4

SECONDARY METABOLIC PROCESSES AND PRODUCTS

The synthesis and degradation of carbohydrates, organic acids, proteins, lipids, pigments, aromatic compounds, phenolics, vitamins and phytohormones are classified as secondary processes (i.e., secondary to respiration and photosynthesis), but the distinction is somewhat arbitrary. The metabolism of most of these products is absolutely essential in both the pre- and postharvest life of a product. During the postharvest period, there is continued synthesis of many compounds (for example, the volatile flavor components of apples and bananas) and a degradation of other compounds to provide energy and precursors for synthetic reactions. Many of these changes occurring after harvest, however, are not desirable. As a consequence, we strive to store products in a manner that minimizes undesirable changes.

Excluding the synthesis of carbohydrates and proteins, there are three primary pathways that lead to the diverse array of chemical compounds found in plants. These include: 1) the shikimic acid pathway that leads to the formation of lignin, coumarins, tannins, phenols and various aromatics; 2) the acetate-malonate pathway which forms the precursors of fatty acids, phospholipids, glycerides, waxes, and glycolipids; and 3) the acetate-mevalonate pathway which results in various terpenoids (gibberellins, carotenoids, abscisic acid) and steroids (Figure 4.1).

This chapter covers the major classes of plant constituents in postharvest products, critiques some of the quantitative and qualitative changes that occur after harvest and outlines the important environmental factors that accelerate these postharvest alterations.

1. CARBOHYDRATES

Carbohydrates are the most abundant biochemical constituent in plants, representing 50–80% of the total dry weight. They function as forms of stored energy reserves and make up much of the structural framework of the cells. In addition, simple carbohydrates such as the sugars sucrose and fructose, impart important quality attributes to many harvested products. The concentration of sugars alone can range from slight, as in lime fruit, to as much as 61% of the fresh weight in the date (Table 4.1).

Carbohydrates are molecules comprised of carbon, hydrogen and oxygen, however, many may also contain other elements such as nitrogen and phosphorous. As a group, they are defined as polyhydroxy aldehydes or ketones, or substances that yield either of these compounds

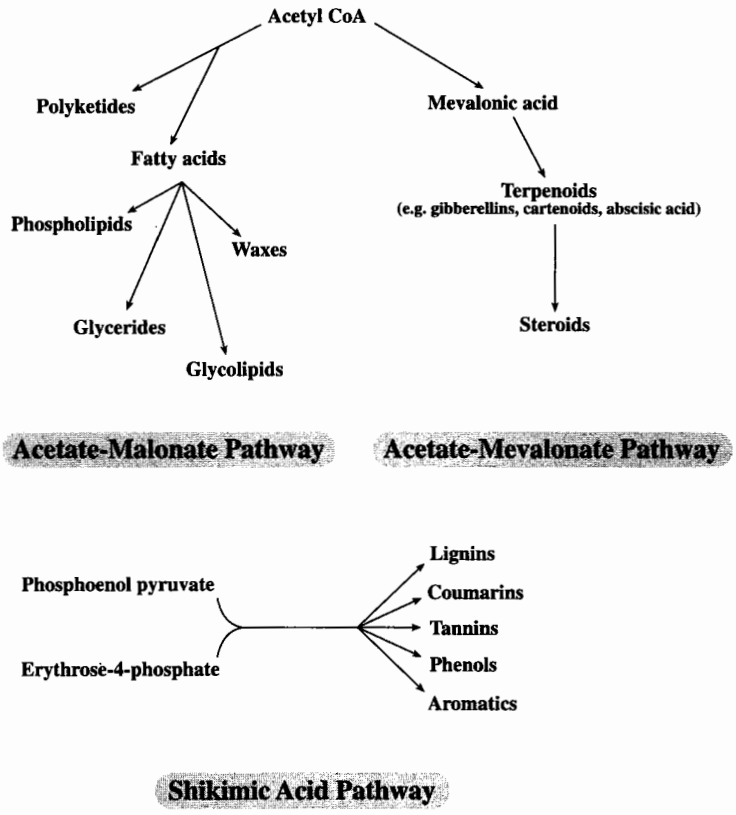
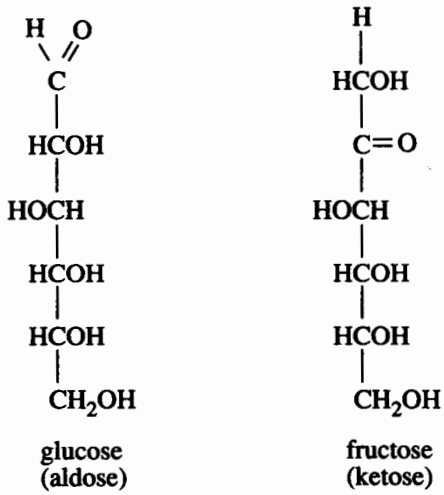


Figure 4.1. The three dominant pathways responsible for the synthesis of secondary plant products.

upon hydrolysis. Glucose and fructose, structural isomers of each other (both are $C_6H_{12}O_6$), illustrate the differences between the two types of sugars, aldoses and ketoses.



In addition, sugars that have a free or potentially free aldehyde group are classified as reducing sugars based on their ability to act as a reducing agent (accept electrons) in an alkaline solution. Most of the common sugars in plants are reducing sugars (e.g., glucose, fructose,

Table 4.1. General Range of Total Sugars and Relative Amounts of Specific Sugars Found in a Cross-Section of Fruits.

		*Specific Sugars** *(% Fresh Weight of Edible Portion)*		
	Total Sugars† *(% Fresh Weight)*	*Glucose*	*Fructose*	*Sucrose*
Apple	11.6	1.7	6.1	3.6
Avocado	0.4			
Currant, red	5.1	2.3	1.9	0.2
Date	61.0	32.0	23.7	8.2
Grape	14.8	8.2	7.3	
Lime	0.7			
Pineapple	12.3	2.3	1.4	7.9
Pear	10.0	2.4	7.0	1.0
Tomato	2.8	1.6	1.2	

**Source:* Widdowson and McCance.[246]

†*Source:* Biale;[16] Money;[167] Money and Christian;[168] Swisher and Swisher;[222] Widdowson and McCance.[246]

galactose, mannose, ribose and xylose). Sucrose and raffinose are the most common nonreducing sugars. The level of reducing sugars is important in several postharvest products such as white potato, to be used for chips (crisps). When the reducing sugar concentration in the potatoes is high, there is a greater incidence of undesirable browning reactions during frying. Improper handling and storage conditions prior to cooking can significantly increase the level of free reducing sugars, leading to a lower quality product.

Carbohydrates can be further classified, based on their degree of polymerization, into monosaccharides, oligosaccharides and polysaccharides. Simple sugars or monosaccharides represent the most fundamental group and cannot be further broken down into smaller sugar units. These basic units of carbohydrate chemistry are subclassed based upon the number of carbon atoms they contain (Figure 4.2). This number ranges from three in the triose sugars to seven in the heptose sugars, although occasionally octuloses are found. Both the glycolytic and pentose pathways are important in the synthesis of these molecules that are the building blocks for more complex carbohydrates. Monosaccharides may also be modified to form several types of compounds that are essential metabolic components of the cells. For example, amino and deoxy sugars, and sugar acids and alcohols, although seldom high in concentration in harvested products, are common (Figure 4.2). Less common are branched sugars such as apiose.

1.1. Monosaccharides

In many products the monosaccharides comprise a major portion of the total soluble sugars present (Table 4.1). Glucose and fructose are the predominant simple sugars found, especially in fruits, however, mannose, galactose, arabinose, xylose and various others are found in a number of harvest products.

1.2. Oligosaccharides

Oligosaccharides are more complex sugars that yield two to six molecules of simple sugars upon hydrolysis. For example, a disaccharide such as sucrose yields two monosaccharides

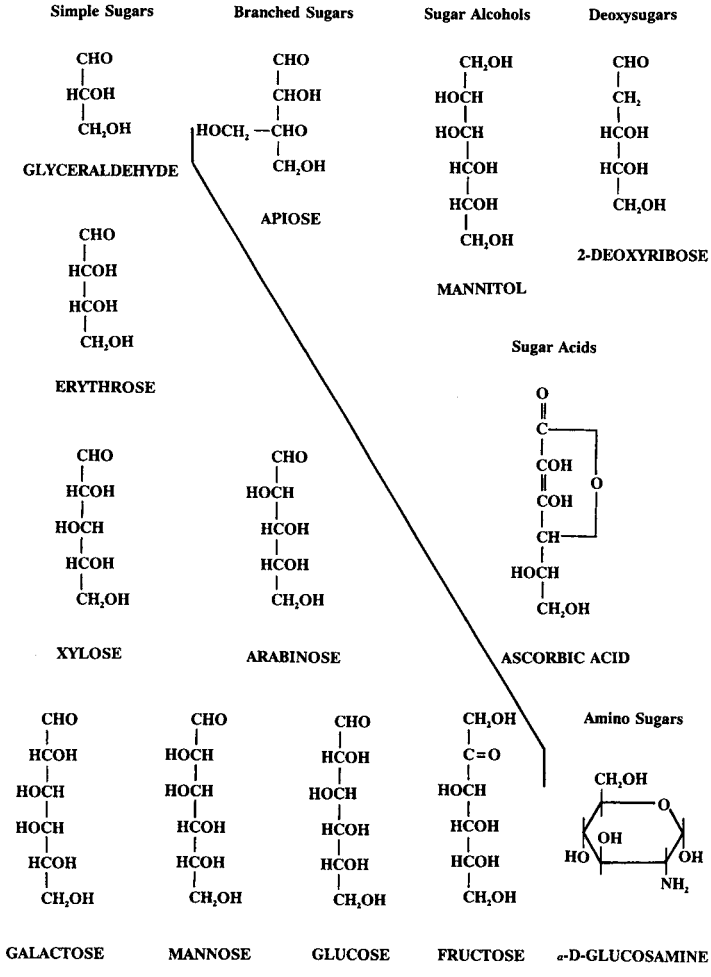


Figure 4.2. Common sugars and sugar derivatives.

(glucose and fructose) upon hydrolysis while a pentasaccharide yields five monosaccharide molecules. Both the monosaccharides and oligosaccharides are water-soluble and together they comprise the total soluble sugars in a product. The most abundant oligosaccharide is sucrose which is the primary transport form of carbohydrate in most plants. Sucrose phosphate synthetase appears to be the prevalent *in vivo* enzyme catalyzing the reversible reaction.[101]

$$\text{UDP-glucose} + \text{fructose-6-P} \rightarrow \text{sucrose-P} + \text{UDP}$$
$$\text{sucrose-P} \rightarrow \text{sucrose} + \text{Pi}$$

Sucrose can also be synthesized by sucrose synthase. This reaction is also reversible; however, the opposite direction (i.e., the hydrolysis of sucrose) is generally favored. In addition to sucrose synthase, sucrose may also be hydrolyzed by the enzyme invertase yielding glucose and fructose. Other common oligosaccharides are the disaccharide maltose found in germinating seeds and the trisaccharide raffinose and tetrasaccharide stachyose act as translocatable sugars in several species (Figure 4.3).

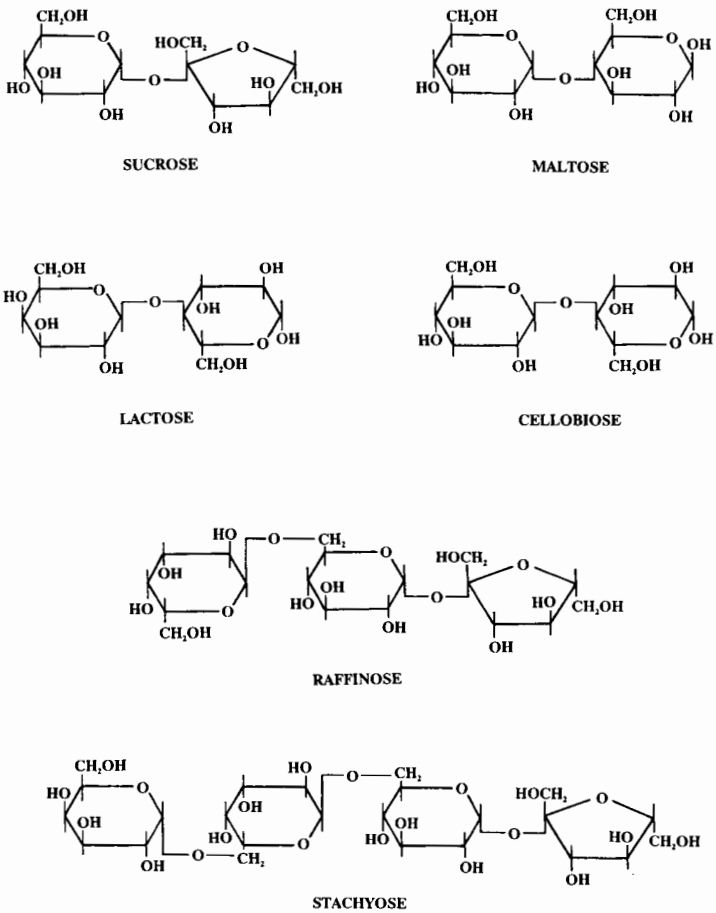


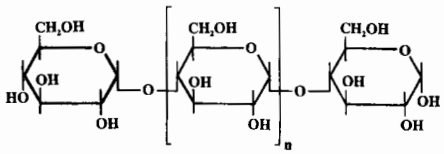
Figure 4.3. Molecular structure of some common oligosaccharides found in plants.

