Metabolomic Profiling of Fruits: Biochemical Pathways and Quality Indices

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Abstract

The fruit ripening process is delineated into distinct phases, each characterized by specific physiological and biochemical transitions that collectively dictate the nutritional profile and sensory attributes of the fruit. The intricacies of the ripening sequence are subjected to rigorous scrutiny to elucidate the determinants of fruit quality. Extensive research has been dedicated to deciphering the biochemical and molecular underpinnings governing the ripening cascade, a pursuit driven by the substantial economic and societal value attributed to this phase. Contemporary discoveries have challenged conventional paradigms of ripening initiation, revealing a more complex interplay wherein ethylene may be implicated in both climacteric and non-climacteric ripening processes. This chapter aggregates the latest insights on pivotal ripening transformations, spotlighting the modulation of ethylene synthesis, pigmentary evolution, and aroma compound generation. The manuscript encapsulates a review of the fundamental metabolic routes integral to fruit quality and proffers a critical analysis of molecular methodologies employed in ripening research.

INTRODUCTION

Fruits and fruit products are rich in an array of vitamins, minerals, and nutrients. They vary greatly across plant species in size, shape, texture, color, flavor, sensory (organoleptic) properties, and nutritional content. Fruits have evolved to attract animals for seed dispersal, exhibiting diverse features to facilitate this. The development of the seed-bearing part and the edible portion of fruits can be quite distinct. For example, strawberries and pineapples develop their fruit from the receptacle tissue, while tomatoes use ovary tissue. In citrus fruits, the ovary walls create structures holding juice sac locules. The physiological processes of fruit growth and maturation, along with the regulatory mechanisms of biochemical and molecular transformations during ripening, share similarities across different fruits and plants. Ripening involves significant changes in color, sugar levels, acidity, softening, texture, aroma and flavor compound synthesis, and increased susceptibility to physiological disorders. These variations demonstrate a broadly conserved regulatory system across species over evolutionary timeframes. The biochemistry of fruit ripening has been extensively documented in scientific literature, such as in the works of Paliyath and Murr (2006), Giovannoni (2004), Seymour et al. (1993), and Knee (2002). During ripening, there are pronounced alterations in biochemical pathways and molecular ripening processes.

REGULATION OF FRUIT RIPENING: THE ROLE OF ETHYLENE

Ethylene is recognized as a pivotal hormone in fruit ripening, influencing various processes from ancient preservation methods to modern postharvest treatments like citrus degreening. The concept of climacteric ripening, introduced by Kidd and West in 1925, delineates fruits based on their ethylene production and respiration increase during ripening. Climacteric fruits exhibit a surge in both ethylene synthesis and respiration, often autocatalytically, while nonclimacteric fruits do not display significant changes in these parameters (Biale & Young, 1981). However, this classification is not absolute, as demonstrated by varying climacteric behaviors within plum types (Abdi et al., 1997), and nonclimacteric fruits may still respond to ethylene treatment.

Ethylene's role extends to System 1 and System 2 pathways, where System 1 relates to basal ethylene production and System 2 to autocatalytic production during climacteric ripening (Yang, 1987). Ethylene biosynthesis involves the conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), followed by the conversion of ACC to ethylene by ACC oxidase (ACO) (Kende, 1993). Gene expression of ACS and ACO is tightly regulated, impacting ethylene levels during fruit development (Barry et al., 2007). Genetic modifications of these genes in various crops demonstrate the nuanced role ethylene plays in fruit maturation (Barry & Giovannoni, 2007; Matas et al., 2009).

Ethylene perception and signal transduction are mediated by ethylene receptors, such as those in tomatoes, which belong to two subfamilies resembling histidine kinases (Klee, 2004). The expression of these receptors varies during ripening and stress responses, with some acting as negative regulators of ethylene signaling (Kevany et al., 2007). Even in nonclimacteric fruits, ethylene influences ripening aspects like anthocyanin synthesis and carotenoid biosynthesis, albeit through different biochemical and molecular mechanisms (Goldschmidt et al., 1993; Chervin et al., 2004).

Finally, studies on transgenic fruits with altered ethylene perception or synthesis, such as ACO-antisense melons, have revealed both ethylene-dependent and independent pathways in ripening, highlighting the hormone's selective role in regulating specific ripening events (Hadfield et al., 2000; Schaffer et al., 2007).

In summary, while ethylene is a key ripening hormone, its role is complex and varies among different fruit types and ripening stages, with some processes being ethylene-independent. The classification into climacteric and nonclimacteric is useful but not definitive, as fruits can exhibit a spectrum of responses to ethylene.
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CARBOHYDRATE METABOLISM

The biochemical transformations during fruit ontogeny are critical determinants of fruit quality attributes. In the initial stages of fruit development, plants utilize solar energy to assimilate inorganic carbon into organic molecules, a process central to photosynthesis. However, the reliance on photosynthesis for carbon allocation diminishes as the fruit progresses from growth to maturation. This is evidenced by the reduction of chlorophyll (Chl) content, leading to a decline in the photosynthetic capacity of fruit tissues. The contribution of photosynthesis to the fruit's carbon economy is variable, accounting for approximately 5% to 15% in many fruit-bearing species (Fluenc, 2007).

Carbon assimilated through photosynthesis is mainly transported as sucrose, which is converted from glycerate-1-phosphate. This transformation occurs in the leaves and bark before translocation to the fruit, where sucrose is often converted into starch for storage. However, sucrose is not typically the primary form of sugar transport within fruits due to the action of invertase, an enzyme that catalyzes its breakdown into glucose and fructose. Instead, sugar alcohols such as mannitol and sorbitol, prevalent in species like apples and olives, are the dominant carbohydrates for transport and storage functions within these fruits.

