1  Biochemistry of Fruit Ripening
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Introduction

This chapter is intended to provide an overview of the key metabolic and regulatory pathways involved in fruit ripening, and the reader is referred to more detailed discussions of specific topics in subsequent chapters.

The quality of fruit is determined by a wide range of desirable characteristics such as nutritional value, flavor, processing qualities, and shelf life. Fruit is an important source of supplementary diet, providing minerals, vitamins, fibers, and antioxidants. In particular, they are generally rich sources of potassium, folate, vitamins C, E, and K as well as other phytonutrients such as carotenoids (beta-carotene being a provitamin A) and polyphenols such as flavonols (Saltmarsh et al., 2003). A similar, but perhaps more disparate, group of nutrients is associated with vegetables. Thus nutritionists tend to include fruits and vegetables together as a single “food group,” and it is in this manner that their potential nutritional benefits are normally investigated and reported. Over the past few decades, the increased consumption of fruits and vegetables has been linked to a reduction in a range of chronic diseases (Buttriss, 2012). This has led the WHO to issue a recommendation for the consumption of at least 400 g of fruits and vegetables per day. This in turn has prompted many countries to issue their own recommendations regarding the consumption of fruits and vegetables. In Britain this has given rise to the five-a-day recommendation. A portion in the United Kingdom is deemed to be around 80 g; so five-a-day corresponds to about 400 g per day. Other countries have opted for different recommendations (Buttriss, 2012), but all recognize the need for increased consumption.

The rationale for the five-a-day and other recommendations to increase fruit and vegetable consumption comes from the potential link between high intake of fruits and vegetables and low incidence of a range of diseases. There have been many studies carried out over the last few decades. The early studies tended to have a predominance of case-control approaches while recently more cohort studies, which are considered to be more robust, have been carried out. This has given rise to many critical and systematic reviews, examining this cumulative...
evidence base, over the years which have sometimes drawn disparate conclusions regarding
the strength of the links between consumption and disease prevention (Buttriss, 2012). One
of the most recent (Boeing et al., 2012) has concluded that there is convincing evidence for
a link with hypertension, chronic heart disease, and stroke and probable evidence for a link
with cancer in general. However, there might also be probable evidence for an association
between specific metabolites and certain cancer states such as between carotenoids and cancers
of the mouth and pharynx and beta-carotene and esophageal cancer and lycopene and prostate
cancer (WRCF and American Institute for Cancer Research, 2007). There is also a possible link
that increased fruit and vegetable consumption may prevent body weight gain. This reduces
the propensity to obesity and as such could act as an indirect reduction in type 2 diabetes,
although there is no direct link (Boeing et al., 2012). Boeing et al. (2012) also concluded there
is possible evidence that increased consumption of fruits and vegetables may be linked to a
reduced risk of eye disease, dementia, and osteoporosis. In almost all of these studies, fruits
and vegetables are classed together as a single “nutrient group.” It is thus not possible in most
cases to assign relative importance to either fruits or vegetables. Similarly, there is very little
differentiation between the very wide range of botanical species included under the banner
of fruits and vegetables and it is entirely possible that beneficial effects, as related to individual
disease states, may derive from metabolites found specifically in individual species.

Several studies have sought to attribute the potential beneficial effects of fruits and vegetables
to specific metabolites or groups of metabolites. One such which has received a significant
amount of interest is the antioxidants. Fruit is particularly rich in ascorbate or vitamin C which
represents one of the major water-soluble antioxidants in our diet and also in carotenoids such
as beta-carotene (provitamin A) and lycopene which are fat-soluble antioxidants (Chapter 4).
However, intervention studies using vitamin C or indeed any of the other major antioxidants,
such as beta-carotene, often fail to elicit similar protective effects, especially in respect of
cancer (Stanner et al., 2004). Polyphenols are another group of potential antioxidants that have
attracted much attention in the past. The stilbene—resveratrol—which is found in grapes, for
example, has been associated with potential beneficial effects in a number of diseases (Baur and
Sinclair, 2006). Similarly, the anthocyanins (Chapter 5), which are common pigments in many
fruits, have again been implicated with therapeutic properties (Zafra-Stone et al., 2007). It is
possible that these individual molecules may be having quite specific nutrient–gene expression
effects. It is difficult to study these effects in vivo, as bioavailability and metabolism both in the
gut and postabsorption can be confounding factors.