1.3. Polysaccharides

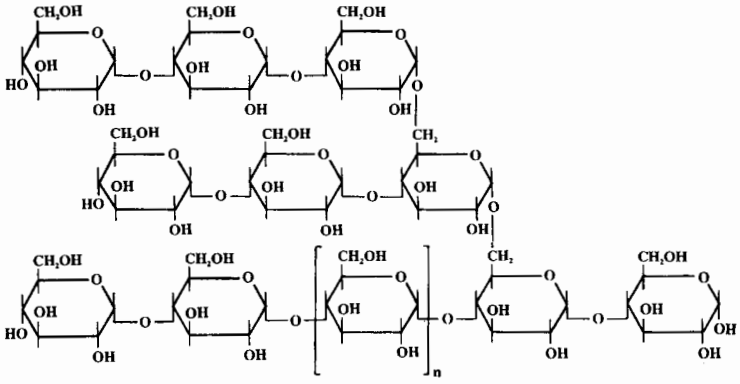
1.3.1. Cellulose

Cellulose is a straight-chain crystalline polymer of glucose and is one of the most abundant compounds in plants. Cellulose is not, however, the major component in many storage organs where storage carbohydrates or lipids often abound. For example, the cellulose content of dates in only 0.8%[35] whereas cotton fibers are 98% cellulose. Individual cellulose molecules can be extremely long, 1,000 to 10,000 glucose subunits, with molecular weights of 200,000 to 2,000,000 Da. The attachment between neighboring glucose molecules in the chain is a β-linkage between carbon 1 of one glucose molecule and carbon 4 of the next glucose and is referred to as a β-(1-4) linkage (Figure 4.4). Cellulose is found largely in the primary and secondary cell walls of the tissue.

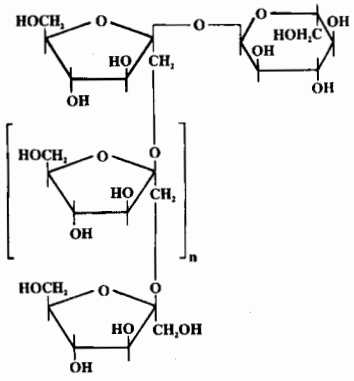
The synthesis of cellulose takes place in a plasma membrane bound protein complex that is guided by the cellular cytoplasm cytoskeleton.[43] The basic subunit for insertion into the chain is cellobiose (two glucoses) rather than individual glucose molecules.[44] UDP-glucose is an essential participant in the synthesis scheme.[36] In most detached products where there is little, if any, growth, cellulose synthesis is usually limited.



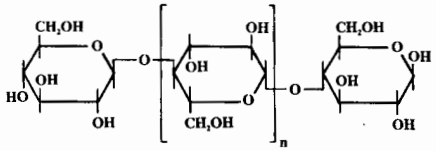
AMYLOSE



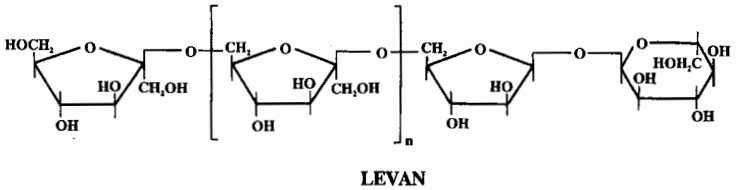
AMYLOPECTIN



INULIN



CELLULOSE



LEVAN

Figure 4.4. The structure of several common polysaccharides found in plants.

Cellulose molecules are extremely stable and can be broken down (hydrolyzed) only with strong acids or by enzymes such as cellulases, found in bacteria and fungi. There is often little change in the cellulose structure in fruits during ripening (e.g., peach[220]). Plants have a glucanase, often referred to as cellulase, that can degrade water-soluble molecules having the same glucose bonding but not crystalline cellulose. Glucanases are known to function during the abscission of leaves or other organs from the parent plant but do not act on cellulose.

1.3.2. Starch

Starch, composed of a mixture of branched and straight-chained glucose polymers, represents the major storage carbohydrate in most postharvest products. It is stored in the form of starch grains, found in specialized storage plastids (amyloplasts) and in leaf chloroplasts. Starch is comprised of two compounds: a straight-chained molecule, amylose that contains 200 to 1,000 glucose subunits and amylopectin, a branched chain molecule that is substantially larger, 2,000 to 200,000 subunits. Amylose has individual glucose molecules linked by α-(1-4) glucosidic bonds (Figure 4.4), the bonding angle of which imparts a helical structure to the molecule. Amylopectin has similar α-(1-4) bonds between glucose subunits, however, every 20 to 25 glucose molecules there is a branch formed through an α-(1-6) glucosidic linkage (Figure 4.4). The ratio of amylose to amylopectin is genetically controlled with amylopectin comprising the dominant form, e.g., 60 to >95%.

Biosynthetic or hydrolytic changes in the starch concentration are extremely important during the postharvest period for many commodities. For example, in banana and many other climacteric fruits, the conversion of starch to sugars in the fruit is an important component of the ripening process, giving the fruit its distinctive sweet flavor as well as precursors for many of the aromatic flavor compounds. On the other hand, in some products (e.g., sweet corn) free sugars can be readily converted to starch after harvest, decreasing the quality of the product. Because of these differing postharvest scenarios, an understanding of starch metabolism is advantageous.

a. Starch synthesis

Starch synthesis occurs in the chloroplasts of leaves and plastids in non-green tissue.[174] In plastids, synthesis probably begins with sucrose, the primary transport carbon source, which is converted to nucleotide diphosphate glucose. Subsequent polymerization is carried out by the enzyme starch synthetase, which requires an existing α-(1-4) glucan primer molecule of at least two glucose residues (e.g., maltose). There are several forms of starch synthetase (e.g., ADP-glucose and UDP-glucose transglucosylase) that catalyze the addition of glucose from either ADP or UDP-glucose nucleotides. Sucrose is converted to ADP-glucose either directly by sucrose synthase or indirectly through the action of invertase. Invertase first produces glucose and fructose with the glucose activated by ATP to ADP-glucose.

- ADP-glucose formation from sucrose

$$\text{sucrose} + H_2O \xrightarrow[\textit{invertase}]{} \text{glucose} + \text{fructose}$$

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose-6-P} \rightarrow \text{glucose-1-P}$$

$$\text{glucose-1-P} + \text{ATP} \rightarrow \text{ADP-glucose} + \text{PPi}$$

- Addition of glucose to an existing glucan chain

$$\text{n ADP-glucose} + \alpha\text{-(1–4) glucan primer} \rightarrow \text{starch} + \text{nADP}$$

The formation of amylopectin, requiring the addition of α-(1-6) branches, proceeds similarly; however, a branching enzyme (amylo-(1,4-1,6)-transglycosylase) catalyzes the branch addition every 20 to 25 glucose residues.

Several mutants of sweet corn have been found that give both higher sugar levels in the endosperm and decreased incorporation of the sugars into starch after harvest.[71,169] In the case of one mutant, both the branching enzyme and starch synthase levels are considerably reduced.[187]

b. Starch breakdown

Starch can be broken down to glucose by at least three different enzymes, α- and β-amylase and starch phosphorylase. The amylases hydrolyze starch into two glucose segments (maltose) that are then further hydrolyzed by the enzyme maltase to glucose.

$$\text{starch} + \text{n } H_2O \xrightarrow[\textit{amylase}]{} \text{n maltose}$$

$$\text{maltose} + H_2O \xrightarrow[\textit{maltase}]{} \text{2 glucose}$$

The α-amylases rapidly hydrolyze the α-(1-4) linkages of amylose at random points along the chain, forming fragments of approximately 10 glucose subunits called maltodextrins. These are more slowly hydrolyzed to maltose by the enzyme. Alpha-amylase also attacks the α-(1-4) linkages of amylopectin, however, in the regions of the α-(1-6) branch points it is inactive, leaving dextrins (>3 glucosyl units).[151] Beta-amylase removes maltose units starting from the nonreducing end of the starch chain and hydrolyses up to a α-(1-6) branching point. This yields maltose and dextrins.

Starch phosphorylase also attacks α-(1-4) linkages, but forms glucose-1-phosphate. Unlike the hydrolysis reactions of the amylases where a single water molecule is used in each bond cleavage, the phosphorylase enzyme incorporates phosphate.

$$\text{starch} + \text{n Pi} \xrightarrow[\textit{starch phosphorylase}]{} \text{n glucose-1-P}$$

Neither starch phosphorylase nor either of the amylases will attack the α-(1-6) branch points of amylopectin, so complete breakdown by these enzymes is not possible. Several debranching enzymes have been isolated from plants; however, neither their importance nor action are well understood.

1.3.3. Pectic Substances

The bulk of the primary cell walls in plants is comprised of dense gel-like, noncellulosic polysaccharides called pectic substances.[29] Pectin is found extensively in the middle lamella where it functions as a binding agent between neighboring cell walls. Each molecule is composed largely of α-(1-4) linked D-galacturonic acid subunits, although a number of other monosaccharides may by present (i.e., xylose, glucose, rhamnose, mannose, galactose, arabinose) (Table 4.2). There are three general classes of pectins based upon their chemical composition: a) homogalacturonans, b) xylogalacturons and c) rhamnogalacturonan I (Figure 4.5A).

Table 4.2. Gross Composition of the Cell Wall for Apple and Strawberry: Monomers Yielded Upon Hydrolysis of Wall Polymers.*

Component Monomers	% Total Accounted for	
	Apple	*Strawberry*
Rhamnose	0.4	1.1
Fucose	0.7	N.D.
Arabinose	19.5	6.5
Xylose	5.9	1.9
Mannose	1.9	0.7
Galactose	5.8	7.6
Glucose	47.5	31.1
Galacturonic acid	16.6	40.3
α-Amino acid	1.7	10.7
Hydroxyproline	0.04	0.1

**Source:* Knee.[129]
ND = not detected.

The structure of pectic substances varies widely with source. For example, jackfruit pectin[8] is comprised of only galacturonic acid residues; however, most species have rhamnose interspersed between segments of galacturonic acid residues. A 3-dimensional structure is conferred to the pectin matrix *via* cross-linking between neighboring pectin molecules by a) glucomannans, b) galactomannans and c) mannans (Figure 4.5B). Calcium (or magnesium) bridges between neighboring pectin molecules are also important in conferring a more ridged structure [Figure 4.5B(d)].

Pectic acids, found in the middle lamella and the primary cell wall, are the smallest of three general size classes (a very heterogenous group based upon solubility) and are usually around 100 galacturonic acid subunits in size. Pectic acids are soluble in water but may become insoluble if many of the carboxyl groups combine with Ca^{2+} or Mg^{2+} to form salts (see Ca^{2+} bridge in Figure 4.5B). Pectins are usually larger than pectic acids (e.g., 200 subunits) and have many of their carboxyls esterified by the addition of methyl groups. They are also found in the middle lamella, primary cell wall and in some cells as constituents of the cytoplasm. Protopectins are larger in molecular weight than pectins and intermediate in the degree of methylation between pectic acids and pectins. They are insoluble in hot water and are found primarily in the cell wall.

a. Synthesis of pectic substances

Pectic substances are synthesized from UDP-galacturonic acid on other UDP-sugars, although several other nucleotides may, in some cases, function in place of uridine diphosphate.[27,73] Methylation occurs after the subunit is placed in the chain and is catalyzed by methyl transferase. The methyl donor is S-adenosylmethionine.

b. Breakdown of pectic substances

The activity of pectic enzymes correlates with increases in softening during the latter stages of ripening of many fruits and a concurrent increase in soluble pectins. For example, the soluble pectin content of apples increases more than 3-fold during a 1.4 kg decrease in fruit firmness.[10] The increase is due to hydrolytic cleavage of the long pectic chains increasing their solubility. The principal pectic enzymes are pectinesterase, endopolygalacturonase and exopolygalac-

turonase. The early stages of fruit softening, however, are not apparently associated with the solubilization of pectin, however, the enzymes are operative in the later stages.