A comprehensive overview of these crucial metabolic pathways and the carbon flux in fruit cells is depicted in Figure 2.2, illustrating the complex interplay between photosynthesis, carbon transport, and storage during fruit development.

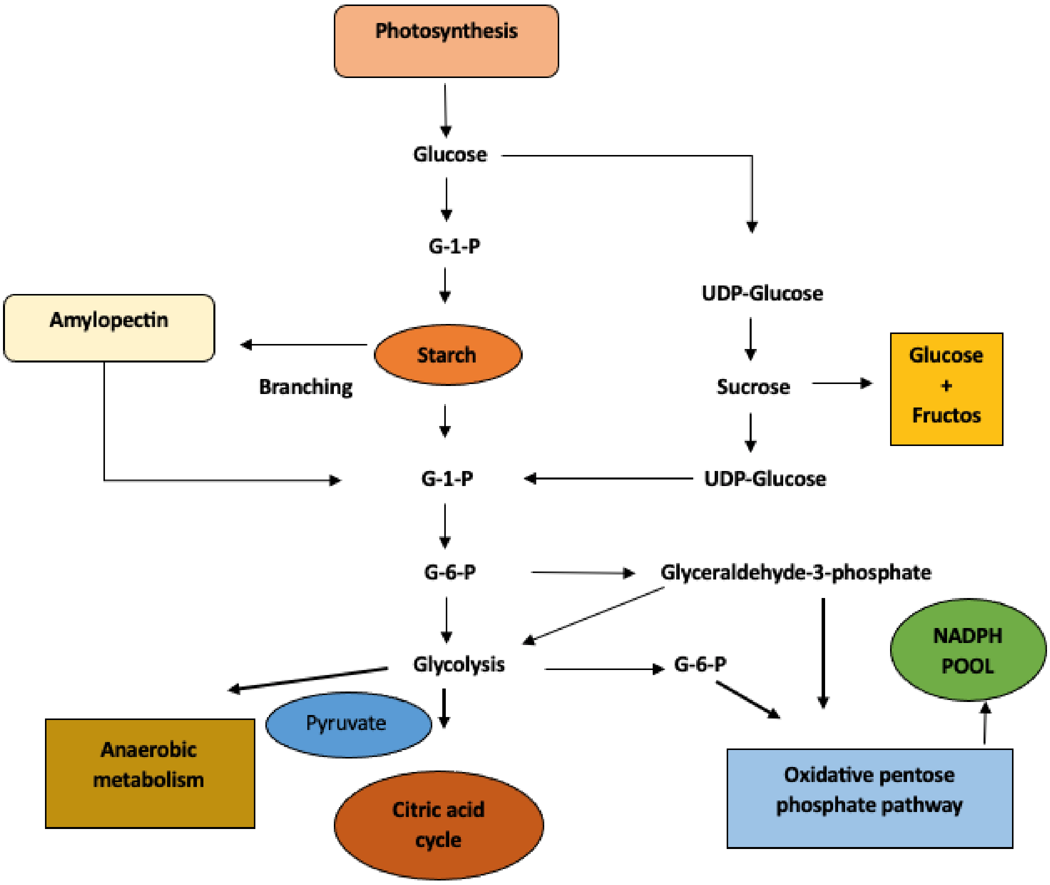

Fig. Pathway of carbohydrates metabolism in fruits

During the maturation of unripe fruits, starch, composed of amylose and amylopectin, is stored within plastids. As fruits approach ripeness, starch is hydrolyzed into reducing and non-reducing sugars such as glucose and fructose, facilitated by catabolic processes within the plastids before being transported to the cytoplasm. This increase in cytoplasmic sugar levels allows their entry into metabolic pathways. Sugars serve as substrates for respiration via glycolysis or as precursors for biosynthesis of amino acids, nucleic acids, and secondary metabolites, processes integral to maintaining cellular homeostasis. These processes are further interconnected through the pentose phosphate pathway, which provides NADPH and pentose sugars for anabolic reactions (Figure 2.2).

During the development phase, fruit carbohydrate metabolism is geared towards biosynthesis, supported by glucose derived from photosynthesis. However, as fruits transition to a stage characterized by increased metabolic activity, known as the ripening climax, there is a metabolic shift towards catabolism to meet the high energy demand. This period is marked by a significant rise in respiration, especially in climacteric fruits, which necessitates an elevated energy input. Such fruits, including those with high oil content like avocados, mangoes, and bananas, are highly perishable post-harvest due to their respiratory demands. To extend their shelf life, controlled atmosphere storage techniques involving low oxygen, high carbon dioxide, and reduced temperatures have been employed.

The distribution of metabolic intermediates between glycolysis, the pentose phosphate pathway, or starch and sucrose degradation pathways is dictated by the cell's requirements at various stages of growth and maturity. For example, variations in sugar content have been observed in citrus fruits, where clementine mandarins exhibit different sugar profiles in their tissues compared to their juice (Tadeo et al., 1987).

Key enzymes involved in sucrose metabolism include invertases, sucrose synthases, and sucrose phosphate synthases. The balance of sucrose accumulation in the fruit is regulated by the interplay between sucrose synthesis and breakdown, influenced by the activities of these enzymes. Sucrose synthase plays a role in synthesizing respiratory substrates, while UDP-glucose is utilized in the synthesis of complex carbohydrates (Sung et al., 1988). Sucrose phosphate synthase activity increases as fruits develop and is crucial during ripening for sucrose synthesis in fruits like muskmelon, while its activity remains constant in tomatoes (Lingle & Dunlap, 1987).

In ripening fruits, the breakdown of starch leads to an increase in sucrose phosphate synthase activity, as observed in bananas and cucurbits (Cordenunsi & Lajolo, 1995; Irving et al., 1997). Transcriptional levels of sucrose phosphatase and synthase are also elevated in ripe citrus, although their enzymatic activities may decrease (Lowell et al., 1989). During the early stages of fruit development, energy demands drive up sucrose synthase activity, which primarily acts as a sucrose-degrading enzyme (Cano-Medrano & Darnell, 1997).

