, 1993; Kanayama & Odanaka, 2000). Citrus fruits following the same pattern accumulate glucose, fructose, and sucrose as they ripen (Ting & Attaway, 1970). These fruits can be harvested at the mature green stage and still attain acceptable flavor on ripening after storage (Tucker, 1993). Other fruits, however, continue to accumulate sugar from the plant during ripening (e.g., strawberry, grape). Sucrose translocated into grapes is hydrolyzed, so that the ripe fruits contain mainly glucose and fructose and only small amounts of other sugars (e.g., less than 0.1% sucrose) (Kanellis & Roubelakis-Angelakis, 1993). Cucurbits also do not accumulate starch and require active translocation of photo-assimilates from leaves during development and ripening (Handley et al., 1983). For satisfactory flavor, cantaloupe melons should contain at least 10% sugar, and if harvested before maturity, the fruits of these species (also grape, strawberry, watermelon and muskmelon [Cucumis melo L.]) are insipid and not sweet even when ripe (Bianco & Pratt, 1977). Even in fruits that accumulate starch, such as tomato, the longer the fruit remain on the plant before harvest the better the flavor will be (Tucker, 1993), as indicated by differences in acid-to-sugar ratios and volatile profiles between fruit ripened on the plant and those ripened after harvest at the green stage.

Tomato is a good example of a fruit that stores carbohydrate in the form of starch before ripening. Sucrose is translocated to the fruit and metabolized by acid invertase and possibly sucrose synthase (SuSy) so as to maintain a low fruit sucrose concentration and therefore a steep sucrose concentration gradient between the phloem and the surrounding cells within the fruit. Glucose and fructose derived from sucrose are phosphorylated by hexokinase and fructokinase respectively for further
metabolism, such as glycolysis and starch synthesis. Two divergent fructokinase genes, Frk1 and Frk2, have been shown to be expressed differentially in tomato plants, with Frk1 playing a role as a “housekeeping enzyme” in carbohydrate metabolism in all plant cells, whereas Frk2 is induced for starch synthesis and seed development (Kanayama & Odanaka, 2000).

By contrast, in young cucumber and melon fruits α-galactosidase cleaves galactose from imported stachyose and raffinose to leave sucrose; whereas galactose is further metabolized via galactokinase, UDP-Gal pyrophosphorylase (PPase), and UDP-Glc-4 epimerase to form UDP-glucose (Gao & Schaffer, 1999). In pome fruits, the translocated assimilates (70% sorbitol and 30% sucrose) are metabolized to form fructose, glucose, sucrose, malic acid, and starch. Sorbitol in apples and Japanese pears (Pyrus pyrifolia [Burm.] Nak.) is converted to fructose by sorbitol dehydrogenase; thus in these fruits, fructose is produced in preference to glucose (Berüter, 2004). Sorbitol oxidase has also been detected as a minor cell-wall bound enzyme in apples (Knee, 1993).

During the later stages of ripening of fruits that accumulate starch, rapid hydrolysis of starch is catalyzed by the enzymes α- and β-amylase and starch phosphorylase. In banana, starch reserves decrease from 25% to less than 1% of the total fruit weight during the climacteric phase, whereas the sucrose content increases 12-fold following by an increase in hexoses (Cordenunsi & Lajolo, 1995). The enzymes responsible for starch degradation are active only against the linear glucose chains of amylase within the starch and are unable to degrade the α-(1–6) branch points also found in the amyllopectin of starch. However, enzymes capable of attacking the branch points (i.e., debranching enzyme, EC 3.2.1.10) have been identified in several tissues, including banana. Although starch phosphorylase hydrolyzes the terminal α(1–4) linkage to give glucose-1-phosphate, amylases produce maltose, which is converted into glucose by the action of α-1,4- and α-1,6-glucosidases. The end products of starch degradation (i.e., glucose and glucose-1-phosphate), are converted to glucose-6-phosphate, by the action of hexokinase or glucose phosphate mutase respectively. Starch degradation is confined to the plastids, but utilization of the breakdown products occurs mainly in the cytoplasm, either in respiration or by reconversion to glucose phosphate and fructose for sucrose synthesis (Tucker, 1993).

In tomatoes, the increased activity of acid invertase may be responsible for the low ratio of sucrose-to-hexose at maturity in commercial cultivars, whereas in wild-type tomatoes, a higher sucrose concentration could result either from the reduced activity of acid invertase (Miron & Schaffer, 1991) or high sucrose phosphate synthase (SPS) activity (Dali et al., 1992). As Nguyen-Quoc et al. (1999) showed, SPS probably affects sucrose turnover and starch synthesis but not sucrose-to-hexose ratios. However, sucrose recycling may also occur via four “futile cycles” involving sugar transport between the cytosol, vacuole, and apoplast (Nguyen-Quoc & Foyer, 2001). In these, there is continuous degradation of sucrose in the cytosol by SuSy, sucrose resynthesis via either SuSy or SPS, sucrose hydrolysis in the vacuole, or apoplast by acid invertase with subsequent transport of the hexoses to the cytosol where they are reconverted into sucrose. In this way, futile cycles of sucrose-hexose interchange govern the fruit sugar content and composition, whereas a constantly high invertase activity during the later stages of ripening maintains high cellular hexose concentrations.

In pomes, starch hydrolysis by α- and β-amylases and starch phosphorylase usually begins at the later stages of fruit growth but before the onset of the climacteric. In the ripe fruit, starch is almost totally hydrolyzed to sucrose (Berüter, 2004), which in turn is slowly hydrolyzed to fructose and glucose. Fruits that accumulate sucrose during ripening (e.g., mango, kiwi, banana, and melon) show an increase in SPS activity and a decrease in acid invertase during rapid sucrose accumulation (Hubbard et al., 1991), although gluconeogenic enzymes fructose-1-6-diphosphatase and glucose-6-phosphatase may also be involved (Cordenunsi & Lajolo, 1995).
**Lipid Metabolism**

Fatty acids are synthesized within the cytosol as well as within certain plastids, such as chromoplasts and chloroplasts. In avocados, saturated or polyunsaturated fatty acids are synthesized during the first weeks of fruit development whereas monounsaturated fatty acids are synthesized throughout fruit growth (Mazliak, 1970). The biosynthesis of fatty acids and triglycerides in avocado has been reviewed by Seymour and Tucker (1993).

In olives, fatty acids accumulate mainly after the period of rapid fruit growth, but later decline. Oleic acid is the principal fatty acid in olives, but palmitic, stearic, and linoleic acids are also present in significant amounts. The concentration of oleic acid increases during low temperature (Mazliak, 1970).

Palmitic, oleic, linoleic, and linolenic are the principal fatty acids formed in the peel and pulp of bananas. A decrease in fatty acid concentration occurs during fruit maturation and ripening, with linoleic acid decreasing and linolenic acid increasing (Wade & Bishop, 1978). Changes in the relative concentrations of fatty acid constituents during maturation are also seen in other fruits. For example, green pumpkins (*Cucurbita pepo* L.) contain linolenic and myristic acid, which are not present in ripe fruits. The lipid content of muskmelon peel does not change significantly during fruit development, but with the start of ripening unsaturated fatty acids accumulate (Forney, 1990).

**Organic Acid Metabolism**

Although green fruits can synthesize organic acids as products of photosynthesis, most organic acids within the fruit are derived from other parts of the plant (Ulrich, 1970), and the organic acid content may be a useful indicator of fruit maturity, for example in apples and tomatoes (Stevens et al., 1979; Kader, 1999), but varies with the cultivar and cultivation conditions. For example, the malic acid-to-citric acid ratio in tomato fruit varies between cultivar and with the stage of ripeness. Early cultivars tend to have more malate than late cultivars, whereas the citrate levels increase with ripening. Moreover, the free and total acid levels in tomatoes relate to the K concentration within the root substrate (Passam et al., 2007). Generally, overripening and aging of fruit results in a decrease in acid content and a concomitant loss of flavor.