Although there are recommendations across many countries regarding the consumption of
fruits and vegetables, in general, the actual intake falls below these recommendations (Buttriss,
2012). However, trends in consumption are on the increase driven potentially by increasing
nutritional awareness on the part of the consumer and an increasing diversity of available
produce. Fruit is available either fresh or processed in a number of ways the most obvious
being in the form of juices or more recently smoothies. The list of fruits and vegetables traded
throughout the world is both long and diverse. The FAO lists over 100 “lines” of which 60 are
individual fruits or vegetables or related groups of these commodities. The remaining “lines”
are juices and processed or prepared material. However, the top five traded products are all
fruits and these are banana, tomato, apple, grape, and orange. In 1982–1984 these five between
BIOCHEMISTRY OF FRUIT RIPENING

Table 1.1  Global production, consumption, and net export of the five major (million tons) fruit commodities in 2002–2004. Data from European Commission Directorate-General for Agriculture and Rural Development (2007).

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Production</th>
<th>Consumption</th>
<th>Net Export</th>
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<tbody>
<tr>
<td>Banana</td>
<td>71</td>
<td>58</td>
<td>12.9</td>
</tr>
<tr>
<td>Tomato</td>
<td>119</td>
<td>103</td>
<td>2.1</td>
</tr>
<tr>
<td>Apple</td>
<td>59</td>
<td>56</td>
<td>3</td>
</tr>
<tr>
<td>Grape</td>
<td>64</td>
<td>59</td>
<td>1.7</td>
</tr>
<tr>
<td>Orange</td>
<td>63</td>
<td>53</td>
<td>2.5</td>
</tr>
</tbody>
</table>

them accounted for around half of global trade in fruits and vegetables; by 2002–2004, this had fallen to around 40% (European Commission Directorate-General for Agriculture and Rural Development, 2007). This probably reflects a growing trend toward diversification in the fruit market, especially in respect of tropical fruit. These figures represent traded commodities and in no way reflect global production of these commodities. In fact only about 5–10% of global production is actually traded. The EU commissioned a report in 2007 to examine trends in global production, consumption, and export of fruits and vegetables between 1980–1982 and 2002–2004. This demonstrated that fruits and vegetables represented one of the fastest growing areas of growth within the agricultural markets with total global production increasing by around 94% during this period. Global fruit production in 2004 was estimated at 0.5 billion tonnes. The growth in fruit production, at 2.2% per annum, was about half that for vegetables during this period. The report breaks these figures down into data for the most commonly traded commodities and the results for production, consumption, and net export in 2002–2004 are summarized in Table 1.1. Not all of the five major fruit commodities increased equally during this period. Banana and tomato production both doubled; apple and orange production both went up by about 50% while grape stagnated or even declined slightly during this period.

Global consumption of fruits and vegetables rose by 52% between 1992–2004 and 2002–2004 (European Commission Directorate-General for Agriculture and Rural Development, 2007). This means that global fruit and vegetable consumption rose by around 4.5% per annum during this period. This exceeded the population growth during the same period and as such suggested an increased consumption per capita of the population. Again the results for the consumption amongst the five major traded crops were variable with increases of banana, tomato being higher at 3.9% per annum and 4.5% per annum, respectively, while grapes (1.6% per annum) and oranges (1.9% per annum) were lower.

The net export figures reported above do not include trade between individual EU countries; however, even taking this into account, it is clear that only a small proportion of fruit production enters international trade. A major problem with trade in fresh fruit is the perishable nature of most of the commodities. This requires rapid transport or sophisticated means of reducing or modifying the fruits’ metabolism. This can be readily achieved for some fruits, such as apple, by refrigeration; however, several fruits, such as mango, are subject to chilling injury that limits this approach. Other methods that are employed are the application of controlled or modified atmospheres (Jayas and Jeyamkondan, 2002). Generally an increase in carbon
dioxide accompanied by a reduction in oxygen, will serve to reduce ethylene synthesis and respiration rate. The application of chemicals such as 1-MCP, an ethylene analog, can also significantly reduce ripening rates (Blankenship and Dole, 2003). Genetically modifying the fruit, for instance to reduce ethylene production, can also lead to an increase in shelf life (Picton et al., 1993).