Ripening involves intense glycolytic activity, breaking down carbohydrates to generate the energy required for this process. Acetyl-CoA, produced through glycolysis, enters the citric acid cycle, which is reversed during fruit development to synthesize sugars from organic acids—a process termed gluconeogenesis.

Additionally, the pentose phosphate pathway, which generates NADPH, and sugar phosphates derived from starch breakdown, can be balanced through irreversible reactions or reversible interconversions. This flexibility allows for the transfer of sugar phosphate intermediates between the pentose phosphate cycle and glycolysis, facilitating the formation of a NADPH pool essential for the functioning of the antioxidant enzyme system.

Postharvest, low temperatures can induce anaerobic respiration in ripe fruits as mitochondrial electron transport is inhibited, preventing ATP generation via the citric acid cycle. Under such conditions, ATP may be generated through lactate dehydrogenase-mediated anaerobic respiration, converting pyruvate to lactate and regenerating NAD+ (Beaudry et al., 1989).

Specific sugars like d-mannoheptulose in avocado are predominant during fruit growth and are essential for respiration, but their levels decrease postharvest as oil accumulates (Liu et al., 1999). In bananas, the regulation of ATP-dependent phosphofructokinase channels carbon through glycolysis during the respiratory climacteric, initiated by starch degradation (Beaudry et al., 1989).

Overall, carbohydrate metabolism during fruit development is linked to postharvest issues such as watercore in apples and pears, where sucrose accumulation is implicated in the disorder (Bowen & Watkins, 1997). Ethylene treatments, known to induce sugar accumulation in fruits like loquat, are thought to enhance fruit sink activity, highlighting the hormonal regulation of carbohydrate metabolism (Hirai, 1982).

ORGANIC ACIDS

Organic acids, notably malic and citric acids, contribute significantly to the sour taste in fruits and are essential for crop harvest timing due to their impact on consumer acceptance (Baldwin, 2002). These acids are predominant in fruits regardless of ripening conditions or agricultural practices, with malic acid found abundantly in apples and citric acid in citrus fruits (Sweetman et al., 2009). The interaction between malic acid and the perceived sweetness of sucrose differs from that of citric acid (Bonnans and Noble, 1993), and their antimicrobial properties are well documented.

The Krebs cycle, a central metabolic pathway involving organic acids, is crucial for fruit ripening, with sugars serving as primary respiratory substrates (Tucker, 1993). Organic acids in the vacuole help maintain cell turgor, which is vital for fruit growth. Citric acid also protects plants from oxidative damage by chelating metals, and malic acid can signal fruit freshness (Guillet et al., 2002).

As fruits mature, organic acid levels and pH undergo significant changes; young fruits are more acidic with a pH below 3, which gradually rises as sugars accumulate during ripening. Malic enzymes, which are either cytosolic and NADP-dependent or mitochondrial and NAD-dependent, facilitate the conversion of malic acid to pyruvate, allowing carbon to enter the Krebs cycle directly, bypassing glycolysis (Sweetman et al., 2009).

Research has indicated that differences in malic acid content between apple genotypes do not correspond to variations in respiration rates or enzyme activity. In low-acid genotypes, reduced vacuolar storage capacity results in lower malic acid accumulation (Berüter, 2004). Malic acid levels also influence the allocation of assimilates to other cellular components, including carbohydrates, with implications for carbon metabolism pathways.

Variations in citric acid levels have been noted between different fruits, such as peaches and citrus, with species-specific influences on organic acid metabolism during ripening. Notably, citric and malic acids in tomatoes degrade more slowly at low temperatures post-harvest (Davies and Hobson, 1981; Gomez et al., 2009).

In apples, malic acid is the predominant organic acid and can decrease by up to half during ripening due to respiration. The enzymes phosphoenolpyruvate carboxylase (PEPC) and NAD-dependent malate dehydrogenase (NDM) are involved in its production (Blanke and Lenz, 1989). Metabolic interventions, such as ethanol biosynthesis inhibitors and transgenic apples with reduced ethylene production, have shown that malic acid metabolism is ethylene-dependent (Defilippi et al., 2004).

Grape juice acidity is crucial for winemaking, prompting studies into malic acid metabolism in non-climacteric grape berries. During grape development, carbohydrate metabolism leads to malic acid accumulation with high PEPC activity, which diminishes as ripening commences (Ruffner et al., 1976; Diakou et al., 2000).

In citrus fruits, mandarin oranges, for instance, lose a significant portion of their citric acid during maturation, while malic acid levels remain stable. Gene expression studies have revealed changes in citric acid metabolism during ripening, including its role in the GABA shunt pathway (Cercos et al., 2006).

Understanding oscillations in organic acid concentrations throughout fruit development requires integrating physiological models of the Krebs cycle, metabolite transport, and associated enzymes (Lobit et al., 2006; Wu et al., 2007). A comprehensive understanding of organic acid formation and degradation will necessitate diverse experimental approaches and high-throughput technologies.

LIPID METABOLISM

In fruit, lipids play a key role, either as structural components (components of cell biomembranes) or as storage components. Fats, diacyl and triacylglycerols, galactolipids, galactolipids, sterols, and wax are all part of the lipid family. Most of the biomembranes are composed of phospholipids, diacylglycerols, and sterols. Phospholipids include phosphatidylcholine and phosphatidylethanolamine. Membranes may include a range of other metabolites in addition to phosphatidic acid, free fatty acids, and diacylglycerol. Signal transmission in response to environmental stimuli is mediated by phosphatidic acid and various phosphorylated derivatives of phospholipids.