Pectinesterase or pectinmethylesterase catalyzes the hydrolysis of methyl esters along the pectic chain producing free carboxyl groups (Figure 4.5C). The enzyme deesterifies in a linear manner, moving down the chain and producing segments with free carboxyl groups. Deesterification by pectinesterase must precede degradation by polygalacturonases that require at least four galacturonic acid groups in sequence without methylation.

The polygalacturonases represent a class of pectolytic enzymes that degrade deesterified pectin chains into smaller molecular weight polymers and component monosaccharides.[81] Often two polygalacturonases are found in fruit tissues. Exopolygalacturonase cleaves single galacturonic acid subunits from the non-reducing end of the protopectin molecule (Figure 4.5C), while endopolygalacturonase attacks the chain randomly. Cleavage within the chain by endopolygalacturonase has a much more pronounced effect on the degree of solubilization of the pectic molecule and the pectin viscosity. Both enzymes are found in a number of fruits and often the increases in their activity tend to parallel the formation of water-soluble pectins and are thought to be involved in the later stages of fruit softening during ripening.[188]

1.3.4. Hemicelluloses

The hemicelluloses represent a heterogeneous group of polysaccharide compounds that are closely associated with cellulose, hence the name hemi (*half*) cellulose. They are stable, a major component of cell walls and normally can only be extracted with a strong base. Hemicelluloses do not represent carbohydrate reserves that can be recycled as an energy source for cells, though an exception may be mannans. These carbohydrates are composed largely of glucose, galactose, mannose, xylose, and arabinose molecules linked in various combinations and with varying degrees of branching. The total number of subunits ranges from 40 to 200. In monocots, the major hemicellulosic components of the cell walls are arabinoxylans[28] while in dicots, they are xyloglucans.[29] Hemicelluloses are synthesized from nucleotide sugars (e.g., UDP-xylose, UDP-arabinose, UDP-glucuronic acid) in reactions catalyzed by transferase enzymes.[27,73]

1.3.5. Fructosans

Some plant species store polymers of fructose as carbohydrate reserves rather than glucose. The polymers include the inulins and levans and are common in the Compositae, Campanulaceae and Graminae families.[186] Inulin is composed of a chain of 25 to 35 fructose subunits joined by β-linkages through C-1 of one molecule and C-2 of the adjacent, i.e., β-(2-1), and is terminated with a sucrose molecule. Inulin represents a population of structurally similar molecules that differ in the number of fructose subunits. Inulin is a straight-chained polymer, however, some fructosans are branched. They are substantially smaller than starch molecules and more soluble in water. Inulins are more commonly found stored in roots and tubers rather than above-ground plant parts. The tubers of Jerusalem artichokes and dahlia, the bulbs of iris and the roots of dandelion and chicory are high in inulin.

The synthesis of inulin begins with the addition of fructose to a terminal sucrose molecule forming a trisaccharide.[49] Complete synthesis of the polymer requires several enzymes:

- sucrose-sucrose 1-fructosyltransferase (SST) catalyzes the formation of the trisaccharide from two sucrose molecules;

$$\text{Glu–Fru} + \text{Glu–Fru} \rightarrow \text{Glu–Fru–Fru} + \text{Glu}$$

- Glucose is subsequently converted *via* several steps into sucrose;
- β-(2-1) fructan 1-fructosyltransferase (FFT) catalyzes the transfer of a fructose subunit from a donor to an acceptor, both of which are trisaccharides or greater in size.

$$\underset{\text{donor}}{\text{Glu–Fru–Fru}_N} + \underset{\text{acceptor}}{\text{Glu–Fru–Fru}_M} \rightarrow \underset{\text{donor}}{\text{Glu–Fru–Fru}_{N-1}} + \underset{\text{acceptor}}{\text{Glu–Fru–Fru}_{M+1}}$$

This reaction is reversible and also functions during depolymerization.

The degradation or depolymerization of inulin can follow one of two possible pathways. During cold storage, inulin is broken down into shorter chain length oligomers. This involves the action of hydrolases, β-(2-1′) fructan 1-fructosyl-transferase (FFT) and the enzymes involved in sucrose synthesis. During sprouting, inulin is degraded completely to fructose by hydrolases and the fructose converted to sucrose for export to the growing apices. Several yeasts have the enzymes required to hydrolyze the inulin polymer and subsequently convert the subunits to alcohol, thus enhancing the attractiveness of using inulin as a carbon substrate for alcohol production.[78]

Levans, another type of fructose polymer, are formed through a β-linkage between C-2 and C-6 of two adjacent fructose subunits, i.e., β-(2-6). As with inulin, levans are also terminated with a sucrose molecule. Levans are found primarily in the Gramineae family; for example, a levan called phlein is found stored in the roots of timothy.

1.3.6. Gums and Mucilages

Gums and mucilages are composed of a wide cross-section of sugar subunits and as a consequence, generalizations about their individual composition cannot easily be made. Hydrolysis of gum from plum fruits yields a mixture D-galactose, D-mannose, L-arabinose, D-xylose, L-rhamnose, D-glucuronic acid and traces of 4-0-methyl glucuronic acid.

These polymers may be found free in the cytoplasm or in some cases sequestered in specialized cells. As a group they are hydrophilic in nature and their function in the plant is not well understood. Gums are thought to be involved in sealing mechanical or pathogenic wounds to the plant while mucilages may function by modulating water uptake in seeds or water loss from some succulent species.

2. ORGANIC ACIDS

A number of harvested plant products contain significant concentrations of organic acids, many of which play a central role in metabolism. In addition, the levels of organic acids present often represent an important quality parameter; this is especially so in many fruits (Table 4.3).

Organic acids are small mono-, di- and tricarboxylic acids that exhibit acidic properties due to the presence of their carboxyl group(s) (COOH) that can give up a hydrogen atom. They exist as free acids or anions, or are combined as salts, esters, glycosides or other compounds. Organic acids are found in active pools that are utilized in the cytoplasm for metabolism and to a greater extent, as storage pools in the vacuole. For example, only about 30% of the malate is in the mitochondria, with the remainder thought to be in the vacuole. In some plant cells, certain organic acids may, to a large extent, be in the form of insoluble salts, e.g., calcium oxalates in rhubarb or potassium bitartrate in grapes. When in the ionized anion form ($-COO^-$), the name of the acid ends in "ate" (e.g., malate); while in the protonated state (-COOH), the ending is "ic" (e.g., malic acid).

Organic acids can be classified or grouped in a number of ways. For example, they may be grouped based on the number of carbon atoms present (typically 2 to 6) or on their specific function(s) within the cell. Another means of separation is based on the number of carboxylic groups present. This nomenclature gives a greater indication of how the acid will act chemically. The organic acids found in postharvest products (Figure 4.6), include monocarboxylic acids, monocarboxylic acids with alcohol, ketone or aldehyde groups, monocarboxylic carbocyclic aromatic and alicyclic acids, dicarboxylic acids and tricarboxylic acids. The type of acid present and the absolute and relative concentration of each, vary widely among different postharvest products (Table 4.3). Most organic acids are found in only trace amounts, however, several, such as malic, citric and tartaric, tend to be found in abundance in some tissues. The concentrations of these abundant acids varies widely among products, for example, lemon fruit contain 70–75 meq of citric acid · 100 g^{-1} fresh weight while the banana fruit has only 4 meq of malic acid · 100 g^{-1} fresh weight. In addition, in the first case (lemon), high acidity is a desirable flavor attribute while in the second it is not.

Organic acids play a central role in the general metabolism of postharvest products. A number of organic acids are essential components of the respiratory tricarboxylic acid cycle and phosphoglyceric acid plays an essential role in photosynthesis. Many organic acids have multiple functions in the plant. In some tissues with high concentrations, organic acids repre-

Table 4.3. The Organic Acids Present in the Fruit of Apple, Pear, Grape, Banana and Strawberry.*

Acids	*Apple*	*Pear*	*Grapes*	*Banana*	*Strawberry*
Glycolic	+	+	+	+	tr
Lactic	+	+	+	+	
Glyceric	+	+	+	+	tr
Pyruvic	+	+	+		
Glyoxylic		+	+	+	
Oxalic	+		+	+	
Succinic	+	+	+	+	+
Fumaric	+		+		
Malic	++	++	++	++	+
Tartaric			++		
Citramalic	+	+		+	
Citric	+	+	+	+	+++
Isocitric	+		+		
Cis aconitic			+		
Oxaloacetic	+		+	+	
α-Oxoglutaric	+	+	+	+	
Galacturonic	+	+	+		
Glucuronic	+		+		
Caffeic	+		+		
Chlorogenic	+	+	+		
p-Coumarylquinic	+				
Quinic	+	+	+	+	+
Shikimic	+	+	+	+	tr

* *Source:* Ulrich[235]; data derived from Hane;[83] Hulme and Wooltorton;[103] Kliewer;[127] Kollas;[132] Steward, et al.;[221] and Wyman and Palmer.[251] Increasing number of + signs denotes increased concentration; tr = trace.

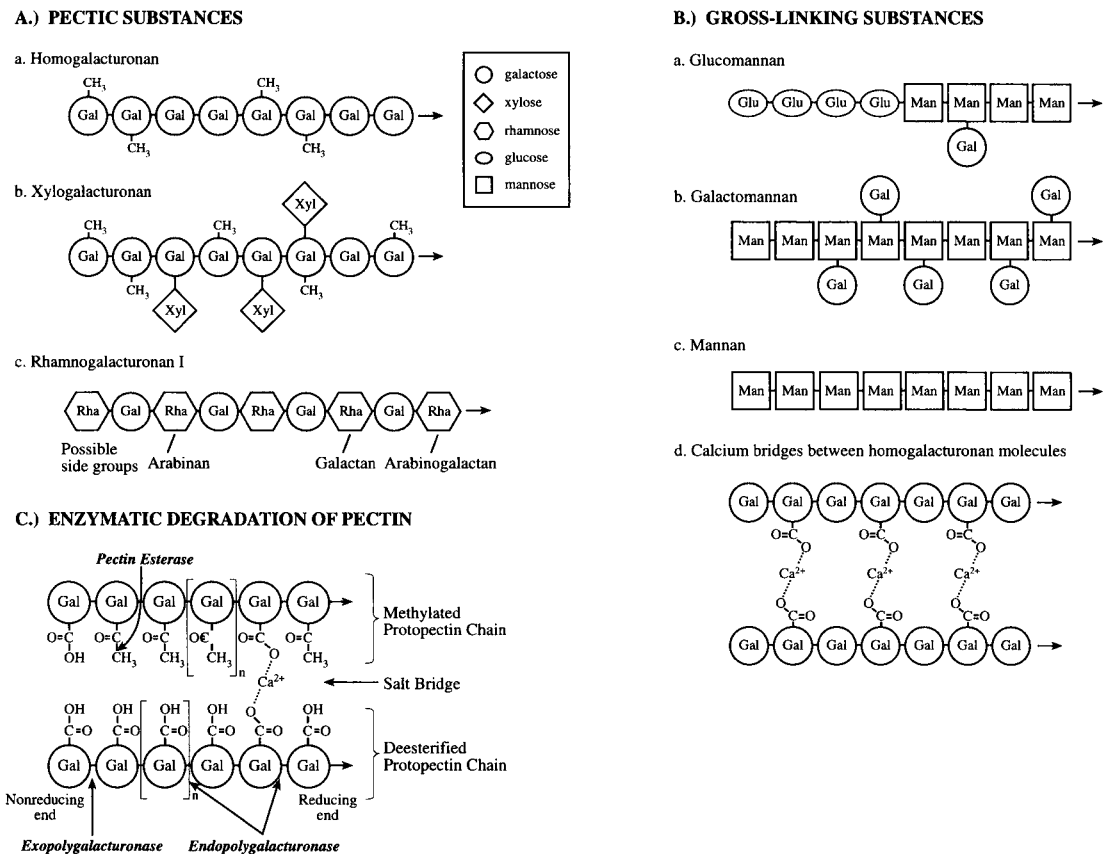


Figure 4.5. The cell wall region is comprised of cellulose, hemicellulose, protein, pectic substances (A) and a number of relatively complex cross-linking compounds (B), the latter of which confer rigidity to the three-dimensional wall structure. Calcium bridges (B-d) can form between carboxyl groups of neighboring pectin chains, tethering the molecules together and enhancing the structure. Degradation of pectic compounds during softening involves three enzymes (C). Pectinesterase removes methyl groups leaving free hydroxyls; exopolygalacturonase removes single galacturonic acid subunits starting at the non-reducing end of the molecule; and endopolygalacturonase cleaves the linkage between neighboring galacturonic acids randomly within the chain.

sent a readily available source of stored energy that can be utilized during the postharvest period. In food products, organic acids may impart a significant portion of the characteristic flavor, both taste and odor. Aromatic compounds, such as esters of organic acids, given off by various plant products are diverse in type (Table 4.4) and in some cases, such as isoamyl acetate in banana fruit, impart a major portion of the characteristic aroma.