In apples, the organic acid content increases during fruit development, but decreases before harvest and during subsequent storage. On the other hand, the organic acid content of pears decreases continually from an early stage of fruit development (Ulrich, 1970). In citrus, except lemons, acidity increases early in fruit development and then decreases during ripening (Samson, 1986), and this can be partly associated with increased fruit size and water content (Kimball, 1984). These changes mainly reflect the change in citric acid, which is the most abundant organic acid, whereas malic acid does not change significantly (Shaked & Hasdai, 1985). During storage, the organic acid content of citrus fruit decreases but the pattern of change varies with the different tissues of the fruit; for example, malate (the second most abundant organic acid of citrus) decreases in the albedo and increases in the flavedo (Sasson & Monseline, 1977). In lemons, the increase in acidity during ripening is associated with an increased concentration of citrate (Ting & Attaway, 1970).

The environmental conditions during fruit growth can significantly affect both the organic acid and the sugar content, and therefore fruit flavor and quality. Although shading did not change the citrate content of strawberries (Watson et al., 2002), the pH and reducing sugar content of peaches were higher in fruits produced under conditions of high light intensity (Geanard & Bruchou, 1992). Moreover, the afternoon exposure of fruits to the sun reduces the concentration of sucrose and
malate and increases the concentration of citrate. Tomatoes produced under low relative humidity are firmer and juicier than those cultivated under high relative humidity (Janse & Schols, 1992). In tomato, too, the sugar, titratable acid, aroma volatile, and vitamin C contents are enhanced by drought stress (Auerswald et al., 1999; Veit-Köhler et al., 1999), apparently due to a combination of osmoregulation and decreased yield, which concentrates the photosynthates into fewer and smaller fruits (Beverly et al., 1993). Stress due to soil salinity can lead to an increased concentration of organic acids in tomatoes (Auerswald et al., 1999; Passam et al., 2007) and enhanced blossom end rot (Reid et al., 1996), whereas high CO₂ concentrations in the greenhouse or in the storage atmosphere lead to a reduction (Huyskens-Keil & Schreiner, 2004). With the notable exception of banana, the organic acid content of fruit decreases during storage, but low storage temperatures may disrupt this process, especially in species that are sensitive to chilling injury.

**Vitamin Metabolism**

Vitamin A is liposoluble and is formed from carotenoids, classified as carotenes with C and H (e.g., α-carotene, β-carotene, lycopene) or xanthophylls (e.g., violaxanthin, zeaxanthin), which are oxygenated derivatives. The carotenoids are terpenoids formed by eight isoprene units that take part in radiation interception (400–500 nm) and transfer to chlorophyll during photosynthesis (Taiz & Zeiger, 2002). They are primarily responsible for the red, yellow, and orange color of fruits and also protect the photosynthetic structures from excessive energy (Grusak & DellaPenna, 1999) through a reversible reaction involving violaxanthin and zeaxanthin (Horton et al., 1996).

Vitamin C (L-ascorbic acid) is synthesized either via a pathway involving L-lactose as precursor or via the galacturonic acid pathway associated with cell wall pectin degradation. L-ascorbic acid is readily oxidized to L-dehydroascorbic acid, which can be irreversibly oxidized to form diketogulonic acid that has no vitamin C activity (Parviainen & Nyyssonen, 1992). L-ascorbic acid degradation is affected mainly by the activity of ascorbate oxidase, which is associated with rapidly growing regions of the plant.

The vitamin C content of fruit varies with genotype and environmental conditions during growth. Irradiation has a definite influence on the amount of vitamin C formed because ascorbic acid is synthesized from sugars supplied through light dependent photosynthesis (Lee & Kader, 2000). In contrast, cool temperatures increase L-ascorbic acid in citrus such as mandarins (*Citrus reticulata* Blanco) and grapefruits (*Citrus × paradisi* Macfад.) (Lee & Kader, 2000) and tomato (Islam & Khan, 2001). High rates of nitrogen application apparently reduce the L-ascorbic acid concentration in citrus, whereas potassium application or reduced irrigation may increase the L-ascorbic acid levels (Nagy, 1980; Lee & Kader, 2000). In addition, L-ascorbic acid concentrations are reduced in tissues under stress (e.g., pathogen or chemical exposure) due to increased ascorbate oxidase activity (Loewus & Loewus, 1987).

The L-ascorbic acid concentration within fruits is significantly affected by maturity. Ripe tomatoes and red peppers have a higher L-ascorbic acid content than unripe (green) ones (Howard et al., 1994). Although L-ascorbic acid accumulation in tomatoes can occur after harvest, higher levels are reached when fruits ripen on the plant. Likewise, L-ascorbic acid content is higher in apricots and peaches ripened on the plant, but apples, mangoes, and citrus have lower levels at maturity (Nagy, 1980; Lee & Kader, 2000).

The postharvest management of fruits is significant for vitamin C content. L-ascorbic acid decreases during the storage of tomatoes and apples, and losses are enhanced by mechanical injury, extended storage, high temperatures, low relative humidity, physical damage, and chilling injury.
(Lee & Kader, 2000). On the other hand, treatment with calcium chloride (CaCl₂) can increase the vitamin C content of apples and tomatoes and the application of modified atmosphere storage reduces the rate of vitamin C loss in apples (Lee & Kader, 2000). The processing of fruits after harvest (e.g., by cooking or freezing) can also lead to significant reductions in vitamin C concentration because of the high sensitivity of L-ascorbic acid to chemical and enzymatic oxidation (Oruna-Concha et al., 1998). Similarly, vitamin E is highly susceptible to oxidation during storage and processing (Vicente et al., 2009).

**Phenolic Compounds Metabolism**

Phenolic compounds are synthesized in several different ways. Most phenolics are derived at least in part from phenylalanine, which is produced from the shikimic acid pathway and leads to the formation of cinnamic acid. The formation of cinnamic acid is catalyzed by phenylalanine ammonia lyase (PAL) and leads to the production of phenolic acids, such as derivatives of benzoic acid (e.g., vanillic and salicylic acids), cumaric acid and simple phenylpropanoids (e.g., caffeic, ferulic acid) (Taiz & Zeiger, 2002). One of the most common phenolic acids in fruits such as apples, pears, and peaches is chlorogenic acid, which is the dominant substrate of enzymatic oxidation and leads to cell blackening after wounding. The antioxidant capacity of the phenolic acid depends on its structure, and it is higher in molecules with a large number of hydroxyls (Vicente et al., 2009).

Fruit phenolic acid content varies with cultivar (Wang & Lin, 2000) and environmental factors during fruit development. High light intensity favors the accumulation of phenolic acids in the peel of tomato (Gautier et al., 2008), and even within the same fruit, the shaded region contains fewer phenolics than the sun-exposed region (Lee & Kader, 2000). The phenolic acid concentration of blueberry fruit decreases during ripening (Castrejon et al., 2008), as does the chlorogenic acid content of tomato, although due to fruit enlargement, the total amount of phenolic acid per fruit may increase.

Flavonoids are synthesized from products of the shikimic acid and malonic acid pathways involving the interaction of at least five different pathways: (1) the glycolytic pathway, (2) the pentose phosphate pathway, (3) the shikimate pathway that synthesizes phenylalanine, (4) the general phenylpropanoid metabolism that produces activated cinnamic acid derivatives (4-coumaroyl [CoA]) and also lignin, and (5) the diverse specific flavonoid pathways (reviewed by Robards & Antolovich, 1997). Flavonoid synthesis is affected by light intensity and wavelength, with fruits exposed to full sunlight containing more flavonoids than those in the shade (Awad et al., 2001). Moreover it can be modified by temperature, humidity, and phytoregulators, as for example in citrus (Arcas et al., 2000).

The flavonoids may affect fruit quality characteristics such as texture, color, flavor, and the nutritional value of fruits (e.g., in apples) through their involvement in the formation of undesirable brown pigments following bruising or cutting, due to the enzymatic oxidation of endogenous phenolics into quinones, which are then polymerized into brown products (Robards & Antolovich, 1997). Flavonoids increase the postharvest resistance of fruits to pathogens (Lattanzio, 2003) but may contribute to the formation of undesirable sediments in fruit juices and wine.