Fruit ripening is highly coordinated, genetically programmed, and an irreversible developmental process involving specific biochemical and physiological attributes that lead to the development of a soft and edible fruit with desirable quality attributes (Giovannoni, 2001). The main changes associated with ripening include color (loss of green color and increase in nonphotosynthetic pigments that vary depending on species and cultivar), firmness (softening by cell-wall-degrading activities), taste (increase in sugar and decline in organic acids), and odor (production of volatile compounds providing the characteristic aroma). While the majority of this chapter will concentrate on central carbon metabolism, it is also intended to document progress in the understanding of metabolic regulation of the secondary metabolites of importance to fruit quality. These include vitamins, volatiles, flavonoids, pigments, and the major hormones. The interrelationship of these compound types is presented in Figure 1.1. Understanding the mechanistic basis of the events that underlie the ripening process will be critical for developing more effective methods for its control.

**Central Carbon Metabolism**

Sucrose, glucose, and fructose are the most abundant carbohydrates and are widely distributed food components derived from plants. The sweetness of fruits is the central characteristic determining fruit quality and it is determined by the total sugar content and by their ratios among those sugars. Accumulation of sucrose, glucose, and fructose in fruits such as melons, watermelons (Brown and Summers, 1985), strawberries (Fait et al., 2008) and peach (Lo Bianco and Rieger, 2002) is evident during ripening; however, in domesticated tomato (*Solanum lycopersicum*) only a high accumulation of the two hexoses is observed, whereas some wild tomato species (i.e., *Solanum chmielewskii*) accumulate mostly sucrose (Yelle et al., 1991). The variance in relative levels of sucrose and hexoses is most likely due to the relative activities of the enzymes responsible for the degradation of sucrose, invertase, and sucrose synthase.

The importance of the supply to, and the subsequent mobilization of sucrose in, plant heterotrophic organs has been the subject of intensive research effort over many years (Miller and Chourey, 1992; Zrenner et al., 1996; Wobus and Weber, 1999; Heyer et al., 2004; Roitsch and Gonzalez, 2004; Biemelt and Sonnewald, 2006; Sergeeva et al., 2006; Lytovchenko et al., 2007). While the mechanisms of sucrose loading into the phloem have been intensively studied over a similar time period (Riesmeier et al., 1993; Burkle et al., 1998; Meyer et al., 2004; Sauer et al., 2004), those by which it is unloaded into the sink organ (the developing organs attract nutrients) have only been clarified relatively recently and only for a subset of plants studied (Bret-Harte and Silk, 1994; Viola et al., 2001; Kuhn et al., 2003; Carpaneto et al., 2005). Recently, in the tomato fruit, the path of sucrose unloading in early developmental stages has been characterized as apoplastic. The study used tomato introgression lines containing
Figure 1.1 Interrelationships of primary and secondary metabolism pathways leading to the biosynthesis of aroma volatiles, hormones, pigments and vitamins (adapted from Carrari and Fernie (2006)).

an exotic allele of LIN5, a cell wall invertase that is exclusively expressed in flower (mainly ovary but also petal and stamen) and in young fruit (Godt and Roitsch, 1997; Fridman and Zamir, 2003), and it has been demonstrated that alterations in the efficiency of this enzyme result in significantly increased partitioning of photosynthate to the fruit and hence an enhanced agronomic yield (Fridman et al., 2004; Baxter et al., 2005; Schauer et al., 2006). Utilizing the reverse genetic approach, Zanor et al. (2009a) reported that LIN5 antisense plants had decreased
glucose and fructose in the fruit proving in planta the importance of LIN5 in the control of the total soluble solids content. The transformants were characterized by an altered flower and fruit morphology, displaying increased numbers of petals and sepals per flower, an increased rate of fruit abortion, and a reduction in fruit size. Evaluation of the mature fruit revealed that the transformants had a reduction of seed number per plant as well as altered levels of phytohormones. Interestingly, a role for apoplastic invertase in the control of sink size has been postulated previously in other species; the apoplastic invertase-deficient miniature1 mutant of maize exhibits a dramatically decreased seed size as well as altered levels of phytohormones (Miller and Chourey, 1992; Sonnewald et al., 1997; LeClere et al., 2008). This raises interesting questions regarding the regulation of carbon partitioning in fruits. Recently, a metabolic and transcriptional study using introgression lines resulting from a cross between S. lycopersicum and S. chmielewskii have revealed that the dramatic increase in amino acid content in the fruit is the result of an upregulated transport of amino acids via the phloem, although the mechanism is still unknown (Do et al., 2010).

