CHAPTER 7

Ripening and Senescence

Enriqueta Alós, Maria Jesus Rodrigo and Lorenzo Zacarias
Instituto de Agroquímica y Tecnología de Alimentos, Consejo Superior de Investigaciones Científicas (IATA-CSIC), Valencia, Spain

7.1 INTRODUCTION

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

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

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

7.2 REGULATION OF FRUIT RIPENING

7.2.1 Climacteric and Nonclimacteric Ripening

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

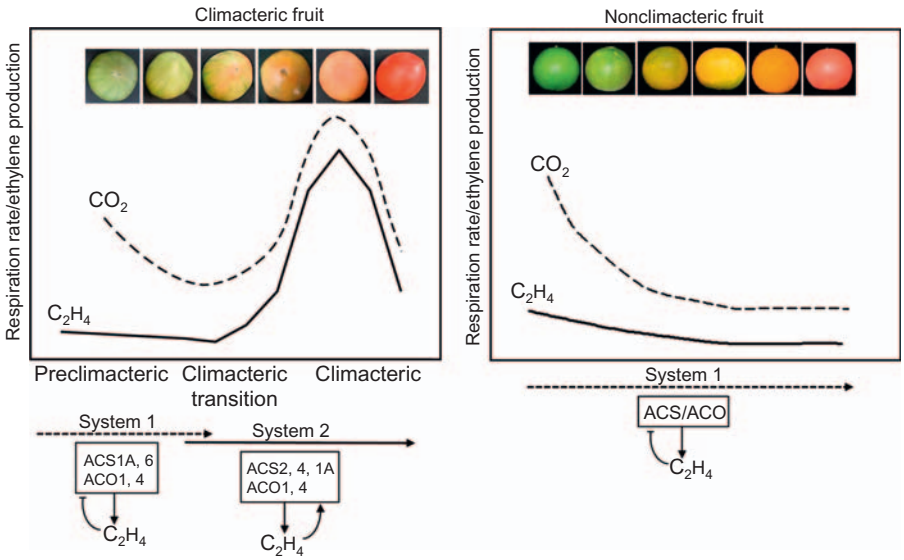

FIGURE 7.1

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

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

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

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

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

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

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

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

7.2.2 Ethylene Biosynthesis and Perception

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

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

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

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

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

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

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

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

7.2.3 Transcriptional and Epigenetic Regulation

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

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

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

7.2.4 Interaction With Plant Hormones

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

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

7.3 ETHYLENE-DEPENDENT AND -INDEPENDENT FRUIT-RIPENING EVENTS

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

7.4 CHANGES IN FRUIT COLOR

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

7.4.1 Chlorophylls

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

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

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

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

7.4.2 Carotenoids

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

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

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

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

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

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

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

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

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

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

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

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

7.4.3 Anthocyanins

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

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

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

7.5 FRUIT SOFTENING

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

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

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

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

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

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

7.6 FLAVOR AND AROMA VOLATILES

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

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

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

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

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

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

7.7 ORGANIC ACIDS

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

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

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

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

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

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

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

7.8 CARBOHYDRATE METABOLISM

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

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

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

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

7.9 VITAMINS

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

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

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

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

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

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

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

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

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

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

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

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REFERENCES

Ahrazem, O., Gómez-Gómez, L., Rodrigo, M.J., Avalos, J., Limón, M.C., 2016. Carotenoid cleavage oxygenases from microbes and photosynthetic organisms: features and functions. Int. J. Mol. Sci. 126, 1781.

Alós, E., Roca, M., Iglesias, D.J., Mínguez-Mosquera, M.I., Ornero, D., Thannhauser, T.W., et al., 2008. An evaluation of the basis and consequences of a stay-green mutation in the navel negra citrus mutant using transcriptomic and proteomic profiling and metabolite analysis. Plant Physiol. 147, 1300–1315.