Anthocyanins are always produced during fruit ripening, but in some fruits such as nectarines (*Prunus persica* [L.] Batsch.), they are produced during the first stages of fruit development. The anthocyanin content of ripe fruit is affected by environmental conditions such as light and temperature (Faragher, 1983; Arakawa et al., 1985) and is enhanced by low rates of nitrogen fertilization. In apples, anthocyanin accumulation is favored by low temperatures, especially at night. Cyanidin is the most common anthocyanidin in fruits such as peaches and pears.
Quercetin, kaempferol, myricetin, and isorhamnetin are the most common flavonol aglycones that have been identified in plants (Robards & Antolovich, 1997). Flavan-3-ols are important constituents of fruits in oligomeric or polymeric forms, such as proanthocyanidins or condensed tannins. However, the monomers found in fruits (e.g., [+] catechin, [2]-epicatechin, [+] gallocatechin, and [2]-epigallocatechin) are important natural products. They are distinguished from other flavonoids because they are present usually in free rather than glycosylated forms. Moreover, catechins are the natural substrates of polyphenol oxidases and are therefore involved in browning phenomena. They are also the monomer units for procyanidins (Robards & Antolovich, 1997).

Although flavanones are generally found in small amounts in fruits, they are the predominant flavonoid of citrus (most common are naringenin, eriodictyol, isosakuranetin and hesperetin), and they are usually present in the glycoside forms. The flavone content of fruit is generally low, except citrus, where they are present as polymethoxylated flavones (e.g., nobiletin, sinensetin, and tangeretin).

In tomato, the most abundant flavonoid is naringenin chalcone. Tomatoes also contain the flavonols quercetin-rutinoside (rutin) and kaempferol-rutinoside. The accumulation of these flavonoids occurs exclusively in the peel during fruit color development (Muir et al., 2001). Cool storage leads to an increase in the anthocyanin content of fruits such as strawberry, blueberry, and grape, but low temperatures may also have negative effects on the fruit phenolics contents (Tomás-Barberan & Espín, 2001).

**Proteins and Amino Acids Metabolism**

Although the protein content of fruits is low and is not considered to be a quality characteristic, proteins nevertheless play an important role as enzymes in the physiological processes of ripening and senescence before and after harvest. Thus, fruit development and ripening are related with quantitative and qualitative changes in protein synthesis that vary between varieties and with the season of cultivation. Moreover, amino acids can serve as precursors for lipid, carbohydrate, and nucleic acid synthesis.

Amino acid metabolism is complex because of the large number of metabolites involved (Buchanan et al., 2000) and may follow different pathways according to the different length of carbon structures. For example, alanine is degraded to pyruvate and can be utilized in gluconeogenesis. Aspartate and asparagines are degraded to oxaloacetate and are closely linked to glutamate and α-ketoglutarate interconversion by amino transferases. Glutamine, proline, and arginine are converted ultimately to glutamate, which is deaminated to α-ketoglutarate. Other nonpolar amino acids, such as methionine and valine, are precursors for the synthesis of odd numbered fatty acids via the intermediate propionyl-CoA.

Free amino acids (e.g., aspartic acid, asparagine, proline, lysine, α-κο κλ β- alanine) are present in fruits and especially in their juices. The concentration of free amino acids alters during fruit development and ripening depending on the species (Burroughs, 1970). The onset of ripening of climacteric fruits is also dependent on amino acid metabolism because of the involvement of L-methionine in ethylene synthesis.

In ‘Valencia’ oranges, proline amounts to as much as 50% of the total amino acid concentration, whereas serine and glutamic acid constitute about 10%. The amino acid content varies with the stage of ripening (Tadeo et al., 1988) and is affected by temperature. In grapes, glutamic acid and arginine are the principal amino acids in the juice. Their concentration depends on the cultivar, rootstock, degree of fruit maturity, temperature, and mineral nutrition. The total amino acid and
total free amino acid content of grapes increase during maturation, and synthesis occurs mainly during the last 6 to 8 weeks of fruit ripening (Kanellis & Roubelakis-Angelakis, 1993). In cherry, proline and asparagine accumulate in the red fruit, whereas in tomato, glutamic acid and aspartic acid increase during fruit ripening, especially following the removal of fruit from the plant, and other amino acids (e.g., arginine and alanine) decline. High levels of nitrogen or phosphate application increase the total amino acid content of tomatoes, but high levels of potassium lead to decreased levels of total amino acids (Saravacos et al., 1958).

**Mineral Metabolism**

The mineral content of fruits greatly contributes to their quality characteristics and nutritional value and may be affected by several factors, such as environment (i.e., temperature, humidity, sunlight) and cultural practices (e.g., organic culture, hydroponic culture). For example, tomatoes grown organically in a compost/soil mix had a higher calcium and lower potassium, magnesium, and sodium concentration than those grown hydroponically (Premuzic et al., 1998).

Nitrogen is a component of free amino acids (about 80% of the total nitrogen content) and can be found in nonprotein nitrogenous combinations (e.g., choline, glutathione, asparagine, purine). The presence of nitrogen within the fruits varies from tissue to tissue because tissues with high metabolic rates, such as the epicarp and core, may have higher requirements for nitrogen (Vicente et al., 2009). High amounts of nitrogen in apples favor the development of green color (Marsh et al., 1996), whereas excess nitrogen in peaches lead to an inhibition of color change from green to yellow (Crisosto et al., 1997) as well as color development in citrus and grapes.

A high nitrogen supply to the plants increases the soluble solids content of tomatoes (Barringer et al., 1999) but decreases that of apples (Dris et al., 1999). Although nitrogen application is positively correlated with fruit size, there is evidence that excessive nitrogen fertilization decreases firmness and flavor ratings of ‘d’Anjou’ pears as well as reducing the vitamin C concentration of some fruits such as citrus (Nagy, 1980) and the incidence of physiological disorders, such as apricot pit burn (Bussi & Amiot, 2003).

Tissues with high metabolic rates (e.g., epicarp, core) may have higher requirements for phosphorus, so that an inadequate supply of this element to the plant can result in a loss of fruit firmness and low calcium content (Sharples, 1980). On the other hand, high phosphorus levels may reduce the soluble solids content of apples (Fallahi et al., 1985).

Potassium is one of the most abundant minerals in fruits and is always present in combination with various organic acids (Vicente et al., 2009). Because fruits are a significant source of potassium for the human body, the potassium content of the fruit may be regarded as an important quality trait. It appears to increase during ripening and is affected by environmental factors and the location of cultivation (Wall, 2006). Low levels of potassium lead to a reduction in the concentration of lycopene and β-carotene in tomatoes and ascorbic acid in citrus (Nagy, 1980). According to Neilsen and Neilsen (2003), potassium deficiency leads to reduced red fruit color of apples.

Calcium affects the quality characteristics of the fruits not only at harvest but also during storage. It plays a significant role in the response of cells to stress conditions such as extreme temperatures, anaerobiosis, wounding, fungal attack, and mineral deficiency. Generally, tissues like fruit peel, which have high metabolic activity, contain higher concentrations of calcium than the less metabolically active pulp (Saure, 2005). The calcium content of fruits may be reduced during vigorous plant growth and in kiwi is higher under sunlight than in the shade (Montanaro et al., 2006).
Because a high calcium content of the fruit increases firmness or reduces softening (e.g., in apples, pears, strawberries, peaches, kiwi), calcium may be applied in the form of foliar sprays or by postharvest dips and impregnation procedures. It has been proposed that in these cases calcium reduces the accessibility of enzymes that degrade the cell wall to their substrates (Vicente et al., 2009). In apples, high calcium content is also correlated with lower fruit soluble solids (Fallahi et al., 1985).

The significance of calcium for fruit quality is also observed by its involvement in fruit disorders such as blossom-end rot in tomato and pepper (Adams, 2002) and bitter pit in pome fruit (Ferguson et al., 1999). Blossom-end rot in tomato due to calcium deficiency is exacerbated when the dominant nitrogen ion in the nutrient medium is NH$_4$ (Saure, 2001; Passam et al., 2007). In papayas, low mesocarp calcium concentrations have been linked with fruit softening (Qiu et al., 1995). Calcium is also associated with the inhibition of chilling injury in muskmelon during long-term cold storage (Combrink et al., 1995) and in peaches stored for four weeks at 5°C (Manganaris et al., 2007).