Organic acids are synthesized primarily through oxidations, decarboxylations and, in some cases, carboxylations reactions in the respiratory tricarboxylic acid pathway. Some, however, are formed from sugars during the early stages of the photosynthetic dark reactions. Therefore, in most cases, the immediate precursors of organic acids are either other organic acids or sugars.

After harvest and during storage, the concentration of total organic acids tends to decline. Postharvest changes vary with the specific acid in question, the type of tissue, handling and storage conditions, cultivar, year and a number of other parameters. For example, the con-

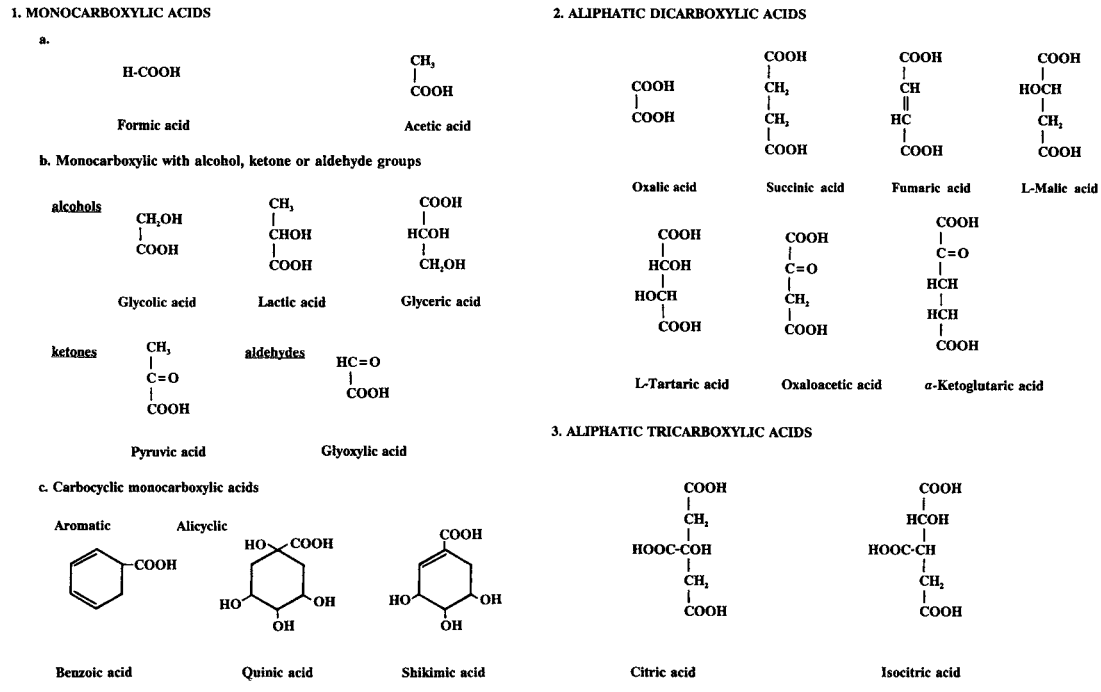


Figure 4.6. Structures of a number of the common organic acids found in plants.

Table 4.4. Organic Acids Emanating as Esters from Apples (cv. 'Cabville blanc').*

Methyl formate	tr	Methyl butyrate	tr
Propyl formate	tr	Ethyl butyrate	
Hexyl formate	tr	Propyl butyrate	tr
Isobutyl formate	tr	Butyl butyrate	
Methyl acetate	tr	Amyl butyrate	
Ethyl acetate		Hexyl butyrate	tr
Propyl acetate		Isopropyl butyrate	tr
Butyl acetate		Isobutyl butyrate	tr
Amyl acetate		Isoamyl butyrate	tr
Hexyl acetate		Ethyl isobutyrate	tr
Isobutyl acetate		Ethyl valerianate	tr
Butyl (second)	tr	Butyl valerianate	tr
Isoamyl acetate		Methyl caproate	tr
Methyl propionate	tr	Ethyl caproate	
Ethyl propionate	tr	Butyl caproate	
Propyl propionate	tr	Ethyl octanoate	tr
Butyl propionate			
Amyl propionate	tr		
Isoamyl propionate			

* *Source:* Paillard;[179] tr = trace.

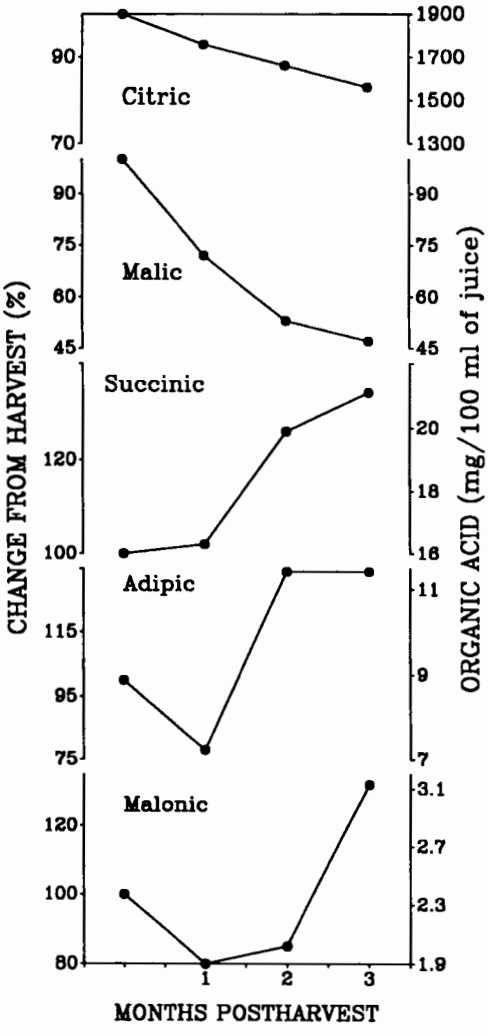


Figure 4.7. Changes in the organic acid composition of the juice of 'Shamouti' oranges during storage at 17°C. Changes are expressed as the % change from harvest and as mg/100 mL of juice (*redrawn from Sasson and Monselise*[201]).

centration of citric and malic acids in the juice of 'Shamouti' oranges declined with storage time while malonic, succinic and adipic acids increased (Figure 4.7). Changes in albedo (white interior portion of the peel) and flavedo (pigmented exterior portion of the peel) organic acids, however, did not parallel changes in the juice.[201] Likewise, both cultivar and year of production can have a pronounced effect on the concentration of specific acids and total titratable acidity (Table 4.5).

Controlled atmosphere storage has been shown to alter the changes in organic acids occurring after harvest. The juice of 'Valencia' oranges stored at 3% O_2 and 5% CO_2 (3.5°C) lost less acid than oranges held in air (0°C). Principal differences were a decreased rate of malic acid loss and an increase in quinic and shikimic acids in controlled atmosphere stored fruit.[132] These effects may in part be due to the dark fixation of CO_2 by the fruit.

Table 4.5. Differences in the Organic Acid Concentration and Titratable Acidity for 12 Muscadine Grape Cultivars (*Vitis rotundifolia* Michx).*

Cultivar	*Malate (%)*	*Tartrate (%)*	*Citrate (%)*	*Titratable acidity (%)*
Albemarle	0.49†	0.25	0.05	0.75
Carlos	0.54	0.19	0.04	0.85
Chowan	0.50	0.23	0.05	0.82
Dearing	0.45	0.23	0.04	0.71
Hunt	0.54	0.32	0.04	0.93
Magnolia	0.33	0.23	0.05	0.64
Magoon	0.43	0.41	0.02	0.94
Pamlico	0.62	0.24	0.04	1.05
Roanoke	0.70	0.29	0.04	0.10
Scuppernong	0.52	0.28	0.04	0.87
Thomas	0.51	0.27	0.05	0.84
Topsail	0.37	0.19	0.04	0.51
Roanoke 1965	0.26	0.23	0.05	0.52
1966	0.67	0.27	0.06	1.22
1967	1.06	0.38	0.02	1.55

**Source:* Carroll et al.[30] and Kliewer.[127]
†Mean of three seasons.

3. PROTEINS AND AMINO ACIDS

Proteins are extremely important components of living cells in that they regulate metabolism, act as structural molecules and in some products, represent storage forms of carbon and nitrogen. Proteins are composed of chains of amino acids each joined together by a peptide bond (Figure 4.8). When there are fewer than 10 amino acids, they are referred to as peptides, 10 to 100 amino acids are polypeptides and more than 100 amino acids are proteins. Many of the properties of a polypeptide or protein are a function of which amino acids are in the chain and their particular sequence in the molecule. In plants, approximately 20 amino acids are commonly found, however, over 100 nonprotein amino acids are known.

Each of the amino acids that make up a protein has distinct properties that, in turn, influence the properties of the polypeptide in which they are found. Amino acids are small in size

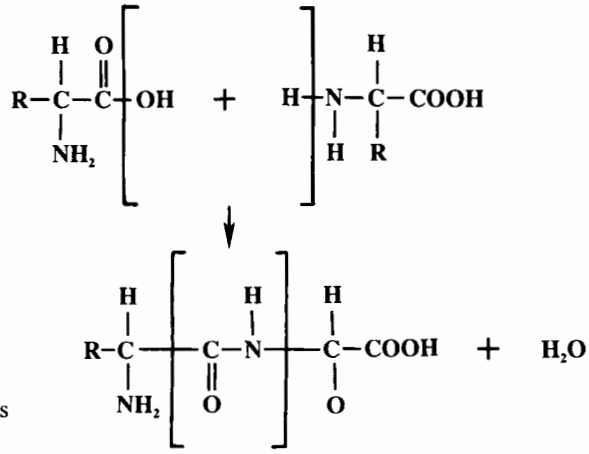


Figure 4.8. Proteins are composed of amino acids joined together in long chains by peptide bonds.