Starch is another carbohydrate that undergoes modifications during ripening. The tomato introgression lines containing the exotic allele of LIN5 (IL 9-2-5) accumulated significantly more starch in both, pericarp and columella tissues (Baxter et al., 2005). This is in agreement with the finding that starch accumulation plays an important role in determining the soluble solids content or Brix index of mature fruit (Schaffer and Petreikov, 1997). Recently, in tomato fruits, reduction of the activities of either mitochondrial malate dehydrogenase (mMDH) or fumarase via targeted antisense approaches have demonstrated the physiological importance of malate metabolism in the activation state of ADP-glucose pyrophosphorylase (AGPase) that is correlated with the accumulation of transitory starch and also with the accumulation of soluble solids at harvest (Centeno et al., 2011).

Organic acid manipulation is highly valuable from a metabolic engineering perspective because the organic acid to sugar ratio defines quality parameters at harvest time in fruits. However, their study has received much less attention than that of the sugars to date. Malate is the predominant acid in many fruits, both climacteric, including tomato (Kortstee et al., 2007), apple (Beruter, 2004), and nonclimacteric, including pineapple (Saradhuldhat and Paull, 2007), cherry (Usenik et al., 2008), strawberry (Moing et al., 2001), and grape (Kliewer et al., 1967). Interestingly, levels of both citrate and malate were also highly correlated to many important regulators of ripening in an independent study that was focused on early fruit development (Mounet et al., 2009). Patterns of malate accumulation differ between plant species and even cultivars (Kliewer et al., 1967). In fruits, patterns of malate accumulation and degradation cannot be explained by the classification of species as climacteric or nonclimacteric, nor can they be attributed to changes in overall respiration rates. Some climacteric fruits such as plum and tomato appear to utilize malate during the respiratory burst (Goodenough et al., 1985; Kortstee et al., 2007), while others such as banana and mango continue to accumulate malate throughout ripening, even at the climacteric stage (Selvaraj and Kumar, 1989a; Agravante et al., 1991). Nonclimacteric fruits also display widely varying malate accumulation and degradation events (Moing et al., 2001; Saradhuldhat and Paull, 2007); some fruits, including mango, kiwifruit, and strawberry display no net loss of malate throughout ripening (Selvaraj and Kumar, 1989a; Walton and De Jong, 1990; Moing et al., 2001). For this reason, the metabolism of malate
has been a strong focus of research on grapes and tomato fruits, in which the acid plays a more metabolically active role (Goodenough et al., 1985). In grapefruit, malate is increasing in earlier stages and then is decreasing during ripening (Ruffner and Hawker, 1977). In earlier stages, malate is accumulated mostly through the metabolism of sugars (Hale, 1962) and during ripening, malate is a vital source of carbon for different pathways: TCA cycle and respiration, gluconeogenesis, amino acid interconversion, ethanol fermentation, and production of secondary compounds such as anthocyanins and flavonols (Ruffner, 1982; Famiani et al., 2000). Work with tomato fruit suggests that in early development, the majority of malate oxidation occurs through the TCA cycle.

The structure of the TCA cycle is well known in plants; however, until recently its regulation was poorly characterized. In our laboratory, several studies have been pursued to determine the role of mitochondrial TCA cycle in plants. Biochemical analysis of the Aco1 mutant revealed that it exhibited a decreased flux through the TCA cycle, decreased levels of TCA cycle intermediates, enhanced carbon assimilation, and dramatically increased fruit weight (Carrari et al., 2003). Nunes-Nesi et al. (2005) produced tomato plants with reduced mMDH activity. Plants showed an increment in fruit dry weight likely due to the enhanced photosynthetic activity and carbon assimilation in the leaves, which also led to increased accumulation of starch and sugars, as well as some organic acids (succinate, ascorbate, and dehydroascorbate). Reduction of fumarase activity has been investigated in tomato plants (Nunes-Nesi et al., 2007), which led to lower fruit yield and total dry weight. Those plants showed opposite characteristics to plants that were impaired for mMDH activity. Additionally, biochemical analyses of antisense tomato mitochondrial NAD-dependent isocitrate dehydrogenase plants revealed clear reduction in flux through the TCA cycle, decreased levels of TCA cycle intermediates, and relatively few changes in photosynthetic parameters; however, fruit size and yield were reduced (Sienkiewicz-Porzucek et al., 2010). All those studies have been performed on the illuminated leaf; recently, it has characterized tomato plants independently exhibiting a fruit-specific decreased expression of genes encoding consecutive enzymes of the TCA cycle, fumarase, and mMDH (Centeno et al., 2011). Detailed biochemical characterization revealed that the changes in starch concentration, and consequently soluble solids content, were likely due to a redox regulation of AGPase. Those plants showed also a little effect on the total fruit yield as well as unanticipated changes in postharvest shelf life and susceptibility to bacterial infection. Despite the fact that much research work is needed to understand the exact mechanism for the increment in the fruit dry matter, manipulation of central organic acids is clearly a promising approach to enhance fruit yield (Nunes-Nesi et al., 2011).