Lipid Biosynthesis Fatty Acid and Glycerolipids Biosynthesis

In the metabolic pathways of fat-storing fruits like avocados and olives, de novo fatty acid biosynthesis has been extensively studied. Acetyl-CoA, a central molecule in this process, is synthesized from a 6-carbon sugar precursor via the action of the enzyme pyruvate dehydrogenase located in plastids and mitochondria (Salas et al., 2000). This enzyme catalyzes the decarboxylation of pyruvate, yielding acetyl-CoA.

Fatty acid synthesis is driven by two key enzymes: acetyl-CoA carboxylase, which produces malonyl-CoA, and fatty acid synthase, which orchestrates a series of elongation and condensation reactions. These reactions repetitively add two-carbon units to the growing fatty acid chain, predominantly yielding C16 and C18 fatty acids. The activity of acetyl-CoA carboxylase is a determinant for the proportion of these fatty acids.

Desaturation processes, which introduce double bonds into the fatty acid chains, can occur within plastids or the endoplasmic reticulum. The level of polyunsaturation of the fatty acids is enhanced through these desaturation steps. Subsequently, in the endoplasmic reticulum, the Kennedy pathway is responsible for esterifying these fatty acids to glycerol backbones, forming glycerolipids (Salas et al., 2000).

Storage Lipids

The mesocarp tissue of avocados and olives contains cells known as idioblasts that specialize in lipid storage, predominantly in the form of triglycerides. These triglycerides are synthesized through a process where glycerophosphate is sequentially converted to phosphatidic acid and then to diglycerides (Kikuta & Eriksson, 1968). Lipid metabolism is essential for the proper maturation of these fruits, and lipid concentration increases progressively throughout their development and ripening.

In the composition of the triglycerides within avocados, up to 95% of the total lipid content can be attributed to these molecules. The fatty acid composition of triglycerides in these fruits is primarily made up of four types: palmitic, palmitoleic, oleic, and linoleic acids. As the fruit matures, the oil content increases and oleosomes—or oil bodies—aid in segregating different types of fats within the cells.

While comprehensive molecular studies on lipid and fatty acid metabolism in fruit are limited, there has been research conducted on other fruits such as mangoes. Specifically, during the growth of mango fruit, there has been a noted increase in transcripts for thiolase, the final enzyme in the β-oxidation pathway of fatty acids, which is indicative of the active metabolism of lipids during fruit development (Bojorquez & Gomez-Lim, 1995).

Membranes

Cellular membranes are critical hereditary structures that regulate the diffusion of water-soluble molecules. Composed principally of proteins and polar lipids, these membranes serve as barriers and platforms for cellular signaling. Lipids, particularly phospholipids, play a pivotal role in membrane architecture and function. Plant cells contain approximately 17 distinct membrane systems, each characterized by a unique lipid composition that varies between different organelles, tissues, and even among plant species. The diversity in lipid composition is crucial for the specialized functions of various membranes within the plant.

The fluid-mosaic model, proposed by Singer and Nicholson in 1972, currently offers the most accepted explanation for membrane structure and fluidity. According to this model, a bilayer of phospholipids serves as a fluid matrix in which proteins are embedded, allowing for lateral movement within the membrane. The fluidity of the membrane is largely determined by the fatty acid composition of the phospholipids; membranes with a higher proportion of unsaturated acyl chains—such as oleic, linoleic, and linolenic acids—are more fluid.

Membrane fluidity is vital for maintaining cellular homeostasis and the functionality of organelles. Surrounding membrane receptors, phospholipids not only contribute to structural integrity but also participate in signal transduction. Upon stimulation of a receptor, specific enzymes can catalyze the conversion of phospholipids into signaling molecules, a process that is integral to plant responses to various stimuli, including stress responses (Meijer & Munnik, 2003).

Wax Synthesis and Deposition

Plant cuticles, composed largely of waxes, serve as the primary barrier for gas and water exchange. The formation of epicuticular layers involves the deposition or embedding of waxes into the cutin matrix, a polymer that is integral to the cuticle structure. Waxes are primarily made up of long-chain fatty acids, which are synthesized through the action of elongases. These enzymes extend the chains by successively adding two-carbon units, typically starting from C16 and C18 acyl-CoAs (Mintz-Oron et al., 2008).

During fruit development and ripening, the composition of waxes on the fruit surface changes. Both biosynthesis and deposition of waxes are accelerated in the initial stages of fruit development. It has been observed that the expression of genes related to wax production and the accumulation of wax metabolites peak early in the ripening process and diminish as the fruit reaches maturity (Baker, 1982; Mintz-Oron et al., 2008).

Lipid Metabolism in Fruit During Ripening and Senescence: Postharvest Changes

The maturation and ripening of fruit involve complex biochemical changes, particularly in their cellular structures. During this phase, there's an increase in ion leakage and calcium permeability in cells. These changes trigger the activation of phospholipase D (PLD), especially under conditions of low pH and high calcium concentrations, exceeding 10 µM. The activation of PLD is a response to alterations in the cell's ionic environment during fruit senescence. This stage is also marked by decreased membrane fluidity due to changes in lipid composition. Such alterations are more pronounced when fruits are still attached to the plant. A significant aspect of these changes is the increased concentration of sterols in the lipid bilayer, a factor associated with the onset of senescence (Fuh et al., 1988).

After harvest, fruits experience a reduction in photosynthetic activity, disorganization of chloroplasts, and degradation of proteins, nucleic acids, and lipids. The aging of cell membranes leads to increased acidity, altering phosphatidylinositol levels. Membrane integrity is further compromised by the heightened activities of lipoxygenase (LOX) and lipid peroxidation, contributing to water loss and diminished quality. In watermelon, post-harvest water soaking disorder is linked to increased activities of phospholipase C (PLC), PLD, and LOX. This disorder is characterized by reduced levels of phosphatidylcholine and phosphatidylinositol, but increased phosphatidic acid (PA). Even with treatment by 1-MCP, PLC and LOX activities are not completely inhibited in watermelons not exposed to ethylene (Mao et al., 2004). Studies in citrus fruits indicate that the development of such disorders involves the oxylipin cascade, independent of ethylene signaling (Alférez et al., 2006).