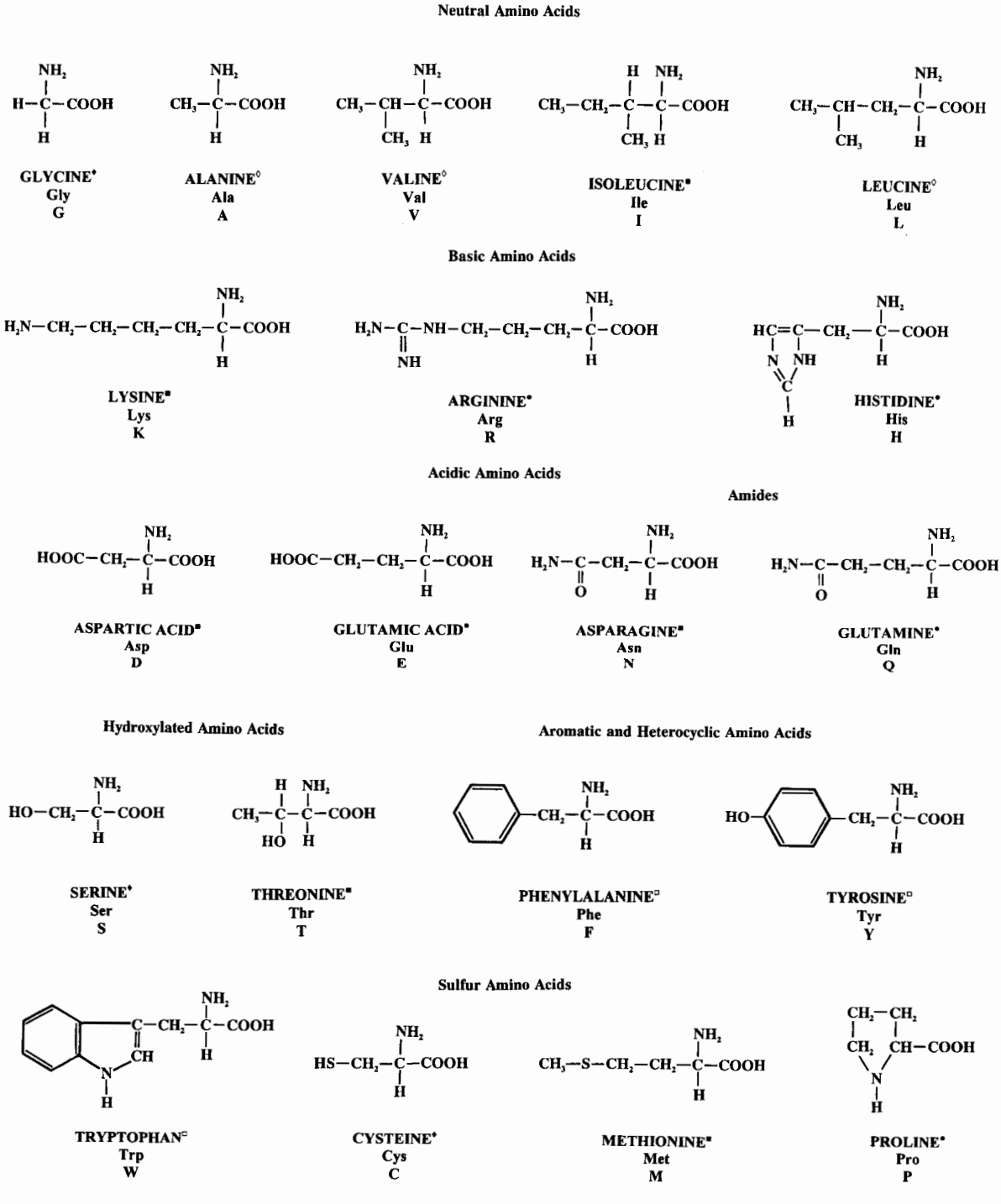
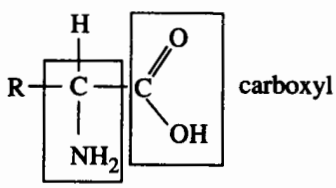


Figure 4.9. The classes and structures of the amino acids found in plant proteins. Plants also contain a relatively diverse cross-section of non-protein amino acids in addition to the above. Symbols indicate precursors: ■ oxaloacetate; ♦ 3-phosphoglycerate; □ phosphoenolpyruvate; ● α-ketoglutarate; and ◇ pyruvate.

Met-Ala-Thr-Lys-Ile-Leu-Ala-Leu-Leu-Ala-Leu-Leu-Ala-Leu-Leu-Val-Ser-Ala-Thr-Asn-Ala-Phe-Ile-Ile-Pro-Gln-Cys-Ser-Leu-Ala-Pro-Ser-Ala-Ser-Ile-Pro-Gln-Phe-Leu-Pro-Pro-Val-Thr-Ser-Met-Gly-Phe-Glu-His-Pro-Ala-Val-Gln-Ala-Tyr-Arg-Leu-Gln-Leu-Ala-Leu-Ala-Ala-Ser-Ala-Leu-Gln-Gln-Pro-Ile-Ala-Gln-Leu-Gln-Gln-Gln-Ser-Leu-Ala-His-Leu-Thr-Leu-Gln-Thr-Ile-Ala-Thr-Gln-Gln-Gln-Gln-Gln-Gln-Gln-Phe-Leu-Pro-Ser-Leu-Ser-His-Leu-Ala-Met-Val-Asn-Pro-Val-Thr-Tyr-Leu-Gln-Gln-Gln-Leu-Leu-Ala-Ser-Asn-Pro-Leu-Ala-Leu-Ala-Asn-Val-Ala-Ala-Tyr-Gln-Gln-Gln-Gln-Gln-Leu-Gln-Gln-Phe-Met-Pro-Val-Leu-Ser-Gln-Leu-Ala-Met-Val-Asn-Pro-Ala-Val-Tyr-Leu-Gln-Leu-Leu-Ser-Ser-Ser-Pro-Leu-Ala-Val-Gly-Asn-Ala-Pro-Thr-Tyr-Leu-Gln-Gln-Gln-Leu-Leu-Gln-Gln-Ile-Val-Pro-Ala-Leu-Thr-Gln-Leu-Ala-Val-Ala-Asn-Pro-Ala-Ala-Tyr-Leu-Gln-Gln-Leu-Leu-Pro-Phe-Asn-Gln-Leu-Ala-Val-Ser-Asn-Ser-Ala-Ala-Tyr-Leu-Gln-Gln-Arg-Gln-Gln-Leu-Leu-Asn-Pro-Leu-Ala-Val-Ala-Asn-Pro-Leu-Val-Ala-Thr-Phe-Leu-Gln-Gln-Gln-Gln-Gln-Leu-Leu-Pro-Tyr-Asn-Gln-Phe-Ser-Leu-Met-Asn-Pro-Ala-Leu-Gln-Gln-Pro-Ile-Val-Gly-Gly-Ala-Ile-Phe

Figure 4.10. The amino acid sequence for one of four distinct groups forming the storage protein zein (approximate molecular weight = 22,000), as determined by sequencing its mRNA (*after Marks and Larkins*[153]).

and soluble in water. Each contains both a carboxyl group (-COOH) and an amino group (-NH) and some may also have hydroxyl groups (-OH), sulfhydryl groups (-SH) or amide groups (-CONH) present.



Typically, amino acids are classified into one of six types based upon the properties of their R group (Figure 4.9) and are designated by their common name, abbreviation or by a single letter. When denoting the amino acid sequence in a large protein, the single letter designation is commonly used.

a) Neutral amino acids—where R is a hydrogen, aliphatic or hydroxyl group (glycine, alanine, valine, leucine and isoleucine).

b) Basic amino acids (arginine and lysine).
c) Acidic amino acids (aspartic acid, glutamic acid), and their amides (asparagine and glutamine).
d) Hydroxylated amino acids (serine and threonine).
e) Aromatic and heterocyclic amino acids (phenylalanine, tyrosine, proline, tryptophan and histidine).
f) Sulfur containing amino acids (cysteine and methionine).

3.1. Protein Synthesis

During the postharvest period, the metabolic processes within plant cells continue, requiring specific proteins to be synthesized at precise times. Synthesis and degradation are the two primary means of modulating the level of a specific protein. As a consequence, protein synthesis, especially the synthesis of specific proteins after harvest, is of interest to postharvest biologists. The sequence for each protein, as illustrated by one of the component groups of the storage protein zein (Figure 4.10), is found coded in the cell's DNA (Figure 4.11). This coded sequence is transcribed by the formation of a special type of ribonucleic acid, the messenger RNAs (mRNA), thus transferring the required amino acid sequence to a molecule that can move from the nucleus into the cytoplasm where the actual synthesis of the protein molecule occurs. The messenger RNA subsequently has a ribosome attached that translates the code from the mRNA and assembles each amino acid in the appropriate sequence to form the polypeptide. Several ribosomes are often attached to an mRNA strand forming a polysome. One protein molecule is assembled per ribosome at a time. In some cases, there are modifications of the amino acids in the protein after assembly of the amino acids; this process is called post-translational modifications.

A very general overview of the steps in protein synthesis is given in Figure 4.12. As one would anticipate, the precise method in which DNA is transcribed to mRNA and mRNA is translated to form the polypeptide is much more complex than indicated by this brief overview. Several references listed in the back of this section give a more detailed account.[21,152]

3.2. Protein Structure

The proteins formed are folded into a three-dimensional structure that is a function of the kind, number and sequence of the amino acids present and the type of nonprotein (prosthetic) groups attached to the amino acids. Protein structure is typically broken down into 4 levels of organization: primary, secondary, tertiary and quaternary (Figure 4.13). The primary structure is the kind, number and sequence of the amino acids present in the chain, while the secondary structure is the conformation of the chain of amino acids due to hydrogen bonding between the oxygen of a carboxyl group and a nitrogen atom of a neighboring amino group. Tertiary structure, which leads to folding or bending of the chain, is due to interactions between side chains on certain amino acids and adjacent portions within the chain and is facilitated by proteins called chaperones. These interactions may be by hydrogen and ionic bonds, and allow the aggregation of polypeptides into a globular, planar, or fibrous forms. Quaternary structure is when two or more different proteins come together to form a complex to carry out a certain function.

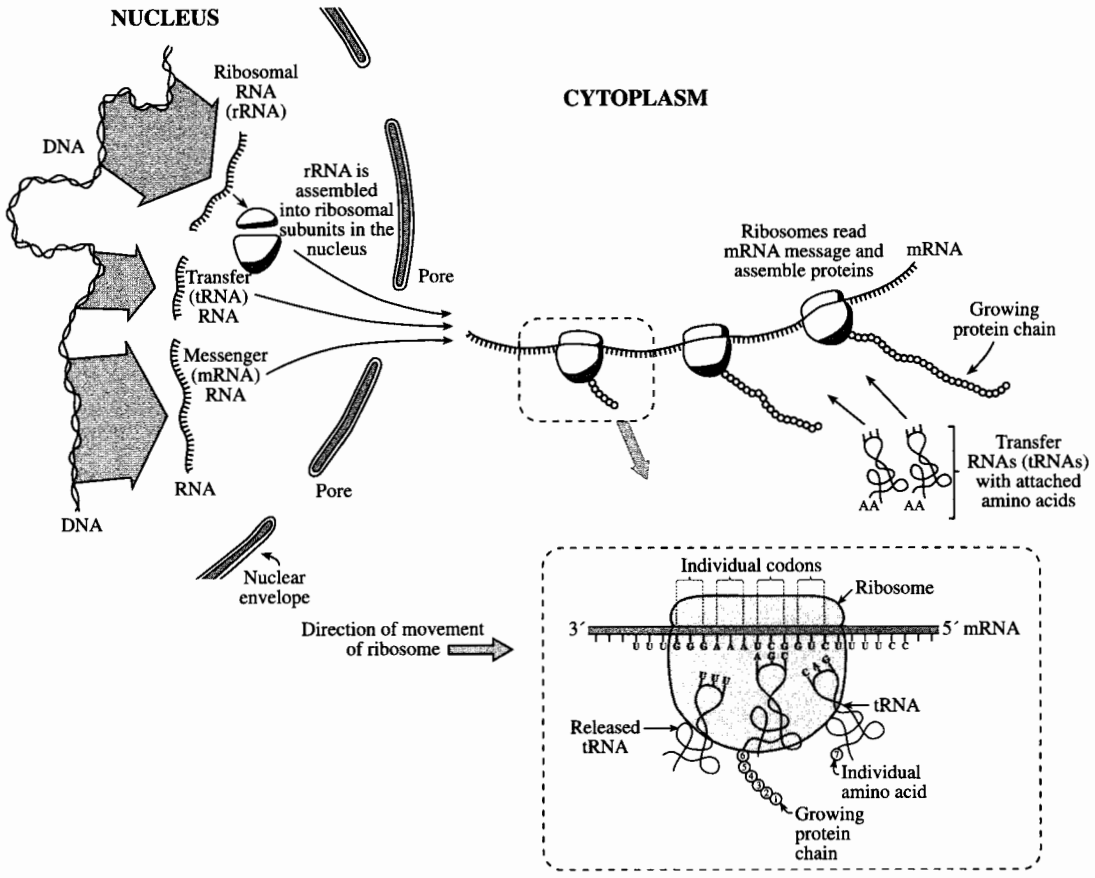


Figure 4.11. Diagrammatic representation of the synthesis of a protein. The amino acid sequence for an individual protein is transcribed with the synthesis of a specific messenger RNA (mRNA) for the protein. Ribosomal RNA (rRNA) and transfer RNA (tRNA), also derived from the DNA molecule, are likewise present. Once in the cytoplasm, several ribosomes attach to an mRNA molecule and moving down the molecule, translating the code. Insertion of the appropriate amino acid, carried to the ribosome by its tRNA, allows assembly of the protein in the appropriate sequence, one amino acid at a time. Many proteins undergo some post-translational modifications once the initial amino acid sequence is assembled.