**Ethylene in Ripening**

Based on the respiratory pattern and ethylene biosynthesis during ripening, fruits have been classified either as “climacteric” or “nonclimacteric.” Climacteric fruits such as tomato show an increase in respiration rate and ethylene formation. Nonclimacteric fruits do not increase respiration, although they produce a little ethylene during ripening and do not respond to external ethylene treatment (Giovannoni, 2001). This difference is one of the main reasons...
that the majority of biochemical research has concentrated on this hormone. The role of ethylene in ripening of climacteric fruits has been known for more than 50 years (see Chapter 3). Since then, considerable effort has been focused on the studies of ethylene biosynthesis (S-adenosylmethionine, SAM; SAM synthetase; 1-aminocyclopropane carboxylic acid; ACC synthase; and ACC oxidase), ethylene perception (ethylene receptors, ETRs); signal transduction (ethylene response factor, ERFs); and ethylene-regulated genes such as cell-wall-disassembling genes (endopolygalacturonase; pectin methyl esterase, PME; and pectate lyase).

The Arabidopsis model system has served as starting point in the knowledge of the steps involved in ethylene perception and signal transduction; however, more efforts in understanding the ethylene response during fruit ripening have focused on the characterization of tomato homologs (Giovannoni, 2007). In this vein, six ethylene receptors have been isolated in tomato (ETHYLENE RECEPTOR1, LeETR1; ETHYLENE RECEPTOR2, LeETR2; ETHYLENE RECEPTOR5, LeETR5; NEVER-RIPE, NR; ETHYLENE RECEPTOR4, LeETR4; and ETHYLENE RECEPTOR6, LeETR6) compared to five members in Arabidopsis (ETHYLENE RECEPTOR1, ETR1; ETHYLENE RECEPTOR2, ETR2; ETHYLENE RESPONSE SENSOR1, ERS1; ETHYLENE RESPONSE SENSOR2, ERS2; and ETHYLENE INSENSITIVE4, EIN4) (Bleecker, 1999; Chang and Stadler, 2001). Five of the six tomato receptors have shown to bind ethylene (Klee and Tieman, 2002; Klee, 2002) but expression studies have been shown different profiles. Transcript levels of LeETR1, LeETR2, and LeETR5 change little upon treatment of ethylene in fruit, where NR, LeETR4, and LeETR6 are strongly induced during ripening (Kevany et al., 2007). Interestingly, analysis of transgenic plants with reduced LeETR4 and LeETR6 caused an early ripening phenotype (Kevany et al., 2007; Kevany et al., 2008). On the other hand, NR mutation resulted in not fully ripened fruit (Wilkinson et al., 1995; Yen et al., 1995). Nevertheless, analysis of transgenic plants with reduction in NR levels suggested that this gene was not necessary for ripening to proceed (Hackett et al., 2000), suggesting that the other fruit-specific member of the receptor family has compensatory upregulation (Tieman et al., 2000). Overexpression of the NR receptor in tomato resulted in reduced sensitivity in seedlings and mature plants (Ciardi et al., 2000). This is in agreement with models where ethylene receptors act as negative regulators of ethylene signaling (Klee and Tieman, 2002; Klee, 2002). Consistent with this model, an exposure of immature fruits to ethylene caused a reduction in the amount of ethylene receptor protein and earlier ripening (Kevany et al., 2007). Recently, further ethylene-inducible (CONSTITUTIVE TRIPLE RESPONSES MAP kinase kinase, CTR) family of four genes have been identified in tomato (LeCTR1, LeCTR2, LeCTR3, and LeCTR4). Unlike NR, LeETR4, and LeETR6, LeCRT1 is also upregulated during ripening (Adams-Phillips et al., 2004). Recently, studies of two-hybrid yeast interaction assay of tomato ethylene receptor and LeCTR proteins have demonstrated that those proteins are capable of interacting with NR (Zhong et al., 2008), reinforcing the idea that ethylene receptors transmit the signal to the downstream CTRe.