Magnesium affects fruit quality indirectly through its involvement in photosynthetic and other metabolic processes (Taiz & Zeiger, 2002). The magnesium content of the fruit is less in vigorously growing plants and its concentration together with that of potassium in apples has been considered indicative for the occurrence of bitter pit (Autio et al., 1986). Low levels of iron limit photosynthesis (Taiz & Zeiger, 2002), and iron also affects carotene metabolism (and therefore pigmentation) because β-carotene hydroxylase requires ferredoxin and iron (Bouvier et al., 1998). Copper, too, may affect fruit quality because it is required for the activity of ethylene receptors (Rodriguez et al., 1999), and as a component of ascorbate oxidase, it promotes the oxidation of L-ascorbic acid to L-dehydroascorbic acid (Saari et al., 1995). In apples, high manganese content is associated with the development of green color, whereas zinc is involved in the maintenance of cell membrane integrity and carbohydrate metabolism (Taiz & Zeiger, 2002). In mango, zinc sulfate fertilization increases the total soluble solids of the fruit (Bahadur et al., 1998). Although fruits contain relatively small amounts of sulfur, it may contribute to the aroma via its involvement in volatile sulfur compounds (e.g., in grapefruit, orange, and pineapple). Sodium and chloride may also affect some quality characteristics of the fruits. For example, a high concentration of sodium chloride in the soil reduces the juice content, total soluble solids, and titratable acidity of lemons, possibly due to the greater accumulation of chloride compared to sodium, leading to a degradation of organic acids for charge balance (Garcia-Sanchez et al., 2003). Furthermore, the presence of small quantities of sodium chloride within the nutrient solution of hydroponically grown tomatoes has been found to improve flavor quality (Passam et al., 2007).

**Cell Wall Metabolism and Fruit Texture**

The texture of the fruit at ripeness is a significant quality aspect and relates to the decomposition of cell walls and softening of the tissues (Wakabayashi, 2000). Typically the plant cell is characterized by the presence of a carbohydrate rich cell wall, which consists of approximately 30% cellulose, 30% hemicellulose, 35% pectin, and 5% protein in dicotyledonous plants (Fry, 1988), but in the fruit cell wall the pectin content is higher and protein content lower (Knee & Bartley, 1981).

According to the biphasic cell wall concept, cellulose microfibrils are coated and cross-linked with hemicelluloses to form a cellulose-xylloglucan skeleton, immersed in a matrix of pectins and hemicelluloses (Brumell & Harpster, 2001). Pectins, which are a structurally diverse group of heteropolysaccharides containing partially methylated D-galacturonic acid residues with side chain appendages of several neutral polysaccharides, are the principal components of the primary cell wall and middle lamella, and thus contribute to fruit texture.
Fruit texture depends on cell integrity and can be ascribed to both the strength of the primary wall and the wall-to-wall adhesion between cells. The latter is considered to be the most critical factor influencing the perception of fruit texture and is dictated by the strength of the middle lamella, the area of cell-to-cell contact and the extent of plasmodesmatal connections (Harker & Hallett, 1994). The mechanical properties of the cell wall mainly depend on the structure of the polysaccharide matrix and the degree of molecular ordering of cellulose (e.g., the ratio of crystalline to amorphous cellulose). For example, the brittle, crisp texture of apples derives from the presence of a largely crystalline form of cellulose (Redgwell & Fischer, 2002).

Fruit softening during ripening results from the hydrolytic breakdown of cell-wall polymers (e.g., celluloses, hemicelluloses, pectins) by hydrolases (e.g., polygalacturonase [PG], pectin methyl esterase, pectate lyase, rhamnogalacturonase, cellulase, β-galactosidase). Softening is further enhanced by expansins, cell wall-localized proteins with no hydrolytic enzymatic activity (Cosgrove, 2000), which break down the multiple polysaccharide networks, thus increasing the accessibility of hydrolases (such as PG or cellulase) to cell wall polymers (Payasi et al., 2009).

The role of the cell wall-modifying enzymes in different fruit species varies due to differences in cell wall composition, the nature, timing, and extent of modification of the cell wall polysaccharides (Wakabayashi, 2000; Brummell & Harpster, 2001). Moreover, there is no obvious correlation between the primary structure of cell wall polysaccharides in different fruit and their texture at maturity (Redgwell & Fischer, 2002). Although the microfibrillar cellulose-xyloglucan network dissolves during fruit softening, immunolocalization studies in kiwi showed that cellulose remained intact and cellulose levels were unaffected by fruit ripening (Sutherland et al., 1999). Cellulose fibrils, however, undergo changes in their physical form and under the influence of expansins the cellulose-xyloglucan network is loosened (McQueen-Mason et al., 1992). Hemicelluloses also undergo solubilization and depolymerization, thus contributing to cell wall loosening and disintegration. In addition, the cleavage of covalent cross-links between either adjacent pectin molecules or pectin and other polysaccharides promotes the destabilization of the matrix (Payasi et al., 2009).

Based on the degree of softening during ripening, fruits can be classified into two groups: (1) those that soften to a melting texture (e.g., strawberry, kiwi, persimmon [Diospyros kaki L.], tomato, and avocado) and (2) those that soften only moderately and retain a crisp texture (e.g., apple, pear). These differences are ascribed primarily to differences in cell-to-cell adhesion, but changes in the internal wall structure are also implicated. In some fruits (e.g., avocado), the molecular weight of solubilized pectin decreases during ripening, but not in others (e.g., plum [Prunus americana Marsh.] and blackberry). Similarly, although the molecular weight of xyloglucan decreased during the softening of strawberry and melon, in tomato it remained either unchanged or decreased to varying degrees (Redgwell & Fischer, 2002).

Cell wall degrading enzymes may be defined as:

1. pectin degrading enzymes (e.g., pectin methyl esterase [PME], PGs, pectate lyase [PL or pectate transeliminase]) and
2. enzymes that degrade other polysaccharides such as cellulose, glucans, xyloglucans, galactosides, (e.g., cellulase [EGase or Endo-P-1,4-glucanase], xyloglucan endotransglycosylase [XET], β-galactosidase, glycosidas, and rhamnogalacturonases [RGs]).

Although PME is a major factor in strawberry and tomato fruit softening and its activity significantly increases during ripening, it is not the only component that determines the loss of firmness because during fruit development and before senescence, cellulases (Trainotti et al., 1999) and expansins (Civello et al., 1999) also contribute to cell wall degradation. In tomato, PME activity is
observed throughout fruit development and ripening and is transcribed by a multigene family (Gaffe et al., 1997). The major isoform PME2 is fruit specific and has a peak of activity at the mature breaker stage. De-esterification of pectins due to PME activity is a prerequisite for further pectin disassembly by PG. Although a reduction in PME2 isoform levels would be expected to promote the retention of fruit firmness, due to a reduction in the substrate availability for endo-PG-mediated hydrolysis, suppression of PME activity in tomato had in fact little effect on fruit firmness (Brummel & Harpster, 2001). In kiwi, ethylene application resulted in decreased esterification of pectins and a concomitant two- to threefold increase in PME activity (Wegrzyn & MacRae, 1992). However, it seems that PME action is not solely responsible for the de-esterification of pectins, which continued throughout fruit softening while PME activity decreased to undetectable levels.

Differences in softening between several varieties of freestone peaches are ascribed to PG activity at the late stages of ripening (Karakurt et al., 2000), and in tomato, peach, and avocado extensive pectin depolymerization is associated with high PG activity. However, PG is not necessarily the principal factor regulating fruit softening. In apples, persimmons, muskmelons, cherries, bananas, and peppers, cell wall breakdown and pectin solubilization during ripening are observed in the absence or at low levels of PG activity (Redgwell & Fischer, 2002). In addition, the correlation of pectin solubilization with PG activity is poor in many fruit species (Redgwell et al., 1997b). In tomatoes, antisense suppression of PG activity resulted in only a modest reduction of polyuronide depolymerization (Brummell & Labavitch, 1997) and a small increase in firmness later in ripening (Langley et al., 1994). Similarly, expression of a chimeric PG transgene in a nonsoftening tomato mutant that normally lacks this activity resulted in polyuronide solubilization, but did not restore softening (Giovannoni et al., 1989). In bananas, the concerted action of at least four PG genes, which are differentially expressed during ripening, is required for softening during ripening (Mehar & Nath, 2005). Additionally, PG activity seems to depend on the action of other pectinolytic mechanisms because it requires several contiguous galacturonosyl residues in the free acid form as substrates (Daas et al., 2000).