3.3. Protein Classification

Proteins can be classified based on: a) their physical and/or chemical properties, b) the type of molecules that may be joined to the protein, or c) by the function of the protein within the cell. The first type of classification, based on chemical and/or physical properties, typically separates proteins into groups based on their size, structure, solubility or degree of basicity (kind and number of basic amino acids, e.g., lysine, arginine and histidine). The second means of classification, based on the prosthetic group attached, separates proteins into classes such as lipoproteins (lipid), nucleoproteins (nucleic acid), chromoproteins (pigment), metalloproteins (metal) and glycoproteins (carbohydrate). Proteins can also be classified based upon their function in the plant. They are typically grouped into three general classes; structural proteins (e.g., membrane-bound proteins, cell wall proteins), storage proteins and enzymes. In some instances, however, the classes overlap. For example, many en-

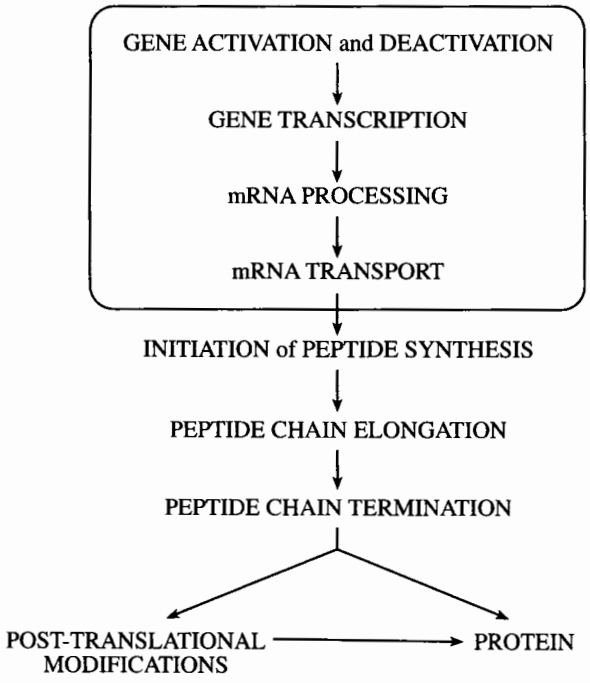


Figure 4.12. The major sequence of steps in protein synthesis and their location in the cell is presented in this flow chart.

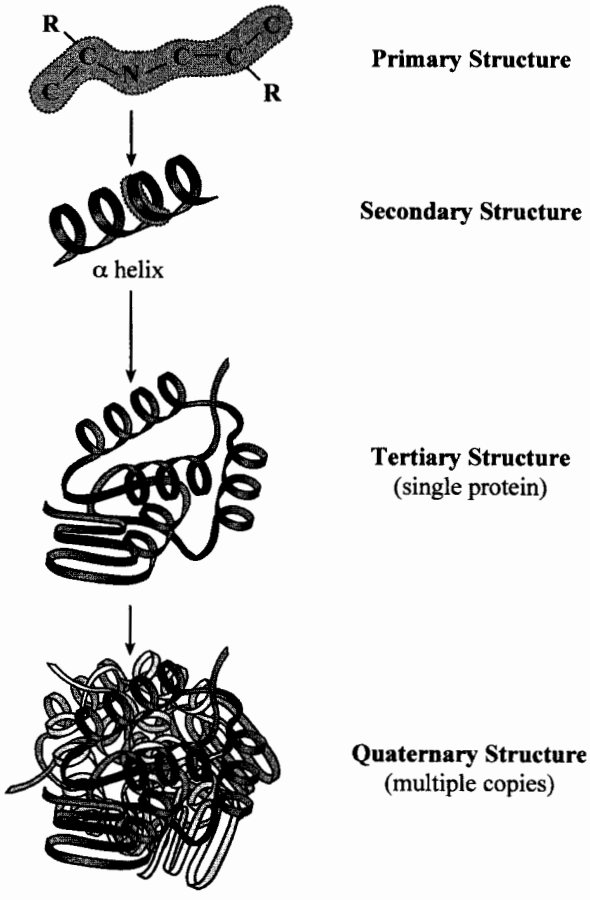


Figure 4.13. The three dimensional structure of proteins is a critical component of their functional specificity. The primary structure is conferred by the peptide bond of each amino acid with the secondary structure, forming an α-helix or pleated sheet (not shown), due to bonding between neighboring amino acids within the chain. The tertiary structure is created by the folding and cross-linking of various parts of the protein, a process facilitated by two classes of molecules called chaperones. The quaternary structure is due to the positioning of multiple copies of the protein forming stacks in a specific orientation. Not all proteins are grouped into a quaternary structure, however, storage proteins such as zein are commonly found in this manner, consolidating the protein and decreasing the space required.

zymes are also components of membranes and may, therefore, have a multiple role within the cell.

Storage proteins, found in abundance in seeds, serve as a source of nitrogen and amino acids during germination. Cereal grains contain on an average 10% protein, largely as storage protein, while legumes 20–30%. Together these make up the major portion of the protein consumed by man, e.g., ~70%. Most other edible crops are lower in protein (Table 4.6).

Enzymatic proteins are extremely important in that they regulate virtually all of the biochemical reactions within cells. Primarily through enzyme synthesis, activation and degradation, control is exerted over the rate of specific processes, thus allowing the plant product to adjust its metabolism to changes in the environment in which it is held and to genetically controlled metabolic shifts (e.g., ripening).

Enzymes are grouped based on their type of catalytic function:

a) Oxidoreductases—catalyze oxidation-reduction reactions, e.g., malate dehydrogenase.
b) Transferases—catalyze the transfer of a specific group from one molecule to another, e.g., methionine transferase in the ethylene synthesis pathway.
c) Hydrolases—catalyze hydrolysis by the addition of water, e.g., amylase.
d) Lyases—catalyze the addition or removal of groups without the involvement of water, e.g., phosphoenolpyruvate carboxylase.
e) Isomerases—catalyze isomerizations, e.g., the conversion of glucose-6-phosphate to fructose-6-phosphate by phosphoglucoisomerase.
f) Ligases—catalyze condensing reactions, e.g., pyruvate carboxylase.

3.4. Protein Degradation

Most of the proteins within cells are in a continuous state of synthesis and degradation. After synthesis, they begin to progress toward eventual degradation and recycling of their component

Table 4.6. Amino Acid Composition of Several Types of Postharvest Products.*

				Amino Acids (mg/g of nitrogen)							
Food Group	*Plant Part Used*	*Common Name*	*Protein Content (g · 100g⁻¹)*	*Isoleucine*	*Leucine*	*Lysine*	*Methionine*	*Cystine*	*Phenylalanine*	*Tyrosine*	*Threonine*
Cereal	Seed	Rice	7.1	306	563	219	225	100	350	300	288
	Seed	Wheat	12.2	204	417	179	94	159	282	187	183
Pulses	Seed	Chickpea	20.1	277	468	428	65	74	358	183	235
	Seed	Bean	22.1	262	476	450	66	53	326	158	248
Roots and Tubers	Tuber	White potato	2.0	236	377	299	81	37	251	171	235
	Root	Sweetpotato	1.3	230	340	214	106	69	241	146	236
Vegetables	Leaves	Lettuce	1.3	238	394	238	112	—	319	169	256
	Stems	Celery	1.1	244	425	150	138	—	281	—	213
	Flowers	Cauliflower	2.8	302	436	356	99	—	225	—	264
	Immature seed	Pea	6.6	260	435	456	58	60	275	194	235
	Immature seed	Green bean	2.4	234	432	344	81	53	266	209	241
Fruits	Fruit	Apple	0.4	220	390	370	49	84	160	94	230
	Fruit	Banana	1.2	181	294	256	125	169	244	163	213
Nuts	Nut	Coconut	6.6	244	419	220	120	76	283	167	212
	Nut	Brazil nut	14.8	175	431	175	363	131	244	169	163
Milk	Cow's milk		3.5	295	596	487	157	51	336	297	278
Eggs	Hen egg		12.4	393	551	436	210	152	358	260	320

* *Source:* Data from FAO.[55]

parts. The length of their life expectancy varies widely among different proteins; it may be from as short as a few minutes to as long as years. The pathway for degradation involves recognition of a protein targeted for degradation, attachment of a small protein (ubiquitin) to the targeted protein and subsequent degradation of the protein by various proteases.[237] The degradation of storage proteins in seeds during germination has been the focus of much research but differs from the process in cells. The turnover of proteins in organs such as leaves, fruits, roots and tubers is under developmental control, though at this time it is not well understood.

Enzymes that cleave the peptide bonds in protein chains (peptidases, proteinases or proteases) are classified as either endopeptidases or exopeptidases. Endopeptidases cleave peptide bonds within the protein chain while exopeptidases remove individual amino acids from either the carbon or nitrogen end of the molecule. These two classes are further broken down into subgroups based on the enzyme's mechanism of action, i.e., there are currently four types of endopeptidases and two types of exopeptidases. The site of degradation may be in the cytoplasm, the vacuole or exterior to the plasma membrane, and individual steps at a specific locale may be sequestered in separate compartments within the cell.

3.5. Changes in Amino Acids and Proteins after Harvest

The relative change in the protein and amino acid content and composition in harvested plant organs is greatest in those products that undergo significant changes during the postharvest period (e.g., leaf senescence). Products such as seeds that are relatively stable when properly stored, do not undergo substantial changes in their protein composition. Two general phenomena that precipitate large changes in protein and amino acid content and composition, are the onset of senescence and fruit ripening.

Leaf tissue has been used widely as a model system for studying senescence, since the changes are substantial and occur quite rapidly. Senescence in leaves is characterized by a decline in photosynthesis and the loss of protein and chlorophyll (Figure 4.14). Proteolysis, the

Table 4.6. (*continued*)

Amino Acids (mg/g of nitrogen)											
Tryptophane	*Valine*	*Arginine*	*Histidine*	*Alanine*	*Aspartic Acid*	*Glutamic Acid*	*Glycine*	*Proline*	*Serine*	*Total Essential Amino Acids*	*Total Amino Acids*
73	463	644	188	—	275	731	381	313	363	2887	—
—	276	288	143	226	308	1866	245	621	287	2049	*6033*
—	284	588	165	271	726	991	251	263	318	2426	*5998*
—	287	355	177	262	748	924	237	223	347	2389	*5662*
—	292	311	94	278	775	639	237	235	259	2082	*4910*
—	283	307	84	298	825	541	234	219	255	1972	*4735*
—	338	281	100	269	719	638	256	325	206	—	—
81	300	289	120	—	—	—	—	—	—	—	—
86	347	250	94	—	—	—	—	—	—	—	—
—	296	548	133	281	620	910	246	240	281	2332	*5591*
—	306	266	147	275	750	669	238	238	334	2253	*5170*
58	250	170	120	280	1300	700	240	200	270	1905	*5205*
—	250	469	469	275	656	575	263	256	244	1968	*5175*
—	339	822	128	279	553	1171	281	233	303	2148	*5918*
119	269	831	144	219	463	1163	275	300	269	2239	*5903*
—	362	205	167	217	481	1390	123	571	362	2947	*6463*
—	428	381	152	370	601	796	207	260	478	3201	*6446*

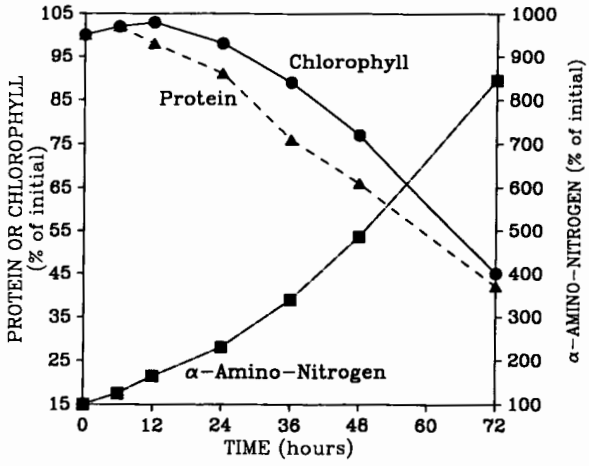


Figure 4.14. Changes in concentration of chlorophyll, protein, and α-amino nitrogen in detached *Avena* leaves held for varying intervals in the dark (*redrawn from Martin and Thimann*[155]).