Recently, genomics approaches have provided insight into primary ripening control upstream of ethylene (Chapter 8). Tomato pleiotropic ripening mutations, ripening inhibitor (rin), non-ripening (nor), and Colorless nonripening (Cnr) have added great insights in this regard. The rin, nor, and Cnr mutations are affected in all aspects of the tomato fruit ripening process that are unable to respond to ripening-associated ethylene genes (Vrebalov et al., 2002; Manning
et al., 2006). Furthermore, in fruits from those mutants, the ripening-associated ethylene genes are induced by exogenous ethylene indicating that all three genes operate upstream of ethylene biosynthesis and are involved in process controlled exclusively by ethylene. The three mutant loci encode putative transcription factors. The rin encoded a partially deleted MADS-box protein of the SEPALLATA clade (Hileman et al., 2006), where Cnr is an epigenetic change that alters the promoter methylation of SQUAMOSA promoter binding (SPB) proteins. Manning et al. (2006) and J. Vrebalov and J. Giovannoni (unpublished results) suggest that the nor loci encodes a transcription factor, although not a member of MADS-box family. The observed ethylene-independent aspect of ripening suggests that RIN, NOR, and CNR proteins are candidates for conserved molecular mechanisms of fruit in both the climacteric and nonclimacteric categories.

Biochemical evidence suggests that ethylene production may be influenced or regulated by interactions between its biosynthesis and other metabolic pathways. One such example is provided by the fact that SAM is the substrate for both the polyamine pathway and the nucleic acid methylation; the competition for substrate was demonstrated by the finding that the overexpression of a SAM hydrolase has been associated with inhibited ethylene production during ripening (Good et al., 1994). On the other hand, the methionine cycle directly links ethylene biosynthesis to the central pathways of primary metabolism.

Polyamines

The most common plant polyamines are the diamine putrescine and the higher polyamines spermidine and spermine and it is known to be implicated in different biological processes, including cell division, cell elongation, embryogenesis, root formation, floral development, fruit development and ripening, pollen tube growth and senescence, and in response to biotic and abiotic stress (Kaur-Sawhney et al., 2003). In plants, putrescine is synthesized from arginine, a reaction catalyzed by arginine decarboxylase, or from ornithine by ornithine decarboxylase. Spermidine is synthesized from putrescine and SAM. SAM as a key intermediate for ethylene (Good et al., 1994; Fluhr and Mattoo, 1996; Giovannoni, 2004) has the potential to commit the flux of SAM either into polyamine biosynthesis, ethylene biosynthesis, or both. The overexpression of a SAM hydrolase has been associated with inhibited ethylene production during ripening (Good et al., 1994) which led to suggestions that changes in the levels of polyamines and ethylene may influence specific physiological processes in the plant (Kaur-Sawhney et al., 2003).

Mattoo et al. (2007) produced tomato fruits with increased SAM decarboxylase, in an attempt to over-accumulate spermidine and spermine whose levels decline during normal ripening process in tomato (Mehta et al., 2002). In the metabolite levels, those fruits showed prominent changes which influence multiple cellular pathways in diverse subcellular compartments such as mitochondria, cytoplasm, chloroplasts, and chromoplasts during fruit ripening. Red fruits showed upregulation of phosphoenolpyruvate carboxylase (PEPC) and cytosolic isocitrate dehydrogenase (ICDHc) as well as increase in the levels of glutamate, glutamine, asparagine, and organic acids; those of aspartate, valine, glucose, and sucrose showed a decrease compared to the wild type. The authors suggested that spermidine and spermine are perceived as signals of carbon metabolism in order to optimize C and N budgets following similar N regulatory
aspects as in roots or leaves (Corruzi and Zhou, 2001; Foyer and Noctor, 2002). Also these data revealed a role of polyamines in mitochondrial metabolic regulation suggested by upregulation of the mitochondrial cytochrome oxidase transcripts, higher respiratory activity as well as higher content of citrate, malate, and fumarate in the ripe transgenic fruits (Mattoo et al., 2006). Polyamines are also postulated to regulate stress responses as is shown in transgenic rice plants overexpressing arginine decarboxylase (Capell et al., 2004). Those plants resulted in activation of SAM decarboxylase and higher levels of spermidine and spermine which triggered drought tolerance. Further support for this role has been provided by a spermine mutant of Arabidopsis that displayed salt sensitivity (Yamaguchi et al., 2006). Various mechanisms have been invoked to explain the effects of polyamines; however, much research work is needed to understand how the plant cells sense threshold levels of polyamines, and what downstream signaling pathways are involved.