Nutrient uptake during crop growth, particularly phosphorus (P), is crucial for maintaining membrane integrity post-harvest. P is a key component of phospholipid structure. In seedless cucumbers, the levels of phosphatidylcholine and phosphatidylethanolamine correlate with P fertilization. Insufficient P nutrition is associated with a decrease in total fatty acids and increased electrolyte leakage (Knowles et al., 2001). Under P deficiency, plants may substitute galactoglycerolipids for phospholipids in extraplastidic membranes (Andersson et al., 2003).

Post-harvest storage conditions can influence phospholipid profiles. The three phospholipase families (PLA, PLC, and PLD) play roles in responding to biotic and abiotic stresses. These enzymes are further divided into subfamilies based on sequence and biochemical properties. For example, PLA2 acts at the sn-2 position of phospholipids, producing lysophospholipids and free fatty acids, including the release of fatty acids from galactolipids in the chloroplast membrane (Matos et al., 2001). LOX enzymes oxidize polyunsaturated fatty acids like linolenic acid to form hydroperoxylinolenic acids. These acids are precursors in metabolic pathways leading to the production of jasmonates in peroxisomes (Blee, 1998; Dhondt et al., 2000). These oxylipins are believed to play a significant role in the post-harvest senescence of fruits and vegetables (Howe & Schilmiller, 2002; Zhuang et al., 1994).

PIGMENTS IN FRUITS

Pigments are molecules containing chromophores that absorb specific wavelengths of visible light, conferring color. Chlorophylls (Chls) are predominantly green, carotenoids range from yellow to red, and anthocyanins display red, blue, and violet, constituting the primary natural pigments. Secondary pigment classes like betalains and quinones, encompassing betalain, phenalone, and phyrone, are also present but less emphasized (Gross, 1987). These pigments not only contribute to the aesthetic quality of fruits, influencing seed dispersal by attracting insects and animals but have also been recognized for their functional roles. They exhibit antioxidant properties, safeguarding DNA, proteins, and lipids against oxidative damage. From a market perspective, pigments are critical as they determine the visual attractiveness of fruits, influencing consumer acceptance (Gross, 1987).

Recent research attributes significant health benefits to pigments, such as carotenoids and anthocyanins, highlighting their role in preventing cardiovascular diseases and various cancers (Duthie et al., 2000; Rao and Rao, 2007). The pigment profile of a fruit, including its concentration and composition, is highly variable among different genera, species, and cultivars and is influenced by environmental and agronomic factors (Gross, 1987; Goldschmidt, 1988; De Pascual-Teresa and Sanchez-Ballesta, 2008). Postharvest processing and storage conditions can further modify pigment levels and composition.

Decades of research have led to the identification of genes encoding enzymes responsible for the biosynthesis of Chls, anthocyanins, and carotenoids, along with the regulatory mechanisms controlling their expression during fruit ripening and development.

Chlorophylls

The green pigment chlorophyll is characterized by porphyrins, which have strong absorption in the blue and red wavelengths of light. The biosynthetic pathway of chlorophyll from glutamic acid involves over a dozen enzymes, and recent advances have provided revisions to this complex process (Eckhardt et al., 2004; Tanaka and Tanaka, 2006; Masuda, 2008). Chlorophyll degradation, a pivotal chemical transformation during the ripening of most fruits and vegetables, results in a loss of green coloration known as degreening. Recent research has elucidated the fundamental steps and associated genes in the catabolic pathway of chlorophyll breakdown (Hortensteiner, 2006; Hortensteiner and Kräutler, 2011).

During the green stages of fruit development, chlorophyll content increases, correlating with chloroplast integrity, but as the fruit matures, chlorophyll levels decline, revealing other pigments and often resulting in the development of new pigments that change the fruit's color. However, some fruits such as certain cultivars of apples, pears, figs, plums, limes, avocados, and kiwis display an atypical ripening process, retaining moderate levels of non-chlorophyll pigments at maturity. "Stay-green" mutations result in these fruits failing to develop additional pigments, leading to a brownish hue at full ripeness. These mutations are categorized into three classes. Classes A and B display chlorophyll degradation but at a reduced rate or with delayed initiation. Class C mutants are deficient in at least one step of the degradation pathway (Matile et al., 1999).

Anthocyanins

Anthocyanins, a class of water-soluble flavonoids, are synthesized in the cytoplasm and sequestered in vacuoles, where their structure and color manifestation are influenced by intracellular pH variations. These molecules are structurally characterized by the presence of at least one sugar moiety attached at various points on their C15 skeleton, particularly on the second chromane ring. The biosynthetic pathways of anthocyanins are well-elucidated, with key enzymes identified and their gene expression patterns analyzed across tissues and developmental stages (Grotewold, 2006; Davies, 2009; Hichri et al., 2011).

Anthocyanin biosynthesis is facilitated by two gene types: structural genes encoding the enzymes directly involved in pigment production, and regulatory genes, particularly transcription factors that modulate the expression of structural genes. It is hypothesized that protein-protein interactions contribute to the formation of a metabolon, a complex of enzymes associated with the endoplasmic reticulum membrane, for efficient synthesis (Ben-Yehuda et al., 2005; Jaakola et al., 2002; Jaakola et al., 2010; Niu et al., 2010; Lo Piero et al., 2005; Boss and Davies, 2009).