Alós, E., Rodrigo, M.J., Zacarías, L., 2014. Differential transcriptional regulation of l-ascorbic acid content in peel and pulp of citrus fruits during development and maturation. Planta 239, 1113–1128.

Alquézar, B., Zacarías, L., Rodrigo, M.J., 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene β-cyclase from *Citrus* and its relation to lycopene accumulation. J. Exp. Bot. 60, 1783–1797.

Alquézar, B., Rodríguez, A., de la Peña, M., Peña, L., 2017. Genomic analysis of terpene synthase family and functional characterization of seven sesquiterpene synthases from *Citrus sinensis*. Front. Plant Sci. 8, 1481.

Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R., Nath, P., 2010. Ripening of fleshy fruit: Molecular insight and the role of ethylene. Biotech. Adv. 28, 94–107.

Barry, C.S., Giovannoni, J.J., 2007. Ethylene and fruit ripening. J. Plant Growth Regul. 26, 143–159.

Bramley, P.M., 2013. Carotenoid biosynthesis and chlorophyll degradation. In: Seymour, G.B., Poole, M., Giovannoni, J.J., Tucker, G.A. (Eds.), The Molecular Biology and Biochemistry of Fruit Ripening. Wiley, Hoboken, pp. 76–112.

Britton, G., 1998. Overview of carotenoid biosynthesis. In: Britton, G., Liaaen Jensen, S., Pfander, H. (Eds.), Biosynthesis and Metabolism, vol. 3. Birkhäuser Verlag, Basel, pp. 13–148.

Britton, G., Khachik, F., 2009. Carotenoids in food: nutrition and health. In: Britton, G., Liaaen-Jensen, S., Pfander, H. (Eds.), Carotenoids, vol. 5. Birkhäuser, Basel, pp. 45–66.

Butelli, E., Garcia-Lor, A., Licciardello, C., Las Casas, G., Hill, L., Recupero, G.R., et al., 2017. Changes in anthocyanin production during domestication of citrus. Plant Physiol. 173, 2225–2242.

Cercós, M., Soler, G., Iglesias, D.J., Gadea, J., Forment, J., Talón, M., 2006. Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization. Plant Mol. Biol. 62, 513–527.

Cholet, C., Claverol, S., Claisse, O., Rabot, A., Osowsky, A., Dumot, V., et al., 2016. Tartaric acid pathways in *Vitis vinifera* L. (cv. Ugni blanc): a comparative study of two vintages with contrasted climatic conditions. BMC Plant Biol. 28, 144.

DellaPenna, D., Pogson, B.J., 2006. Vitamin synthesis in plants: tocopherols and carotenoids. Ann. Rev. Plant Biol. 57, 11–38.

Dismore, M.L., Haytowitz, D.B., Gebhardt, S.E., Peterson, J.W., Booth, S.L., 2003. Vitamin K content of nuts and fruits in the US diet. J. Am. Diet. Assoc. 103, 1650–1652.

Dudareva, N., Klempien, A., Muhlemann, J.K., Kaplan, I., 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 198, 16–32.

El Hadi, M.A., Zhang, F.J., Wu, F.F., Zhou, C.H., Tao, J., 2013. Advances in fruit aroma volatile research. Molecules 11, 8200–8229.

Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D., Bugaud, C., 2013. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J. Exp. Bot. 64, 1451–1469.

Famiani, F., Paoletti, A., Battistelli, A., Moscatello, S., Chen, Z.H., Leegood, R.C., et al., 2016. Phosphoenolpyruvate carboxykinase, pyruvate orthophosphate dikinase and isocitrate lyase in both tomato fruits and leaves, and in the flesh of peach and some other fruits. J. Plant Physiol. 1, 34–44.

FAOSTAT. 2014. http://www.fao.org/faostat/.