Volatile

Metabolism in the fruit involves the conversion of high-molecular-weight precursors to smaller compounds that help to obtain viable seeds and to attract seed-dispersing species (Chapter 6). The flavor of fruit is generally determined from tens and hundreds of constituents, most of them generated during the ripening phase of the fruit growth and development process. The content of sugars and organic acids and the ratios between them play a significant role in the overall flavor of fruit. Indeed, sugar content has previously been regarded as the major quantitative factor determining this parameter (Park et al., 2006). Amino acids are other soluble components that contribute significantly to fruit flavor. In the case of tomato fruit, flavor—a valuable trait—is the sum of the interaction between sugars (principally glucose and fructose), acids (citric, malic, and ascorbic), and glutamate and approximately 400 volatile compounds (Petro-Turza, 1987; Buttery, 1993; Buttery and Ling, 1993; Fulton et al., 2002), although a smaller set of only 15–20 are made in sufficient quantities to have an impact on human perception (Baldwin et al., 2000). Any study on the metabolic pathways leading to their synthesis must be considered in the context of this developmental process. Thus, it is known that the rapid growth phase of the fruits act as strong sinks that import massive amounts of photoassimilates from photosynthesizing organs. The translocation of metabolites occurs in the phloem. Sucrose is the metabolite mostly translocated, although in some species other compounds are predominant as polyalcohols like mannitol or sorbitol, and even oligosaccharides. These translocated compounds, which are the result of the primary metabolism, are the precursors of most of the metabolites that account for the fruit flavor, generally classified as secondary metabolites. Thus, the synthesis of these compounds is necessarily supported by the supply of the primary photoassimilates.

Flavor perception is often described as a combination of taste and smell. Some of these primary metabolites can be essential components of taste since they might be, depending on the species, main components of the harvested fruits, being recognized by sweet taste receptors. Recently, a metabolomic approach was used to describe the phenotypic variation of a broad range of primary and volatile metabolites, across a series of tomato lines, resulting from crosses between a cherry tomato and three independent large fruit cultivar (Levovil, VilB, and VilD)
The results of the most highly abundant primary metabolite analysis of cherry and large-fruited tomato lines were largely in accordance with those obtained from previous studies (Causse et al., 2002). The low sugar and high malate content of the Levovil parental and the corresponding very low sugar/acid ratio could explain the lower acceptance of the fruit by the food panel tasters, especially given that malate is perceived as sourer tasting than citrate (Marsh et al., 2003). In addition to the changes observed in sugars and acids in cherry tomatoes, the glutamate level, known to be sensed as the fifth basic taste (umami) which evokes a savory feeling, was found to be considerably higher in the cherry variety than in the large-fruited varieties. This finding is, additionally, in accordance with the fact that cherry tomatoes were found to be tastier than the other parental lines used in this study. Additionally, in this study considerable correlation within the levels of primary metabolites and volatile compounds, respectively, were also observed. However, there was relatively little association between the levels of primary metabolites and volatile compounds, implying that they are not tightly linked to one another with the exception of sucrose which showed a strong association with a number of volatile compounds (Zanor et al., 2009b).

A broad profiling of tomato volatiles on a tomato introgression line population harboring introgressions of the wild species Solanum pennellii yielded over 100 QTL that are reproducibly altered in one or more volatiles contributing to flavor (Tieman et al., 2006b). These QTL have been used as tools to identify the genes responsible for controlling the synthesis of many volatile compounds. Very few genes involved in the biosynthetic pathways of tomato flavor volatiles have been identified, although the detection of malodorous, a wild species allele that affects tomato aroma, allowed the identification of a QTL that is linked with a markedly undesirable flavor within the S. pennellii IL8-2 (Tadmor et al., 2002). A complementary approach, utilizing broad genetic crosses, has been used to identify QTL for organoleptic properties of tomatoes (Causse et al., 2002). The lines identified as preferable by consumer could now be comprehensively characterized with respect to volatile and nonvolatile compounds alike. By using a combination of metabolic and flux profiling alongside reverse genetic studies on IL8-2, it was possible to confirm the biological pathway of a set of phenylalanine-derived volatiles, 2-phenylacetaldehyde and 2-phenylethanol, important aromatic compounds in tomato (Tieman et al., 2006a). A combined metabolic, genomic, and biochemical analysis of glandular trichomes from the wild tomato species Solanum Habrochaites identified a key enzyme in the biosynthesis of methyl ketones that serve this purpose (Fridman et al., 2005). In recent years, there have been dramatic improvements in the knowledge of volatiles; however, there is still work to be done before it can be claimed that the understanding of their biosynthesis is comprehensive.