The MYB-bHLH-WD40 (MBW) protein complex has been implicated in the regulation of anthocyanin biosynthesis genes. WD40 proteins are believed to stabilize protein interactions, while MYB and bHLH proteins are involved in DNA binding and protein complex formation. MYB transcription factors, in particular, are influential in fruit anthocyanin accumulation, acting as inducers or repressors. The trans-activation efficiency of MYB proteins, their DNA-binding specificity, and interaction dynamics are determined by critical residues in the N-terminal region, while the functional significance of consensus motifs in the C-terminal region remains under investigation (Espley et al., 2009).

Color variations in fruits such as grapes have been associated with transposable elements affecting the stability of transcription factor genes. The primary anthocyanidins responsible for coloration are cyanidin, peonidin, pelargonidin, delphinidin, petunidin, and malvidin, which confer orange, pink, red, purple, mauve, and blue hues respectively. The diversity of anthocyanins is further amplified by species-specific modifications including acyl moieties and glycosylation, methylation, and coumarylation processes. Copigmentation, a mechanism involving flavones, alters the hue of anthocyanin pigments (Gross, 1987).

In some cultivars with high anthocyanin content, pigments are predominantly deposited in the skin and subepidermal cells, although other tissues may also contain pigments. For example, pomegranates and peaches exhibit anthocyanin accumulation around the seeds, while blood oranges have concentrations in the flesh. Cyanidin is the most prevalent aglycone in fruits, found in 90% of common fruits and 82% of angiosperm species fruits (Macheix et al., 1990). Cyanidin-3-glucoside is commonly found across fruits, while malvidin glucosides are notably present in red grape varieties. The anthocyanin profiles can range from simple, as in passion fruit, to complex, as seen in some grape and orange cultivars with over 20 different anthocyanins (Kidoy et al., 1997). Anthocyanin accumulation intensifies during fruit ripening, culminating at full maturity where the variety of anthocyanidins and glycosylation patterns become more intricate. Red berries and grapes are primary dietary sources of anthocyanins, providing approximately 0.25 mg per gram of fresh weight (Gross, 1987).

Carotenoids

Carotenoids are a diverse group of approximately 700 lipophilic compounds, giving fruits and vegetables their red, orange, and yellow colors, residing within plastids in association with lipid-protein complexes due to their hydrophobic nature. These molecules are typically composed of forty carbon atoms arranged in a polyene chain with up to fifteen conjugated double bonds, derived from eight isoprenoid units with an inverted arrangement at the molecule's midpoint. Carotenoids are categorized into carotenes, which are purely hydrocarbons, and xanthophylls, which are oxygenated derivatives of carotenes.

Diverse structural modifications such as hydrogenation, dehydrogenation, cyclization, oxygenation, bond migration, methylation, and chain length alterations give rise to various carotenoids, including lycopene derivatives. Biosynthesis of carotenoids begins with the formation of phytoene, a colorless compound, in the plastids via the methylerythritol phosphate (MEP) pathway from the C5 precursor isopentenyl diphosphate. Progress in gene isolation over the past decade has illuminated the biosynthetic pathways and key regulatory controls of carotenoid formation (Fraser and Bramley, 2004).

The synthesis involves the coordination of structural genes and regulatory mechanisms, including chromoplast-specific enzyme variants, suggesting a specialized chromoplast-centric pathway for carotenoid biosynthesis, as observed in citrus, papaya, and other fruits (Galpaz et al., 2006; Alquézar et al., 2009; Devitt et al., 2010). Carotenoid biosynthesis is primarily regulated at the transcriptional level, with a strong correlation between the expression of critical genes, such as phytoene synthase (PSY), and the carotenoid content in fruits like citrus (Rodrigo et al., 2004; Bramley, 2002). Additional regulatory mechanisms include the orange (or) gene in cauliflower, which affects plastid differentiation and -carotene accumulation (Li et al., 2001). The sequestration capacity of plastids, the presence of binding proteins, and the esterification of hydroxycarotenoids are also known to influence carotenoid levels (Egea et al., 2010).

Although several genes and proteins associated with carotenoid biosynthesis and storage have been identified, the specific mechanisms that control carotenoid accumulation and degradation in fruit remain incompletely understood. Carotenoid profiles shift during fruit ripening into distinct patterns. For instance, in fruits like tomatoes with high carotenoid accumulation, levels increase during ripening, while in fruits such as berries or grapes, which rely on pigments other than carotenoids, levels may decrease (Gross, 1987).

Chloroplastic carotenoids, including lutein, carotene, neoxanthin, and zeaxanthin, are associated with chlorophyll-binding proteins during the early and green stages of fruit development. Upon maturation, chromoplasts in ripened, non-green fruits amass significant amounts of carotenoid compounds, yet the content and composition vary widely. While carotenogenic fruits exhibit pigmentation throughout their tissues, the outer pericarp is typically the most pigmented, although carotenoid profiles can differ within the same fruit (Gross, 1987). For instance, in red-fleshed grapefruit varieties, the flesh contains higher levels of the red carotene lycopene compared to the peel, which may accumulate only trace amounts of colorless carotenes (Alquézar et al., 2009).

In addition to the primary carotenoids, fruits may contain various minor carotenoids in trace or very low concentrations. Goodwin and Britton (1988) identified eight major patterns of carotenoid distribution across different fruits and vegetables, ranging from minimal amounts in fruits like grapes, to complex assortments featuring lycopene and -carotene, epoxides, and unique or species-specific carotenoids like lutein and zeaxanthin, with certain fruits displaying a combination of these patterns.

VOLATILE AROMA COMPOUNDS

Fruit flavor, a key determinant of fruit quality, can be modified through selective breeding, with flavor being a subjective experience influenced by a complex interplay of metabolites such as sugars, organic acids, and volatile aroma compounds (Klee, 2010; Baldwin, 2002). This discussion will focus on the biochemistry of major aroma compounds, as detailed sensory aspects of fruit flavor are covered in Chapter 3. Fruit aroma arises from a broad spectrum of volatile compounds present at minute concentrations, with a subset of 10-20 volatiles predominantly defining the aroma profile of ripened fruits (Schieberle & Hofmann, 1997; Baldwin et al., 2000).