Furie, B., Bouchard, B.A., Furie, B.C., 1999. Vitamin K-dependent biosynthesis of gamma-carboxyglutamic acid. Blood 93, 1798–1808.
Gapper, N.E., McQuinn, R.P., Giovannoni, J.J., 2013. Molecular and genetic regulation of fruit ripening. Plant Mol. Biol. 82, 575–591.
Giovannoni, J., Nguyen, C., Ampofo, B., Zhong, S., Fei, Z., 2017. The epigenome and transcriptional dynamics of fruit ripening. Annu. Rev. Plant Biol. 68, 61–84.
Giuliano, G., 2017. Provitamin A biofortification of crop plants: a gold rush with many miners. Curr. Opin. Biotechnol. 44, 169–180.
Grierson, D., 2013. Ethylene and the control of fruit ripening. In: Seymour, G.B., Poole, M., Giovannoni, J.J., Tucker, G.A. (Eds.), The Molecular Biology and Biochemistry of Fruit Ripening. Wiley, Hoboken, pp. 43–73.
Gross, J., 1987. Pigments in Fruits. Academic Press, London.
Hanson, A.D., Gregory, J.F., 2011. Folate biosynthesis, turnover, and transport in plants. Ann. Rev. Plant Biol. 62, 105–125.
Hichri, I., Barrieu, F., Bogs, J., Kappel, C., Delrot, S., Lauvergeat, V., 2011. Recent advances in the transcriptional regulation of the flavonoid biosynthetic pathway. J. Exp. Bot. 62, 2465–2483.
Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, W., Shima, Y., Nakamura, N., et al., 2017. Re-evaluation of the *rin* mutation and the role of RIN in the induction of tomato ripening. Nat. Plants 3, 866–874.
Johnson, K.L., Gidley, M.J., Bacic, A., Doblin, M.S., 2017. Cell wall biomechanics: a tractable challenge in manipulating plant cell walls 'fit for purpose'. Curr. Opin. Biotechnol. 49, 163–171.
Klee, H.J., Giovannoni, J.J., 2011. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59.
Koch, K., 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr. Opin. Plant Biol. 7, 235–246.
Kumar, R., Khurana, A., Sharma, A.K., 2014. Role of plant hormones and their interplay in development and ripening of fleshy fruits. J. Exp. Bot. 65, 4561–4575.
Lado, J., Zacarías, L., Rodrigo, M.J., 2016. Regulation of carotenoid biosynthesis during fruit development. In: Stange, C. (Ed.), Carotenoids in Nature: Biosynthesis, Regulation and Function. Springer International Publisher, Switzerland, pp. 161–198.
Leegood, R.C., Walker, R.P., 2003. Regulation and roles of phosphoenolpyruvate carboxykinase in plants. Arch. Biochem. Biophys. 414, 204–210.
Liu, M., Pirrello, J., Chervin, C., Roustan, J.P., Bouzayen, M., 2015. Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol. 169, 2380–2390.
Lorence, A., Nessler, C.L., 2007. Pathway engineering of the plant vitamin C metabolic network. In: Verpoorte, R., Alfermann, A.W., Johnson, T.S. (Eds.), Applications of Plant Metabolic Engineering. Springer, pp. 189–209.
Marowa, P., Ding, A., Kong, Y., 2016. Expansins: roles in plant growth and potential applications in crop improvement. Plant Cell Rep. 35, 949–965.
Mazza, G., 2018. Anthocyanins in Fruits, Vegetables, and Grains. CRC Press, 135108660X, 9781351086608.
McMurchie, E.J., McGlasson, W.B., Eaks, I.L., 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237, 235–236.
Mellidou, I., Kanellis, A.K., 2017. Genetic control of ascorbic acid biosynthesis and recycling in horticultural crops. Front. Chem. 11 (5), 50.
Pech, J.C., Bouzayen, M., Latche, A., 2008. Climacteric fruit ripening: ethylene-dependent and independent regulation of ripening pathways in melon fruit. Plant Sci. 175, 114–120.
Quadrana, L., Almeida, J., Otaiza, S.N., Duffy, T., Corrêa da Silva, J.V., de Godoy, F., et al., 2013. Transcriptional regulation of tocopherol biosynthesis in tomato. Plant Mol. Biol. 81, 309–325.
Rambla, J.L., Granell, 2013. Biosynthesis of volatile compounds. In: Seymour, G.B., Poole, M., Giovannoni, J.J., Tucker, G.A. (Eds.), The Molecular Biology and Biochemistry of Fruit Ripening. Wiley, Hoboken, pp. 135–161.