In strawberries, it has been shown that genes encoding PL are major factors controlling both pectin degradation and the loss of firmness during ripening (Medina-Escobar et al., 1997). These genes are strongly and predominantly expressed at the full ripe stage (Benitez-Burraco et al., 2003). Suppression of the PL mRNA during ripening in transgenic strawberry plants (Jiménez-Bermúdez et al., 2002) and antisense inhibition of pectate lyase gene expression (Sesmero et al., 2007) resulted in significantly firmer fruits at ripeness. In banana (Domínguez-Puigjaner et al., 1997) and mango (Chourasia et al., 2006), the expression of PG genes appears to be closely associated with pectin degradation and softening during ripening.

Cellulase, together with the pectic enzymes, plays an important role in fruit softening (Payasi et al., 2009) and is also involved in fruit abscission (Sexton et al., 1997). Cellulase activity increases during the ripening of strawberry, pepper, peach, olives, tomato, raspberry, and especially avocado (Redgwell & Fischer, 2002). Two cellulases, Cel1 and Cel2, show an increase in mRNA during ripening, but antisense suppression of either of these genes did not detectably affect tomato softening (Lashbrook et al., 1998).

Although XET has been implicated in ripening-related changes to the fruit cell wall of kiwi (Redgwell & Fry, 1993), tomato (Maclachlan & Brady, 1994), and persimmon (Cutillas et al., 1994), its role in relation to xyloglucan degradation is still obscure (Payasi et al., 2009). However, XET activity and mRNA accumulation increase during ripening and show good correlation with the softening of tomato and kiwi (Arrowsmith & de Silva, 1995), but not apple (Percy et al., 1996). XET activity also seems to be regulated by ethylene because in kiwi its activity increased significantly after ethylene treatment (Percy et al., 1996) and in pear decreased after treatment with the inhibitor of ethylene perception, 2-Methylcyclopropene (1-MCP) (Hiwasa et al., 2003).
In fruits, β-galactosidase acts as a constitutive enzyme for the turnover of cell wall-associated galactose during growth and maturation and subsequently as a catalyst for the degradation of galactose-containing polysaccharides during senescence (Redgwell & Fischer, 2002; Payasi et al., 2009). During ripening, up to 70% of the cell wall galactose is lost, mainly due to the solubilization of the pectic polysaccharides and by cleavage from the galactan side-chains (Redgwell et al., 1997a). β-galactosidases isolated from kiwi (Ross et al., 1993), papaya (Ali et al., 1998), and tomato (Carey et al., 1995) have been shown to remove galactose residues from a range of pectic and hemicellulosic polysaccharides. Both the suppression of activity (Brummel & Harpster, 2001) and the down-regulation of β-galactosidase (Smith et al., 2002) during the early stages of tomato fruit ripening significantly reduced fruit softening, indicating the importance of pectic galactan side-chains for cell-wall integrity and fruit firmness. β-galactosidase may also catalyse polysaccharide depolymerization during the later stages of fruit softening (Carrington & Pressey, 1996).

The activity of glycosidases, such as β-hexosaminidase, α-mannosidase, and α-galactosidase, increases during the later stages of tomato ripening (Payasi et al., 2009), but in bell pepper, although the activity of β-hexosaminidase increased significantly during ripening, α-mannosidase activity was prominent during fruit development (Jagadeesh et al., 2004). Mannan transglycosylase, a cell wall enzyme that acts on mannan-based polysaccharides in the primary cell wall, has been detected in tomato fruits, and its activity increases during fruit ripening up to the red ripe stage (Schröder et al., 2004).

There is evidence that highly galactosylated polymers exist in distinct domains within the cell wall (Roberts, 2001) and their depolymerization by rhamnogalacturonase or β-galactosidase may produce quite different changes in wall integrity compared with those resulting from endo-PG-mediated depolymerization of homogalacturonan pectin zones (Redgwell & Fischer, 2002). RGase A is present in Aspergillus aculeatus as well as in fruit (e.g., apples, grapes, tomatoes), suggesting that it is a potentially important hydrolase both in fruit softening and fungal decay (Gross et al., 1995).

Expansins are small proteins (28 kDa) that are directly correlated with fruit softening and cell wall degradation by increasing the accessibility of cell wall-modifying enzymes to structurally important cell wall polymers, although they themselves have no hydrolytic enzymatic activity (Cosgrove, 2000). Ripening-related expansin protein concentration has been found to closely correlate with fruit softening during ripening (Payasi et al., 2009) and may restrict or control the activities of ripening-related enzymes involved in fruit softening (Brummel & Harpster, 2001). During the course of fruit ripening, it is likely that EXP1 protein acts on the first component of softening, the loosening of noncovalent linkages between unidentified polymers in the hemicellulose–microfibril matrix, to give PG or other enzymes access to polyuronide substrate sites before senescence (Redgwell & Fischer, 2002). However, expansins are not responsible for the second and major component of softening, the depolymerization of structurally important hemicelluloses.

### The Metabolism of Volatiles that Contribute to Fruit Aroma

Fruit aroma is derived from volatile components within the fruit tissues that are present in concentrations that can be perceived by the human nose (Baldwin, 2002). Aroma is not only important for the actual scent of the fruit but also for the appreciation of flavor. Although the aroma of some fruits depends largely on a single volatile compound (e.g., 3-methylbutylacetate in banana or γ-undecalactone (the so-called “peach aldehyde”) in peach (Berger, 1991), in other fruits such as tomato (Krumbein & Auerswald, 1998), citrus (Shaw, 1991), and apple (Cunningham et al., 1985),
a multitude of compounds contribute to aroma, with no one compound alone being responsible for the characteristic aroma of the fruit.

The typical flavor and aroma of most fruits develop at the later stages of ripening, but decrease with senescence, and are often coupled with ethylene synthesis in climacteric fruits such as tomato (Baldwin et al., 1991), apples (Fan & Mattheis, 1999), and melons (Wyllie et al., 1996). Volatile compounds are formed during this period from major plant constituents through various biochemical pathways and although concentrations may vary with variety, the trends of changes are generally similar (Krumbein et al., 2004). The most important volatiles in fruits belong to the following chemical groups: aldehydes, esters, ketones, terpenoids, and sulfur-containing substances (Gomez & Ledbetter, 1997). In tomato, heavy rain before harvest reduced volatile levels due to dilution from excess water (Baldwin et al., 1995), whereas the application of nitrogen and potassium fertilizers increased several volatiles (Wright & Harris, 1985).

In most cases, the flavor or aroma of fruits after cutting, maceration, chewing, and mild heat treatment, seriously differs from that of the intact fruit, due to the release of aroma compounds upon cell disruption, and the formation of new substances when enzymes and substrates, that were formerly compartmentalized, come into contact (Buttery, 1993). Cooking for a long time or at high temperature can result in the formation of a whole new group of volatile flavor compounds that usually result from the breakdown of carbohydrates, proteins, lipids, and carotenoids, and which may completely mask key flavor or aroma compounds (Christensen et al., 2007). Some volatiles are glycosylated and only exert a sensory effect when released from the sugar, as in grapes, which show an increase in free and glycosylated aroma compounds at the end of the ripening period (Coombe & McCarthy, 1997).

Aroma compounds formed during ripening include fatty acid derivatives, terpenes, and phenolics, whereas those formed during tissue damage through enzymatic degradation or autoxidation reactions of primary or secondary metabolites, include lipids, amino acids, glucosinolates, terpenoids, and phenolics (Christensen et al., 2007).

Aldehydes are important to the aroma of tomato, cucumber, peppers, and other fruiting vegetables (Kays, 1991; Krumbein & Auerswald, 1998). They are formed from linoleic and linolenic acids via the lipoxygenase (LOX) pathway in which LOX together with hydroperoxide lyase (HPL) and a hydroperoxy cleavage enzyme convert linoleic (18:2) and linolenic (18:3) acids to hexanal and cis-3-hexenal, respectively, via 9- and 13-hydroperoxy-C18:2 and -C18:3 intermediates. Hexanal and cis-3-hexenal can then be reduced to hexanol and cis-3-hexenol, respectively, whereas isomerization of cis-3-hexenal to trans-2-hexenal can occur, either enzymatically or nonenzymatically (Riley et al., 1996).