Terpenoids, along with aliphatic and branched esters, and short-chain aldehydes and alcohols, form the core volatile components in fruits. Additionally, apocarotenoids and furan-related compounds significantly contribute to aroma. Volatile compounds, due to their diverse nature, originate from various biosynthetic pathways involving different cellular compartments and organelles. Terpenoids, composed of acetyl-CoA and pyruvate, represent a substantial portion of fruit fragrance molecules. Various prenyl transferases and terpene synthases (TPS) catalyze the synthesis of isoprenoids (C5), monoterpenes (C10), and diterpenes (C20) in the cytosol and plastids (Dudareva et al., 2004). The multiproduct nature of TPS enzymes accounts for the vast diversity of terpenes observed in nature (Degenhardt et al., 2009).

Citrus fruits are exemplary in their significant terpenoid content, with specific cellular localization of terpene synthesis. Terpenoid composition in citrus is influenced by species, variety, fruit tissue, and ripening stage, with the monoterpene limonene dominating the essential oils' volatile profile, followed by various minor monoterpenes and sesquiterpenes (Weiss, 1997; Sawamura, 2000). Recent studies have characterized numerous TPS and their association with limonene accumulation in citrus peels (Lucker et al., 2002; Shimada et al., 2005a). Bioinformatics analysis of citrus fruit EST databases has revealed potential gene clusters for TPS, which may account for the diversity of terpenes in citrus (Takita et al., 2007).

Citrus juices contain a distinct volatile profile from peels, with various aliphatic esters, aldehydes, and alcohols contributing to their aroma. Other fruits, such as strawberries, also exhibit significant terpene profiles affecting their scent (Aharoni et al., 2004). In grapes and apples, TPS enzymes are responsible for the production of aromatics like valencene and germacrene D, with their expression patterns linked to fruit development stages (Lucker et al., 2004; Martin & Bohlmann, 2009).

The biosynthesis of C6 and C9 aldehydes and alcohols involves multiple enzymes, including lipoxygenases (LOX), hydroperoxide lyases (HPL), and alcohol dehydrogenases (ADH), with tomato LOX genes demonstrating varied expression during ripening (Chen et al., 2004). Aliphatic esters, synthesized from oxidized lipids and further processed by AAT enzymes, are pivotal in enhancing fruit aromas (Olias et al., 2002; Beekwilder et al., 2004; Defilippi et al., 2005a). Ethylene plays a significant role in the modulation of aldehyde and fatty acid reduction and ester biosynthesis in fruits like apples (Flores et al., 2002).

Branched-chain aldehydes, alcohols, and esters, derived from amino acids, impart characteristic fruity aromas, such as banana and strawberry (Dudareva et al., 2006). The esterification of branched-chain alcohols, as observed in bananas and apples, is mediated by specific AATs and aminotransferases (Schaffer et al., 2007; Gonda et al., 2010).

Apocarotenoids, also known as norisoprenoids, contribute to fruit aroma with their distinct flavor characteristics and low odor thresholds. The cleavage of carotenoids by carotenoid cleavage dioxygenases (CCD) leads to the production of volatile apocarotenoids, which are significant in fruits like tomatoes, melons, and grapes (Simkin et al., 2004; Ibdah et al., 2006; Aubert et al., 2003).

Finally, the generation of furanones and pyrones, which impart unique scents to fruits such as pineapple and strawberry, involves specific metabolic pathways, although the complete biosynthetic steps are not fully elucidated (Bood & Zabetakis, 2002; Wein et al., 2001). These compounds arise from complex transformations that involve enzymes like enone oxidoreductases and O-methyltransferases.

OTHER COMPONENTS

Fruits are repositories of a multitude of phytochemicals, enzymes, minerals, dietary fibers, and other bioactive molecules alongside essential nutrients. Phytochemicals, a diverse group of compounds with antiviral, antibacterial, and antioxidant properties, are crucial for scavenging free radicals and thus impacting biological systems including immune response, lipid and steroid hormone metabolism, and blood pressure regulation (Jongen, 2002). Due to the absence of these phytochemicals in the human body, dietary intake from fruits becomes essential for health.

Current research indicates that the health benefits of fruits may also arise from the synergistic interactions among their various components. Factors influencing the concentration of these bioactive compounds in fruits include fertility of the soil, irrigation practices, maturity at harvest, harvesting techniques, and postharvest handling. Consequently, reported levels of these compounds can vary significantly, leading to potential discrepancies in published data. This variability underscores the importance of considering agricultural and postharvest factors when evaluating the nutritional and health benefits of fruits.

Vitamins

Vitamins are essential biochemical compounds required in small quantities for proper physiological function. The antioxidant vitamins, A, C, and E, are of particular interest due to their potential roles in preventing heart disease and cancer. Vitamin A, as retinol, is vital for reproduction, vision, and immune response. Dietary sources of vitamin A include preformed vitamin A from animal products and carotenoids from plant-based foods, with the latter being less likely to cause hypervitaminosis due to their lower toxicity. Vitamin A is measured in retinol equivalents (RE), with recommended daily allowances ranging from 210 µg/day for infants to 800 µg/day for lactating women. Among the carotenoids, only about 50 exhibit provitamin A activity, the most significant being β-carotene, α-carotene, and β-cryptoxanthin (with half the provitamin A activity of β-carotene). Fruits like strawberries and pineapple are poor in provitamin A due to low β-carotene content.