Rodrigo, M.J., Alquezar, B., Alferez, F., Zacarias, L., 2012. Biochemistry of fruits and fruit products. In: Sinha, N.K., Sidhu, J.S., Barta, J., Wu, J.S.B., Cano, P. (Eds.), Handbook of Fruits and Fruit Processing. Wiley Blackwell, Oxford, UK, pp. 13–34.
Rodriguez-Casado, A., 2016. The health potential of fruits and vegetables phytochemicals: notable examples. Crit. Rev. Food Sci. Nutr. 56, 1097–1107.
Ruiz-May, E., Rose, J.K., 2013. Cell wall architecture and metabolism in ripening fruit and the complex relationship with softening. In: Seymour, G.B., Poole, M., Giovannoni, J.J., Tucker, G. A. (Eds.), The Molecular Biology and Biochemistry of Fruit Ripening. Wiley, Hoboken, pp. 163–187.
Schweiggert, R.M., Carle, R., 2017. Carotenoid deposition in plant and animal foods and its impact on bioavailability. Crit. Rev. Food Sci. Nutr. 57, 1807–1830.
Slewinski, T., 2011. Diverse transporters and their homologs in vascular plants: a physiological perspective. Mol. Plant 4, 641–662.
Spicher, L., Kessler, F., 2015. Unexpected roles of plastoglobules (plastid lipid droplets) in vitamin K1 and E metabolism. Curr. Opin. Plant Biol. 25, 123–129.
Su, L., Diretto, G., Purgatto, E., Danoun, S., Zouine, M., Li, Z., et al., 2015. Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. BMC Plant Biol. 15, 114.
Toivonen, P.M.A., 2016. Postharvest physiology of fruits and vegetables. In: Pareek, S. (Ed.), Postharvest Ripening Physiology of Crops. CRC Press, Boca Raton, FL, USA, pp. 49–80.
Uluisik, S., Chapman, N.H., Smith, R., Poole, M., Adams, G., Gillis, R.B., et al., 2016. Genetic improvement of tomato by targeted control of fruit softening. Nat. Biotechnol. 34, 950–952.
USDA, 2017. https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/.
Watkins, C., 2008. Overview of 1-methylcyclopropane trials and uses for edible horticultural crops. HortScience 43, 86–94.
Wheeler, G.L., Jones, M.A., Smirnoff, N., 1998. The biosynthetic pathway of vitamin C in higher plants. Nature 393, 365–369.
Xiao, Y.Y., Kuang, J.F., Qi, X.N., Ye, Y.J., Wu, Z.X., Chen, J.Y., et al., 2017. A comprehensive investigation of starch degradation process and identification of a transcriptional activator MabHLH6 during banana fruit ripening. Plant Biotechnol. J. 16, 151–164.
Zhong, S., Fei, Z., Chen, Y.R., Zheng, Y., Huang, M., Vrebalov, J., et al., 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat. Biotechnol. 31, 154–159.
Zhu, X., Chen, J., Qiu, K., Kuai, B., 2017. Phytohormone and light regulation of chlorophyll degradation. Front. Plant Sci. 8, 1911.

FURTHER READING

Owinio, O.W., Ambuko, J., 2006. Advances in ethylene signal transduction in fruits and vegetables. In: Pareek, S. (Ed.), Postharvest Ripening Physiology of Crops. CRC Press, Boca Raton, FL, USA, pp. 339–358.