The LOX pathway is also responsible for the conversion of C1 to C20 fatty acids to alcohols, which are in turn converted to esters using acetyl-CoA and alcohol acyltransferase (AAT), as in apple, banana, melon, and strawberry. Lactones, which are important for peach flavor, are formed by LOX (Baldwin, 2002). Fatty acids are also degraded during ripening by β-oxidation, resulting in saturated and unsaturated lactones, esters, alcohols, ketones, and acids (Aguedo et al., 2004).

Amino acids are involved in volatile synthesis. Leucine, isoleucine, or valine is converted into branched chain alcohols and esters in muskmelon (Yabumoto et al., 1977) and banana (Tressl & Drawert, 1973). In tomatoes, valine is reported to be a precursor of 1-N-2-methylpropane, 3-methylbutan-1-ol, 3-methylbutanol, 3-methylbutylnitrile, 1-N-3-methylbutane, 3-methylbutyric acid, and 2-isobutylthiazole (Buttery & Ling, 1993); isoleucine is a precursor of 2-methylbutanol and 2-methylbutyric acid; and phenylalanine is a precursor of phenylacetaldehyde, 2-phenylethanol, 1-N-2-phenylethane, and phenylacetonitrile. Isoleucine and alanine are converted into esters in apple (Hansen & Poll, 1993) and strawberry (Peréz et al., 1992), respectively. Similarly,
phenylalanine is a precursor of phenylethanol, phenethylacetate, phenethylbutanoate, and phenolic ethers in banana (Tressl & Drawert, 1973).

Aromatic terpenes including limonene, valencene, γ-terpinene and 3-carene, α-copaene, α- and β-pinene, and myrcene are important aroma components of citrus fruits (Wilson & Shaw, 1981) and mango (Malundo et al., 1996). Oranges also contain enzymes for the conversion of mevalonic acid to isopentenyl pyrophosphate and dimethylallyl pyrophosphate and for the synthesis of linalool and its cyclization to 2,8-menthadiene-1-ol, α-terpineol, and D-limonene (Baldwin, 2002).

Some important volatile compounds in tomato are thought to be breakdown products of pigments such as lycopene and other carotenoids (Buttery & Ling, 1993). These compounds include β-ionone, cyclocitral, and β-damascenone from cyclic carotenoids, and geranylacetone, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and pseudoionone from open chain carotenoids (Buttery & Ling, 1993).

**Pigment Metabolism and Fruit Color Changes**

In many fruits, color is the single most important indicator of quality and color defects usually indicate deterioration or inappropriate pre- or postharvest practices. The main pigments conferring color on fruits are classified as chlorophylls, carotenoids, and anthocyanins (Artés et al., 2002).

Carotenoids, tetraterpenes that give plant tissues a red-yellow color, are either carotenes (hydrocarbons) or xanthophylls (derived from carotenes with additional oxidation). At the early stages of fruit development, carotenoids are located with chlorophyll mainly in the form of chlorophyll-carotenoid-protein complexes within the chloroplasts and assist in photosynthesis. However, during ripening, carotenoids accumulate in high amounts in the chromoplasts (transformed chloroplasts) in lipid-rich structures, the plastoglobules (e.g., in fruits of the genus Capsicum), or in cases of overproduction, they form pigment, crystals (carotenoid esterification) within the chromoplast, without affecting the chromophore properties of the pigment, as in tomato, carrot, or pumpkin (Tucker, 1993). Changes in the esterification profile of the xanthophylls of red peppers have been proposed as a maturity index (Hornero-Méndez & Mínguez-Mosquera, 2000).

Fruit carotenoids show great diversity and exotic structures may be found. For example, in citrus fruits apocarotenoids (e.g., citaurin, citranaxanthin) are observed (Farin et al., 1983). In Capsicum annuum more than 25 carotenoids have been detected (Matus et al., 1991), although the typical color of many fruits is derived from only a small number of carotenoids (e.g., lycopene in tomato, cryptoxanthin, and zeaxanthin in mango and persimmon, capsanthin and capsorubin in pepper). Fruit peel contains higher amount of carotenoids than the pulp, and in some fruits (e.g., tomato and peach), the carotenoid concentration increases after harvest (Vicente et al., 2009). During the ripening of tomatoes, oranges, mangoes, and other fruits, carotenoids are synthesized in chromoplasts, derived from chloroplasts on chlorophyll breakdown. However, in some fruit (e.g., grapefruit) carotenoid synthesis may occur even before the initiation of chlorophyll disappearance, whereas in some early orange or mandarin varieties, fruit maturity at high temperatures is not associated with a higher carotenoid concentration because chlorophyll degradation is inhibited.

In tomatoes, the concentration of β-carotene increases during ripening both on the plant and after harvest until just before full color development, whereas the parallel formation of acyclic carotene phytoene from genarygenaryl pyrophosphate conversion results in lycopene accumulation and enhanced red color (Hobson & Grierson, 1993). In peppers, the red coloration relates to the conversion of xanthophyll epoxides to the ketoxanthophylls capsanthin and capsorubin during the transformation of chloroplasts into chromoplasts (Camara et al., 1995).
Carotenoid formation in ripening fruits is affected by environmental factors both before and after harvest. These include temperature, light, and the oxygen concentration within the atmosphere. For example, lycopene synthesis in tomato is inhibited by temperatures higher than 30°C, whereas in red watermelons inhibition is not observed up to 37°C. On the other hand β-carotene can be synthesized even at temperatures as high as 40°C. High nitrogen application favors carotene synthesis (Mozafar, 1993), whereas foliar application with gibberellins can inhibit carotene and xanthophyll synthesis in citrus. Nitrogen application has also been shown to increase the vitamin B1 content of fruit tissues.

Anthocyanins, the most important group of water-soluble pigments in plant tissues, are phenolic compounds with diverse chemical structure, localized within the vacuole of the plant cell, giving rise to colors from red to blue-purple or even black (Artés et al., 2002). They are present mainly in mature epidermal cells (e.g., apple, apricot, eggplant [Solanum melongena L.], fig, nectarine, peach, pear, plum, and pomegranate) as well as in the flesh (e.g., apple, blackberry, blueberry, cherry, cranberry, red- and black-currant, fig, grape, peach, plum, pomegranate, olive, ‘Sanguine’ orange, raspberry, strawberry) (Tomás-Barberan & Espín, 2001). The occurrence and accumulation of anthocyanins vary with the fruit species, cultivar, tissue structure, geographical location, position of the fruit on the tree, and cultivation conditions (Artés et al., 2002). For example, the total amount of anthocyanins in the juice of pomegranate cv. ‘Mollar’ fruits picked from the external part of the tree and that show a reddish husk are usually 60% lower than those in juice from fruits located within the canopy and that show a yellowish husk (Gil et al., 1995b). Moreover, pomegranates grown in low nutrient status soils with high salt concentrations produced less anthocyanin than those cultivated in more fertile soils, although the same profile and relative amounts of anthocyanins were found (Gil et al., 1995a). Nasunin (delphinidin 3-p-coumarylhamnoside) is the major anthocyanin within the peel of purple, but not white or green, eggplant varieties (Tateyama & Igarashi, 2006). It has been isolated in crystalline form and shows strong antioxidant properties (Passam & Karapanos, 2008).

Based on the mechanism of color change and the pigment composition at ripeness, fruits can be classified as follows (Tucker, 1993; Artés et al., 2002):

1. Fruits that lose all their chlorophylls, unmasking previously synthesized carotenes and xanthophylls and usually having a characteristic yellow color (e.g., banana, plantain, lemon).
2. Fruits with marked de novo biosynthesis of carotenoids, referred to as carotenogenic fruits (e.g., tomato, red pepper, orange, persimmon).
3. Fruits with marked de novo biosynthesis of anthocyanins (e.g., grape, apple, olive, pomegranate, red cherry, raspberry, cranberry).
4. Fruits that retain chlorophyll during ripening (e.g., kiwi, avocado, green cultivars of red pepper, green fleshed tomato).