**Cell Wall Metabolism**

Fruit growth and ripening are complex developmental processes that involve many events contributing to the textural and constitutional changes in the fruits and determining their final composition. The metabolic changes during ripening include alteration of cell structure (Chapter 7), involves changes in cell wall thickness, permeability of plasma membrane,
hydration of cell wall, decrease in the structural integrity, and increase in intracellular spaces (Redgwell et al., 1997). Cell wall disassembly rate and extent are crucial for the maintenance of fruit quality and integrity (Matas et al., 2009). For this reason, maintenance of firmness has long been the target for breeders in many crops to minimize postharvest decay.

The major textural changes resulting in the softening of fruit are due to enzyme-mediated alterations in the structure and composition of cell wall, partial or complete solubilization of the major classes of cell wall polysaccharides such as pectins and cellulose (Seymour et al., 1987; Tucker and Grierson, 1987; Redgwell et al., 1992), and hydrolysis of starch and other storage polysaccharides (Fuchs et al., 1980; Selvaraj and Kumar, 1989b). The activity of these enzymes is directly linked to the shelf life of the fruits and it is why those genes have been frequent targets for genetic engineering (Goulao and Oliveira, 2007; Vicente et al., 2007). Among cell wall hydrolases, pectin-degrading enzymes are mostly implicated in fruit softening. Increased solubilization of the pectin substances, progressive loss of tissue firmness, and a rapid rise in the polygalacturonase (PG) activity accompany normal ripening in many fruits (Brady, 1987; Fisher and Bennett, 1991). A positive correlation between PG activity and initiation of softening is known in a number of fruits like guava (El-Zoghbi, 1994), papaya (Paull and Chen, 1983), mango (Roe and Bruemmer, 1981), strawberry (Garcia-Gago et al., 2009; Quesada et al., 2009). However, experiments with transgenic tomatoes have shown that even though PG is important for the degradation of pectins, it is not sole determinant of tissue softening during ripening (Gray et al., 1992). PME catalyzes the de-esterification of pectin, and its activity together with PG increase remarkably during ripening in peach, tomato, pear, and strawberry (Tucker and Grierson, 1987; Osorio et al., 2010). The loss of neutral sugar side chains from pectin is one of the most important features occurring during ripening. Substantial variation in the cell wall composition among fruits exists and their metabolism in relation to softening also varies (Gross and Sams, 1984), that is, neutral sugar side chains are not lost in ripening plum and cucumber fruits (Gross and Sams, 1984). The mutant rin containing little or no PG activity showed a substantial loss of galactose from cell wall, suggesting that this loss is not due to the action of PG. This evidence suggests that other cell wall hydrolases play an important role in the texture softening during ripening (Gray et al., 1992). In parallel to these changes in the cell wall, in many fruits a dramatic increase in susceptibility to necrotrophic pathogens has been reported (Prusky, 1996). It is now accepted that cell wall disassembly can be a key component of this susceptibility (Flors et al., 2007; Cantu et al., 2008).

The regulation of texture and shelf life is clearly far more complex than was previously envisaged (see Chapter 7), and so new approaches are needed; a better understanding of the relationship between changes in the texture properties of specific fruit tissues as well as intact fruit “firmness” and shelf life. Polysaccharide degradation is not the sole determinant of fruit softening and other ripening-related physiological processes also play critical roles. The cuticle has a number of biological functions that could have an important impact on fruit quality and shelf life that include the ability to maintain fruit skin integrity (Hovav et al., 2007), restrict cuticular transpiration (Leide et al., 2007), and limit microbial infection. Other reports also highlight other processes that contribute to fruit softening such as turgor pressure (Saladie et al., 2007; Thomas et al., 2008; Wada et al., 2008) and the possible associated developmental changes in apoplastic solute accumulation (Wada et al., 2008).
Concluding Remarks

Metabolomics allows the identification of changes in chemical composition with agronomic value. The shift from single metabolite measurements to platforms that can provide information on hundreds of metabolites has led to the development of better models to describe the links both between metabolites and between metabolomes. It is likely that the combination of molecular marker sequence analysis, PCR amplification and sequencing, analysis of allelic variation, and evaluation of co-responses between gene expression and metabolite composition traits will allow the detection of both expression QTL (wherein the mechanism underlying the metabolic change is an alteration in transcript and by implication, in protein amount), as well as change in function in which the level of expression is unaltered. It is hoped that in the future, this approach will allow a comprehensive understanding of genetic and metabolic networks that govern fruit metabolism and its effect on compositional quality.

References


BIOCHEMISTRY OF FRUIT RIPENING