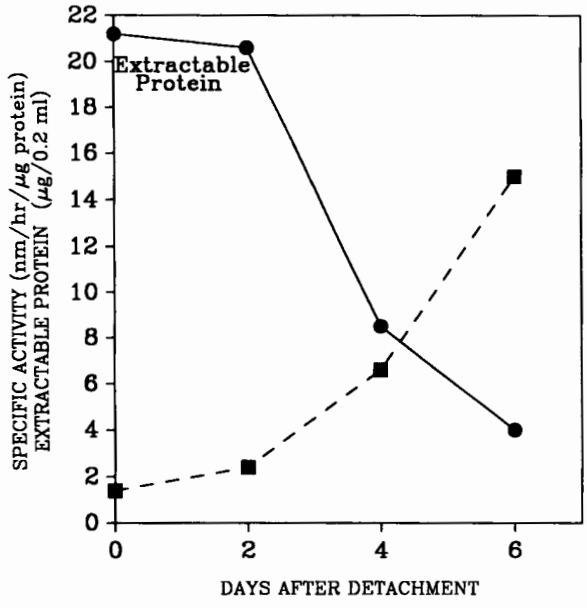


Figure 4.15. The increase in peptidase (protease) specific activity and concurrent decrease in protein concentration with time in detached *Avena* leaves held in the dark (*redrawn from Martin and Thimann*[155]).

breakdown of protein, begins fairly rapidly after the individual leaf is detached from the parent plant.[155,156] Peptidases (proteinases) which cleave proteins are always present within the leaves, however, their concentrations increase substantially at the onset of senescence (Figure 4.15). While most enzymes are declining, certain specific enzymes increase in activity and/or concentration during senescence.[22] For example, glutamate dehydrogenase activity increases by as much as 400% in spinach leaves held in the dark[110] where it appears to stimulate the deamination of amino acids.

Therefore, while proteins are being broken down and the component amino acids recycled, a small but extremely important number of specific proteins are also being synthesized.[22] Their importance in the development of proteolysis and senescence can be inferred from the fact that inhibitors of protein synthesis strongly decrease the rate at which senescence proceeds. The amino acids formed are largely transported, often after conversion to glutamine, to other parts of the plant and this transport is greatest to areas that represent strong sinks (high demand) such as reproductive organs.[34] In leaves detached at harvest, transport out of the organ is not possible thus the protein degradation products tend to accumulate.

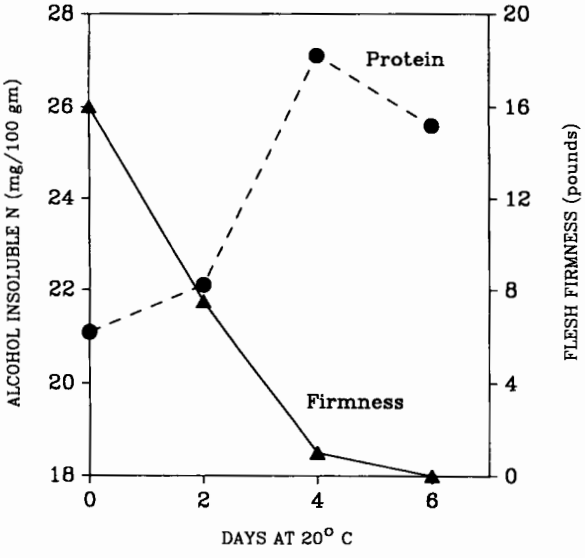


Figure 4.16. Changes in protein synthesis and firmness of Bartlett pears during ripening *(redrawn from Frenkel et al.*[66]).

During the onset of ripening of several climacteric fruits, it has been shown that the actual concentration of protein increases (Figure 4.16). In apples, avocados and several other climacteric fruits, enhanced synthesis of both RNA and protein occurs.[102,194,195] The net effect is an enhanced activity of certain enzymes during ripening (Table 4.7). As with protein synthesis during the onset of leaf senescence, these new proteins appear to be essential since ripening is inhibited if protein synthesis is inhibited. The increase in synthesis of specific enzymes has been monitored using labeled amino acids, then identifying the enzymes that are radioactive and by determining what genes are expressed during ripening.[81] Malic enzyme, which catalyzes the decarboxylation of malic acid, the primary organic acid in apples and certain pear cultivars, is an example of one enzyme that increases markedly during the climacteric. The increase in malic enzyme activity increases the concentration of pyruvic acid, the product of the reaction, which can then enter the respiratory tricarboxylic acid cycle.

4. LIPIDS

Plant lipids represent a very broad group of compounds that play diverse roles in the physiology and metabolism of harvested products. In addition, the absolute concentration of these compounds also varies widely among different species and plant parts. Most postharvest products, however, are relatively low in total lipids, exceptions include avocados, olives and seeds that are high in lipids (Table 4.8). A majority of lipids present are in the form of storage compounds that, in the case of seeds, can be used as an energy source during germination. Plant lipids, in addition to representing a storage form of carbon, also function as components of cellular membranes, as cuticular waxes forming a protective surface on many products and in some cases, as vitamins, pigments, sterols and secondary products.

Biochemically, lipids are normally grouped into neutral lipids, phospholipids, glycolipids, waxes, and terpenoids. Neutral lipids are comprised of fats and oils and represent primarily carbon storage compounds. Phospholipids and glycolipids are components of cellular membranes. Waxes are typically long-chain fatty acids or esters of fatty acids and long-chain alcohols, although numerous other compounds may be found.[130] These compounds form the thin waxy layer on the surface of leaves, fruits and other plant parts. Terpenoids are primarily water-insoluble acyclic and cyclic compounds such as steroids, essential oils and rubber.

Table 4.7. Changes in the Activity of Various Enzymes and Isoenzymes During the Development and Ripening of a Tomato Fruit.*

Enzyme	Stage with Peak Activity	State with Maximum Number of Bands	Activity Trend During Development	Number of Bands at		
				SG	MG	OR
Tyrosinase	LG†	MG	Decrease	1	4	2
Peroxidase	OR	MG-OR	Increase	1	4	4
Esterase	MG	MG	Peak at MG	11	13	8
Acid phosphatase	SG	SG	Decrease	8	6	3
Glycerophosphatase	SG	red	Decrease	1	3	2
ATPase	SG	SG	Decrease	6	5	5
$NADH_2$-diaphorase	MG	MG	Peak at MG	10	12	11
Fumarase	MG	MG	Peak at MG	1	4	2
Malate dehydrogenase	LG red	all	Maximum LG-OR	4	4	4
$NADP^+$ malic enzyme	MG	MG	Peak at MG	3	4	2
Iso-citrate dehydrogenase	SG	MG-OR	Decrease	1	2	2
Glutamate dehydrogenase	MG	SG-MG	Peak at MG	6	6	1
Phosphofructokinase	MG?	MG	Peak at MG?	2	4	3
6-Phosphogluconate dehydrogenase	MG	MG	Peak at MG	2	6	2
Phosphoglucomutase	MG	MG	Peak at MG	3	6	1
Phosphohexose isomerase	SG-MG	MG	SG-MG then a decrease	6	10	6
Glucose-6-phosphate dehydrogenase	SG-MG	MG	Peak at MG	3	4	3
Glutamate-oxaloacetate transaminase	SG-MG	all	Decrease	4	4	4
Leucine aminopeptidase	red?	all	Peak at red?	3	3	3

**Source:* After Hobson.[97]

†*Notes:* SG, small green; LG, large green (preclimacteric); MG, mature green (close to the beginning of the climacteric rise); red, nearly fully ripe; and OR, overripe (postclimateric).

4.1. Fatty Acids

A substantial portion of the physical and chemical properties of lipids are due to the long chains of fatty acids present. These fatty acids may be saturated (no double bonds) or unsaturated to varying degrees. The most common fatty acids in plants range from 4 to 26 carbons in size (Table 4.9), with oleic and linoleic being the most prevalent in nature. Their structure has a zigzag configuration (Figure 4.17) and double bonds, as seen in the example of oleic and linoleic acids, tend to result in curvature of the molecule.

Several methods are utilized to designate fatty acids. They may be referred to by their common name, systematic name or quite commonly by the use of an abbreviation. The short or abbreviated form simply denotes the number of carbon atoms in the molecule and the number of double bonds present. For example, linoleic acid (18:2) has 18 carbons with 2 double bonds. In some cases, the position of the double bond may be designated next to the common name of the unsaturated fatty acids (Table 4.9).

Most fatty acids have an even number of carbons although trace amounts of straight-chain, odd-numbered carbon compounds from C_7 to C_{35} have been detected. For example in pecan kernels, $C_{15:0}$, $C_{15:1}$, $C_{15:2}$, $C_{17:0}$, $C_{17:1}$, $C_{17:2}$ and $C_{21:0}$ fatty acids have been found (Table 4.10) but their concentration represents only approximately 2.1% of the total fatty acids.[209,210]

Table 4.8. Lipid Content of Several Types of Harvested Products.*

	Lipid Content of Edible Portion	
Product	*% Dry weight*	*% Fresh weight*
Fruits		
avocado	63.0	16.4
banana	0.8	0.2
olive	69.0	13.8
Seeds		
peanut	50.3	47.5
rice	0.5	0.4
walnut	61.2	59.3
Leaves		
amaranth	3.8	0.5
cabbage	2.6	0.2
lettuce	2.2	0.1
Roots and tubers		
parsnip	*2.4*	*0.5*
potato	0.4	0.1
radish	1.8	0.1

* *Source:* Data from Watt and Merril.[242]

Table 4.9. Common Fatty Acids in Harvested Plant Products.

Abbreviation	*Systematic Name*	*Common Name*	*Formula*
Saturated Fatty Acids			
4:0	Butanoic	Butyric acid	$CH_3(CH_2)_2COOH$
6:0	Hexanoic	Caproic acid	$CH_3(CH_2)_4COOH$
8:0	Octanoic	Caprylic acid	$CH_3(CH_2)_6COOH$
10:0	Decanoic	Capric acid	$CH_3(CH_2)_8COOH$
12:0	Dodecanoic	Lauric acid	$CH_3(CH_2)_{10}COOH$
14:0	Tetradecanoic	Myristic acid	$CH_3(CH_2)_{12}COOH$
16:0	Hexadecanoic	Palmitic acid	$CH_3(CH_2)_{14}COOH$
18:0	Octadecanoic	Stearic acid	$CH_3(CH_2)_{16}COOH$
20:0	Eicosanoic	Arachidic acid	$CH_3(CH_2)_{18}COOH$
22:0	Docosanoic	Behenic acid	$CH_3(CH_2)_{20}COOH$
24:0	Tetracosanoic	Lignoceric acid	$CH_3(CH_2)_{22}COOH$
Unsaturated Fatty Acids			
16:1	9-Hexadecenoic*	Palmitoleic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
18:1	9-Octadecenoic	Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
18:2	9, 12-Octadecadienoic	Linoleic acid	$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$
18:3	9, 12,15-Octadecatrienoic	Linolenic acid	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$

*The double bond is between carbons 9 and 10.

4.2. Triacylglycerols

Triacylglycerols (previously called triglycerides) are comprised of three fatty acids linked through ester bonds to a glycerol molecule. Mono- and diacylglycerols may also be present with the fatty acid-free position(s) on the glycerol molecule being bonded to other compounds, thus yielding a wide range of other types of lipids.

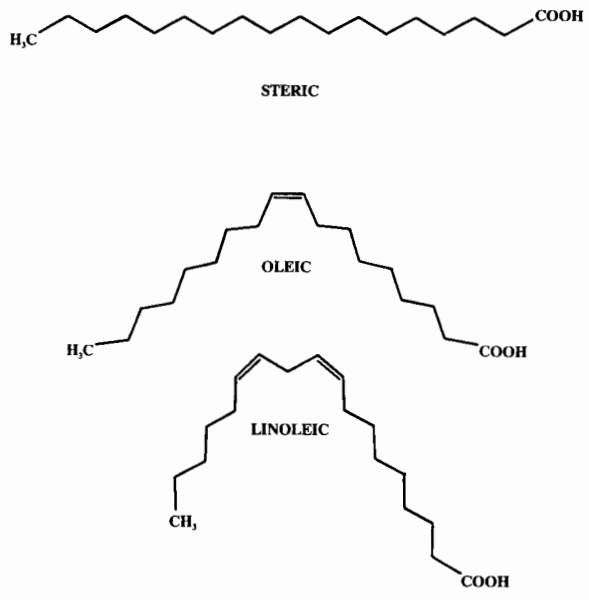
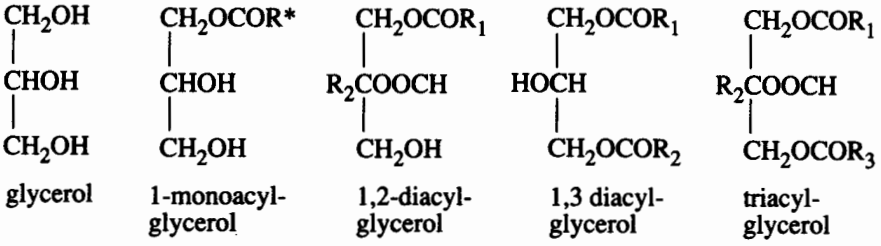
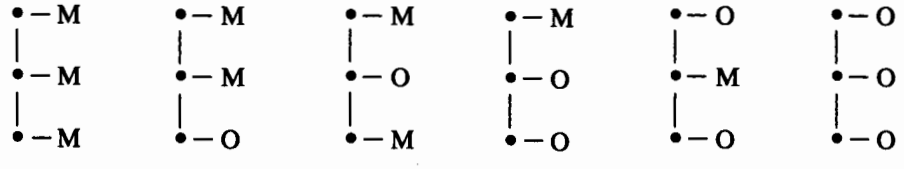


Figure 4.17. The structure of three fatty acids. Note the change in geometry with the addition of each double bond.