The loss of chlorophyll and the biosynthesis of pigments are probably independent processes because in the tomato ripening mutant greenflesh (gf), with impaired chlorophyll degradation, normal carotenoid biosynthesis is observed during ripening (Tucker, 1993). Although chlorophylls are naturally decomposed during the photosynthesis process, in most fruits the rate of chlorophyll loss greatly increases from the onset of ripening, so that at full ripeness no chlorophyll appears (Artés et al., 2002). Carotenoids are derived from isopentenyl diphosphate (IPP) via the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway rather than the mevalonic acid pathway as was assumed for many years, although both pathways produce IPP (Bramley, 2002). IPP is isomerized by IPP isomerase to its allylic isomer dimethylallyl diphosphate (DMAPP), the activated substrate for the formation of the C20 geranylgeranyl diphosphate (GGPP) by GGPP synthase, and two molecules
of this are joined tail-to-tail by phytoene synthase (PSY), to give 15-cis phytoene as the first product with the C40 carotenoid skeleton (Artés et al., 2002; Bramley, 2002). Of the two isoforms of the Psy gene (Psy-1 and Psy-2), which are found in tomato, only Psy-1 has a role in carotenogenesis in ripening fruit (Fraser et al., 1999). Phytoene, which is colorless, is further desaturated to give the more familiar carotenoid pigments. The end-product is lycopene, produced via the successive intermediates phytofluene, ζ-carotene and neurosporene (Artés et al., 2002).

Xanthophylls are formed by the oxygenation of carotenes, typically by the addition of hydroxyl, epoxy, or keto groups. Hydroxylation and oxygenation of α-carotene leads to the formation of the major leaf xanthophyll lutein, and β-carotene to the formation of zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, and so on (Hirschberg, 2001).

Carotenogenesis in ripening fruit is controlled by regulatory mechanisms that are distinct from those in photosynthetic tissues (Thelander et al., 1986). In tomato, the concentration of carotenoids increases 10- to 14-fold in the course of ripening, due mainly to the accumulation of lycopene. Lycopene begins to appear at the breaker stage of ripening, together with a higher expression of isoprenoid genes, especially DOXP synthase (Lois et al., 2000), an increase in the mRNA levels of Psy-1 and Pds (Fraser et al., 1994) and the disappearance of the mRNAs of both lycopene cyclases (Lcy-β and -ε) (Ronen et al., 1999).

Anthocyanins are produced by the transformation of dihydroflavonols through the action of dihydroflavonol 4-reductase (DFR) to produce flavan-3,4-cis-diols (leucoanthocyanidins). The various leucoanthocyanidins are further oxidated, dehydrated, and glycosylated, resulting in pelargonidin (brick-red), cyanidin (red), and delphinidin (blue) anthocyanin pigments, respectively (Holton & Cornish, 1995). In apples, the concentration of red anthocyanin pigments in the skin increases up to threefold during ripening. Both anthocyanin concentration and the activity of PAL were found to be induced by the initiation of endogenous ethylene production, or the application of exogenous ethylene to unripe fruits (Faragher & Brohier, 1984).

Respiration in Relation to Fruit Metabolism and Ripening

Respiration is a property of all living cells and is central to the metabolic processes involved in fruit development, maturation, and senescence. The rate of respiration is an indicator of the rate of metabolism and therefore the speed with which the metabolic changes associated with ripening proceed (Kays, 1991). It thus indicates the potential storage life and relative perishability of fresh fruit and is a guide to the storage conditions that should be employed after harvest. In general, the higher the respiration rate (e.g., banana, avocado) the shorter its postharvest life (Kader, 2002).

Kidd and West (1925) first introduced the term climacteric to describe the rise in respiration rate that accompanied the maturation phase in apple. Subsequently, fruits were defined as climacteric or non-climacteric on the basis of the presence or absence of a respiratory rise during ripening (Biale & Young, 1981). However, respiration rate itself is not a criterion for characterizing a fruit as climacteric or non-climacteric because there are climacteric fruits with low respiration rates (5–10 ml CO₂ kg⁻¹ h⁻¹ at 5°C [apple, kiwifruit, papaya, and pear]) and nonclimacteric ones with high respiration rates (20–30 ml CO₂ kg⁻¹ h⁻¹ at 5°C [blackberry, raspberry, and strawberry]) (Kader, 2002). Hence, the presence or absence of a climacteric is determined on the occurrence of autocatalytic ethylene production rather than respiration per se.

Nonclimacteric fruits produce small quantities of ethylene, and they respond to ethylene treatment by a transient increase in respiration rate the magnitude of which depends on the concentration and duration of ethylene application (Kays, 1991). Ethylene treatment does not induce ripening, but
it will promote the breakdown of chlorophyll and so can be used for degreening (e.g., in citrus). Moreover, in some nonclimacteric fruits (e.g., strawberry) an increase in ethylene production and a concomitant rise in respiration rate have been observed at the red ripe stage and may relate to changes in cellular integrity and senescence (Iannetta et al., 2006). Similarly, in citrus (also nonclimacteric) some autocatalytic ethylene synthesis is observed in harvested immature fruits, possibly due to injury during the detachment of fruits from the trees (Barry & Giovannoni, 2007). Neither of these responses to ethylene, however, relate to the climacteric, which is associated with the induction of ripening.

Climacteric fruits produce much larger quantities of ethylene at the onset of ripening, and exposure to ethylene treatment will result in faster and more uniform ripening (Kader, 1999). In climacteric fruit, the exogenous application of ethylene will advance the timing of the climacteric, autocatalytic production of endogenous ethylene will continue after the removal of the exogenous source, and the magnitude of the respiratory rise is independent of the concentration of applied ethylene above a threshold level (Watkins, 2002).

This difference in ethylene associated ripening induction has far-reaching consequences for fruit harvesting, handling, and marketing. Nonclimacteric fruits do not ripen after removal from the plant and so are harvested at the stage of commercial ripeness. In contrast, climacteric fruit that have completed their growth (i.e., are physiologically mature) can be harvested, stored, and subsequently ripened. For example, bananas are harvested green, transported to market, and treated with 10–100 ppm ethylene at their destination to initiate ripening. Similarly, ethylene may be applied to avocado, kiwi, mango, melon, nectarine, papaya, peach, pear, persimmon, and plum (Knee et al., 1985) and ripening before marketing is increasingly used to provide consumers with the choice of purchasing ready-to-eat ripe fruits or fruits that can be ripened at home. Nonclimacteric fruits include strawberry, cherry, citrus (grapefruit, lemon, lime [Citrus aurantifolia {Christm.} Swingle], orange, mandarin and tangerine [Citrus × tangerina Tanaka]), cucumber, grape, lychee (Litchi chinensis Sonn.), olive, pineapple, pomegranate, and watermelon, whereas climacteric fruits include apple, apricot, avocado, banana, cantaloupe and honeydew melons (Cucumis melo L. inodorus group), fig, pear (European and Chinese [Pyrus bretschneideri R.]), guava [Psidium spp. L.], nectarine, peach, plum, kiwifruit, mango, papaya, tomato, and cherimoya [Annona cherimola Mill.] (Kader, 2002; Watkins, 2002). In pepper, although most cultivars are non-climacteric a few exhibit a climacteric tendency (Villavicencio et al., 1999; Passam & Karapanos, 2008), whereas kiwi shows a differential climacteric behavior related to temperature (climacteric when ripened at 20°C, but not at 10°C) (Antunes et al., 2000).

Apples stored under modified or controlled atmospheres are more susceptible to low O₂ or high CO₂ injuries (e.g., internal browning) when harvested at later stages of ripening (Lau, 1997) and this susceptibility is increased in fruit from trees with a light load (Elgar et al., 1999). Similarly, responses to altered gas concentrations in storage, possibly arising from respiration, are associated with maturity, cropping factors, skin, and gas diffusion properties (Ferguson et al., 1999).

The Role of Ethylene in Fruit Ripening and Quality

All fruits (both climacteric and non-climacteric) produce ethylene during ripening and two distinct ethylene biosynthesis pathways have been described (McMurchie et al., 1972): System 1, common to both climacteric and nonclimacteric fruit, is responsible for low ethylene production in the preclimacteric period of climacteric fruit and is responsible throughout the development and ripening of nonclimacteric fruit. It is also responsible for increased biosynthesis of ethylene in both climacteric and
nonclimacteric fruit in response to wounding. System 2 is unique to climacteric fruit and is responsible for the autocatalytic increase in ethylene production accompanying the ripening of these fruit.