The vitamin B complex comprises essential vitamins including B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6, B9 (folate), and B12, although B12 is not present in fruits. Commonly found B vitamins in fruits include thiamine, riboflavin, niacin, and pantothenic acid, with fruits like avocado, orange, and pineapple containing them in varying amounts. Folate, crucial for nucleic acid and amino acid metabolism, has recommended intakes varying with pregnancy stages, from 65 µg/day to 520 µg/day of dietary folate equivalents. Avocado is a folate-rich fruit that contributes significantly to dietary needs.

Vitamin C exists as ascorbate and its oxidation product, dehydroascorbic acid. Rich sources include citrus fruits, kiwis, plums, and raspberries, with acerola having the highest ascorbic acid content among studied fruits (16.77 mg/g fresh weight). Vitamin E, comprising tocopherols and tocotrienols, is crucial nutritionally, especially α-tocopherol due to its high vitamin E activity. However, vitamin E levels in fruits are generally low.

Vitamin K exists primarily as phylloquinone (vitamin K1), with fruits like avocado, kiwi, papaya, and blackberries being good sources. These fruits contribute to the intake of vitamin K1, which is prevalent in various berries.

Table 2.1

	Vitamin									
	A	**B1**	**B2**	**B3**	**B5**	**B6**	**B9**	**C**	**E**	**K**
Avocado	7.0	0.07	0.13	1.74	1.39	0.26	81.0	8818.34	0.10	0.05
Banana	3.0	0.03	0.07	0.67	0.33	0.37	20.0	8733.33	0.10	0.05
Apricot	96	0.03	0.04	0.60	0.24	0.05	8.57	10,000.00	0.89	3.43
Kiwi (green)	4.0	0.03	0.03	0.34	0.18	0.06	25.0	92,763.16	1.46	40.26
Tangerines	34	0.06	0.04	0.38	0.22	0.08	16.0	26,700	0.20	0.00
Blackberries	11	0.02	0.03	0.65	0.28	0.03	25.0	20,972.22	1.17	19.79
Papaya	55	0.02	0.03	0.36	0.19	0.04	38.1	61,809.21	0.73	2.60
Mango	54	0.03	0.04	0.67	0.20	0.12	43.0	36,400	0.90	4.20
Plum	17	0.03	0.03	0.42	0.13	0.03	5.00	9545.45	0.26	6.36
Oranges	11	0.09	0.04	0.28	0.25	0.06	30.0	53,222.22	0.18	0.00
Squash	10	0.05	0.14	0.49	0.16	0.22	29.2	16,991.15	0.12	3.01
Tomato	42	0.04	0.02	0.59	0.09	0.08	15.0	13,700	0.54	7.90
Strawberry	1.0	0.20	0.02	0.39	0.13	0.05	24.1	58,795.18	0.28	2.22
Pineapple	3.0	0.08	0.03	0.50	0.21	0.11	18.0	47,806.45	0.02	0.71

Fiber

Dietary fiber encompasses a variety of indigestible plant components, with its composition differing across food types. The gelation properties of fruit fibers can contribute to a feeling of fullness. Soluble fibers are known to inhibit digestive enzymes, facilitating enhanced nutrient absorption. Both soluble and insoluble fibers are important for maintaining intestinal health and have been associated with a reduced risk of heart disease and breast cancer by modulating the flow of lipids, bile acids, and hormones from the gastrointestinal tract to the liver. Due to the distinct physiological effects of different fiber types, the daily intake necessary to achieve specific health outcomes can vary. Fruits such as avocados, blackberries, kiwis, and bananas, as well as apples and pears when consumed with their skin, are recognized as being high in dietary fiber.

Minerals

Minerals serve multifaceted roles within the human body. Magnesium and calcium are crucial as both cofactors in myriad enzymatic reactions and as structural elements that enhance bone density and integrity. Bananas are particularly noted for their high potassium content, which is significantly greater in comparison to many other fruits (refer to specific data in Table 2.2). Fruits also provide substantial amounts of calcium, magnesium, and phosphorus, all of which are integral to nucleic acid formation and cellular function.

Trace minerals, including manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), and sodium (Na), are present in fruits in smaller quantities but are essential for various biological functions. However, the bioavailability of some minerals in fruits can be limited. For instance, non-heme iron, which is the form of iron found in plant sources including fruits, and calcium that is bound to oxalates, a form in which it can be present in certain fruits, may not be fully absorbable by the body due to the presence of compounds that inhibit their absorption.

Table 2.2

	Minerals									
	Dietary Fiber	**Ca**	**Fe**	**Mg**	**P**	**K**	**Na**	**Zn**	**Cu**	**Mn**
Avocado	6.80	13	0.61	29	54	507	8	0.68	0.17	0.15
Banana	2.60	5	0.26	27	22	3358	1	0.15	0.08	0.27
Apricot	2.00	13	0.39	10	23	259	1	0.20	0.08	0.08
Kiwi (green)	3.00	34	0.31	17	34	312	3	0.14	0.13	0.10
Tangerines	1.80	37	0.15	12	20	166	2	0.07	0.42	0.04
Blackberries	5.30	29	0.63	20	22	162	1	0.53	0.17	0.65
Papaya	1.88	24	0.10	10	5.0	257	3	0.07	0.02	0.00
Mango	1.60	11	0.16	10	17	186	1	0.09	0.11	0.06
Plum	1.40	6	0.17	7.0	16	157	0	0.10	0.06	0.05
Oranges	2.40	40	0.10	10	14	181	0	0.07	0.05	0.03
Squash	1.10	15	0.35	17	38	262	2	0.29	0.05	0.18
Tomato	1.20	10	0.27	11	24	237	5	0.17	0.06	0.11
Strawberry	2.00	16	0.41	13	24	153	1	0.14	0.05	0.39
Pineapple	1.40	13	0.29	12	8	109	1	0.12	0.11	0.93

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