Ethylene is synthesized from methionine in three steps: (1) conversion of methionine to S-adenosyl-L-methionine (SAM; [AdoMet]) catalyzed by the enzyme SAM synthetase, (2) formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS) and (3) conversion of ACC to ethylene, catalyzed by ACC oxidase (ACO). The formation of ACC also leads to the production of 5'-methylthioadenosine (MTA), which is recycled via the methionine cycle to yield a new molecule of methionine. Due to the methionine cycle, increased ethylene production does not necessarily require high levels of intracellular methionine, even though increased respiration is needed to provide ATP (Alexander & Grierson, 2002). The genes encoding ACS and ACO have been studied in more detail than those for other enzymes in the ethylene synthesis pathway, although there is evidence that several other genes involved in methionine synthesis and the methionine salvage pathway are differentially expressed during ripening and in response to ethylene (Alba et al., 2005).

Regardless of its production, the biological activity of ethylene requires the presence of receptors, unsaturated bonds adjacent to a terminal carbon atom in the ligand, which are bound to a metal-containing receptor site. CO₂ is considered to be an inhibitor of ethylene perception by displacing ethylene from these receptor sites, whereas oxygen appears to promote ethylene binding to the receptor sites (Watkins, 2002).

Although the involvement of ethylene and its receptors in the ripening of climacteric fruits has been reaffirmed (Klee, 2004), other hormones and ethylene-independent pathways are also involved in the ripening process. For example, in strawberries, grapes, and citrus, auxins have been shown to retard fruit ripening (Trainotti et al., 2005).

The ethylene concentrations required to affect fruit physiology vary with species and maturity. Threshold, half-maximal, and saturating doses are 0.01, 0.1, and 10 μl l⁻¹ respectively (Abeles et al., 1992) and the senescence of mature fruit is promoted by an ethylene concentration of 0.1 μl l⁻¹ (Knee et al., 1985). The fact that ethylene is potentially effective even at nl l⁻¹ levels means it is crucial for the postharvest handling of fruits, since Wills et al. (2000) found average ethylene concentrations of 0.06 μl l⁻¹ in wholesale markets and distribution centers, 0.017 to 0.035 μl l⁻¹ in supermarkets, 0.029 μl l⁻¹ in domestic refrigerators without apples, and 0.20 μl l⁻¹ in refrigerators with apples. It was estimated that the ethylene present in market areas could reduce the storage life of nonclimacteric fruit by 10 to 30% (Watkins, 2002).

Apart from promoting ripening in climacteric fruit, the accumulation of ethylene is associated with enhanced senescence and decreased postharvest life. Ethylene can also increase susceptibility to chilling injury, decay associated with stem-end rots and the incidence of off-flavors. Hence, the commercial management of fruit will vary greatly depending on the commodity and whether ethylene action is to be promoted or delayed. Ethylene is increasingly used to ensure fresh fruit uniformity in the market, as well as to promote a uniform product for processors (Watkins, 2002).

Numerous techniques have been applied with varying success to reduce the internal ethylene concentration of fruit during storage and prior to ripening (e.g., by enhancing the rate of diffusion with low-pressure storage or inhibiting the synthesis or action of ethylene using cold storage or controlled or modified atmospheres), together with the avoidance of ethylene accumulation around fruits by ventilation, adsorption on carbon, oxidation by permanganate, ozone, UV, or catalysts (Saltveit, 1999). For instance, the internal ethylene concentrations of apples must be less than 0.1 ml l⁻¹ to delay initiation of softening (Stow et al., 2000), whereas ethylene concentrations in the storage atmosphere of <2 ml l⁻¹ are necessary to retard softening and superficial scald (Knee & Hatfield, 1981). Additionally, the deleterious effects of ethylene on fruit senescence during storage may be
restricted by blocking ethylene receptors. It is believed that low O₂ and high CO₂ levels during modified or controlled atmosphere storage extend the storage life of many crops, mainly due to the inhibition of ethylene action, rather than the inhibition of respiration. Although low O₂ restricts ethylene production (Abeles et al., 1992), elevated CO₂ acts as an ethylene receptor blocker. 1-MCP, an unsaturated cyclic olefin, may bind to the ethylene receptor and so compete with ethylene for the available binding sites. The concentration of 1-MCP required to saturate the ethylene binding sites is greatly influenced by the species, cultivar, tissue, and mode of ethylene biosynthesis induction, as well as by temperature and the stage of fruit maturity, with later harvested fruits being less responsive to 1-MCP (Watkins, 2002). In tomato, 1-MCP delays fruit ripening (Guillén et al., 2007) and also prevents fruit abscission, which is important for cherry tomatoes that are marketed in trusses (Lichter et al., 2006).

Molecular techniques have led to the development of transgenic fruits with inhibited ethylene formation, either by reducing the availability of the ethylene precursor ACC by down-regulating the enzyme ACC synthase or expressing an enzyme that removes ACC, or by inhibiting the enzyme ACC oxidase that converts ACC to ethylene (Seymour & Manning, 2002). For example, the inhibition of ethylene biosynthesis in melon by antisense down-regulation of ACC oxidase, resulting in delayed ripening of fruits on the plant, improved flavor and increased storage-life (Ayub et al., 1996). However, irrespective of the value of ethylene application techniques to fruit ripening, it is an incontrovertible fact that fruits (e.g., tomatoes) ripened on the mother plant invariably have better flavor and aroma characteristics than those that are ripened after harvest. For example, apple fruit flavor is intensified by late harvest (Cliff et al., 1998) and both the tomato-like flavor of tomatoes and the level of volatiles are increased (Maul et al., 1998); mangos, too, have better aroma when harvested more mature (Baldwin et al., 1999).

Conclusion and Future Perspectives

The biological basis of fruit quality is a complex interchange of molecular, physiological, and biochemical processes including carbohydrate, protein and lipid metabolism, the synthesis and breakdown of pigments and structural components, and the formation of volatiles, that all contribute to the development, maturation or ripening, and senescence of the fruit. A deficiency in any one or more of these processes can adversely affect fruit quality through an alteration in fruit composition, texture, aroma, or appearance. True quality is not adequately expressed simply by the product’s appearance, as in current market quality standards, but by a combination of optical, textural, and organoleptic values: shape, size, color, sheen, texture, odor, flavor, and more.

Although considerable advance in our knowledge of the physiology and biochemistry of fruit ripening has been achieved over recent decades, there are still many areas that require further research. In particular, although ethylene is central to ripening and senescence the molecular basis of ethylene action is still far from clear, as is the role of other hormones and their likely interactions. Even though the terms climacteric and non-climacteric are so frequently employed, the molecular basis for this distinction is relatively obscure. Despite innumerable attempts to extend the postharvest life of fruits by manipulation of the storage environment or the application of chemical or physical treatments (Passam et al., 2007), to what extent are they practically applicable? Breeders have performed wonders in improving yields, resistance to disease, and the extension of storage life. But how significant are these advances for fruit quality? How often today do we hear market complaints that cucumbers or tomatoes no longer taste like they used to, and why has the fragrance of old tomato or strawberry varieties been lost?
Nowadays there is increasing consumer awareness that all that glitters is not gold, that the current obsession with market appearance and uniformity does not guarantee high flavor and aroma, which are the biological basis of true quality. It is our opinion that research must seriously take into account these market preferences. In developed markets there is an increasing demand for naturally ripened fruit, not to mention organic produce. Research must aim at improving flavor, aroma, and vitamin and antioxidant levels. Although breeding for ethylene control of ripening will continue (e.g., to enable year-round availability of tomatoes from a single season crop), this should not be done at the expense of quality, and in setting our objectives for future research, we should not forget that while EU and U.S. agricultural policies and quality standards have led to the massive dumping of fruit and other agricultural produce, there is an ever increasing population elsewhere in our world that is undernourished and ill-fed and to whom research and development should also be addressed.

References


INTRODUCTION


INTRODUCTION