*R = alkyl group

Triacylglycerols that are present in nature are normally mixtures and these mixtures are often very complex due to the different fatty acids that can be esterified to each of the three hydroxyl positions. For example, the combination of only two fatty acids at all possible positions on the molecule yields six potential triacylglycerol compounds.



4.3. Neutral Lipids

The neutral lipids are largely triacylglycerols that make up the fats and oils found in plants. Waxes are also grouped here; however, due to their distinctly different physiological role in harvested products, they will be treated separately. As a group, neutral lipids do not have

Table 4.10. Fatty Acid Composition of Pecans.*

Abbreviation	*Systematic Name*	*Common Name*	*Concentration (g/100 g nut meat)*
Saturated fatty acids			
10:0	decanoic	capric	<0.20
12:0	dedecanoic	lauric	<0.20
14:0	tetracecanoic	myristic	1.20
15:0	pentadecanoic		<0.2
16:0	hexadecanoic	palmitic	4.09
17.0	heptadecanoic	margaric	0.27
18:0	octadecanoic	stearic	1.51
20:0	eicosanoic	arachidic	0.44
21:0	heneicosanoic		<0.20
Unsaturated fatty acids			
12:1	dedecenoic		<0.20
14:1	tetradecenoic		<0.20
14:2	tetradecadienoic		<0.20
15:1	pentadecenoic		<0.20
15:2	pentadecadienoic		<0.20
16:1	hexadecenoic	palmitolic	0.42
16:2	hexadecadienoic		<0.20
17:1	heptadecenoic		0.26
17:2	heptadecadienoic		<0.20
18:1	octadecenoic	oleic	37.90
18:2	octadecadienoic	linoleic	22.53
18:3	eicosenoic		0.54
20:2	eicosodienoic		<0.20

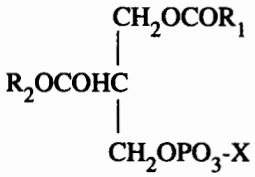
**Source:* Data from Senter and Horvat.[209, 210] Data for the major fatty acids represent means of six cultivars.

charged functional groups attached. The distinction between fats and oils is based simply on their physical form at room temperature. Oils are liquid and tend to contain a larger percentage of unsaturated fatty acids (e.g., oleic, linoleic and linolenic) than fats that are solid at room temperature. Fats have saturated fatty acids as their major component.

The role of these storage lipids has been studied most extensively in oil containing seed crops (linseed, canola, castor bean). Here they represent a source of energy and carbon skeletons during the germination period. In the avocado, which has high levels of lipids in the mesocarp tissue, existing evidence suggests that it does not represent a source of respiratory substrate during the ripening process.

4.4. Phospholipids and Glycolipids

Phospholipids and glycolipids are important components of cellular membranes. Phospholipids are often diacylglycerols that yield inorganic phosphate upon hydrolysis. They are characteristically high in the fatty acid linoleic. The fatty acid portion forms the hydrophobic tail which strongly influences the orientation of the molecule. Common examples of phospholipids in plants are phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl glycerol and phosphatidyl inositol. They represent important components of both the cytoplasmic and mitochondrial membranes.



R = fatty acids, e.g., linoleic acid

X = serine, ethanolamine, choline, glycerol, or inositol

Base structure of phospholipids

Glycolipids, in contrast, have carbohydrate substitutions without phosphate and are important components of the chloroplast membranes. Common examples would be monogalactosyldiglyceride and digalactosyldiglyceride. Approximately 70% of the fatty acid component in these photosynthetic membranes is linolenic acid.

4.5. Waxes, Cutin and Suberin

The outer surface of plants is protected by three general types of lipid compounds.[111] Waxes, typically esters of a larger molecular weight fatty acid and a higher aliphatic alcohol, and cutin, hydroxy fatty acid polymers, act as a protective coating on much of the above ground parts of the plant. Similarly, suberin, a lipid-derived polymeric material, is found on underground plant parts and on healed surfaces of wounds. Like cutin, suberin is often imbedded with waxes.[238]

Plant waxes are extremely important during the postharvest storage and marketing of plant products in that they limit water loss from the tissue and impede pathogen invasion. As a group, waxes are chemically very heterogeneous. In addition to being esters of a higher fatty acid and a higher aliphatic alcohol, waxes contain alkanes, primary alcohols, long chain free fatty acids and other groups. Alkanes may represent over 90% of the hydrocarbons in waxes of apple fruit and *Brassica oleracea.*[131,162] Some plant products (e.g., rutabaga, orange, pineapple) may benefit from the application of supplemental wax after harvest due to insufficient natural waxes on the product, inadvertent removal of surface waxes during washing operations, or the desire to enhance surface glossiness.

The precise chemistry and structure of both cutin and suberin are not fully elucidated, although substantially more is known about the former. Both represent very complex heterogeneous compounds. Cutin, a polyester, is composed primarily of C_{16} and/or C_{18} monomers.[111] General differences in the composition of cutin and suberin are given in Table 4.11 with tentative models in Figure 4.18.

4.6. Fatty Acid and Lipid Synthesis

Fatty acids are synthesized within the cytosol of the cell and in many cases within certain plastids (e.g., chloroplasts, chromoplasts). There are two distinct pathways, one forming saturated

Table 4.11. Differences in the Monomer Composition of Cutin and Suberin.*

	Cutin	*Suberin*
Dicarboxylic acids	Minor	Major
In-chain-substituted acids	Major	Minor
Phenolics	Low	High
Very long-chain (C_{20}-C_{26}) acids	Rare and Minor	Common and Substantial
Very long-chain alcohols	Rare and Minor	Common and Substantial

**Source:* Kolattukudy.[130]

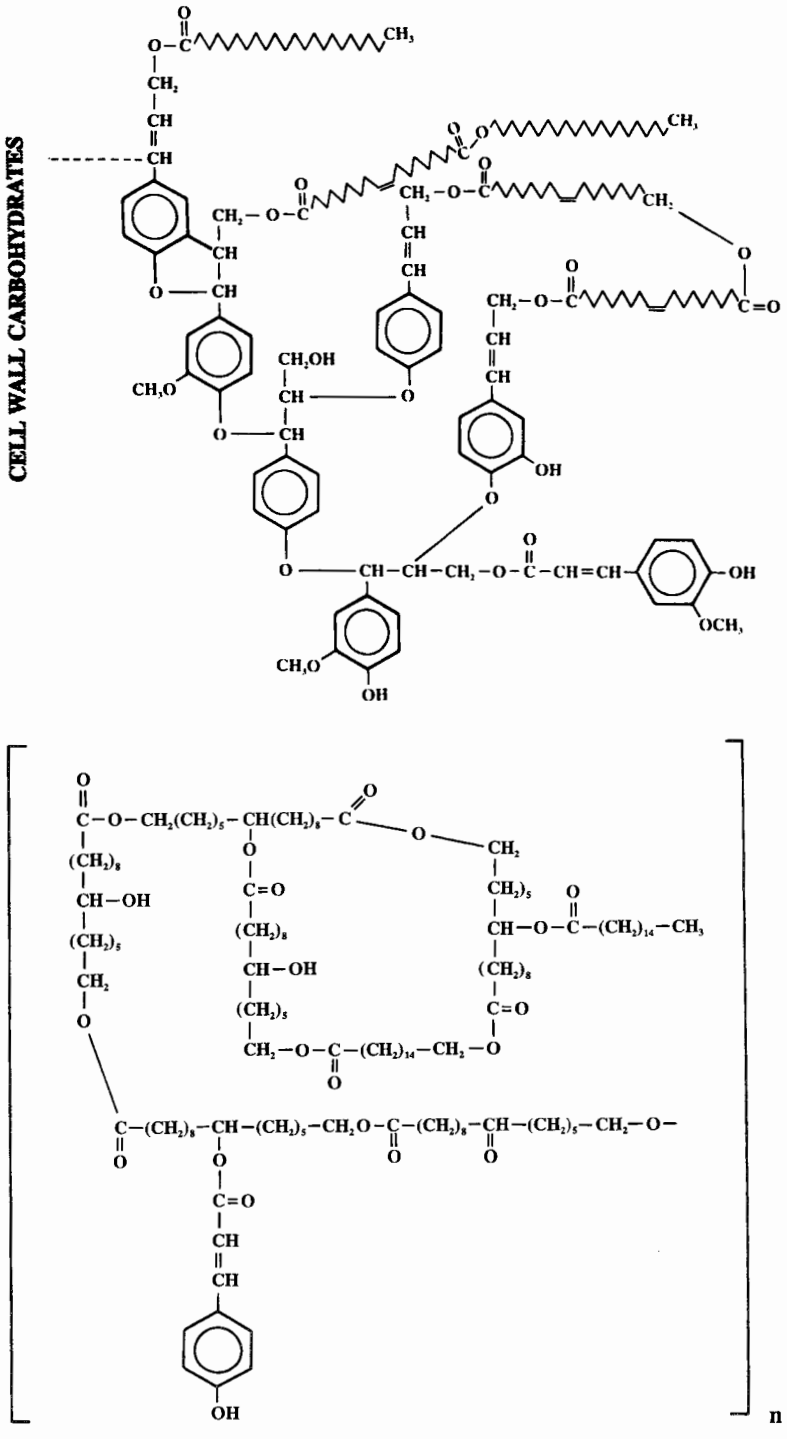


Figure 4.18. Models proposed for the structure of suberin (top) and cutin (bottom) (*redrawn from Kolattukudy*[130]).

fatty acids and the other unsaturated fatty acids.[86] To form saturated fatty acids, acetyl subunits are condensed *via* a series of enzymatically controlled steps forming fatty acids of up to 16 carbons in length.[203] Longer fatty acids require a separate chain lengthening sequence, usually building from a palmitic acid base unit. With the synthesis of unsaturated fatty acids, the introduction of the first double bond forming oleic acid is fairly well established. This can occur *via* one of two options, an anaerobic system or an aerobic one. Introduction of the second and third double bonds forming linoleic and α-linolenic acids, however, is not well understood.[86]

Triacylglycerols are synthesized in plants either directly from carbohydrates or through the modification of existing glycerides.[177,178] The glycerol backbone is derived from glycerol-3-phosphate from either the glycolytic or pentose phosphate pathway, or by direct phosphorylation of glycerol. Esterification of positions 1 and 2 (addition of a fatty acid) is by specific acyltransferases. In some cases the monoacyl (position one) is formed from dihydroxyacetone phosphate in a separate two-step sequence; however, the number 2 position is esterified by a 2-specific acyltransferase. The phosphate moiety is then cleaved from the molecule by phosphaitidate phosphohydrolase to yield diacylglycerol that then serves as a precursor for triacylglycerols, phosphoglycerides and glycosylglycerides. The final step in the synthesis of a triacylglycerol is the esterification of the 3-hydroxy position by a 3-specific transferase.[176]

4.7. Lipid Degradation

Each lipid class undergoes varying degrees of degradation during the postharvest period as the product approaches senescence or in the case of seeds, as they begin to germinate. Of the constituent lipids, the storage lipids are known to undergo marked changes in many products. Storage lipids are composed primarily of triacylglycerols that represent the most common lipids found in the plant kingdom, although they are not the predominant form in all plants. In that their degradation is fairly well understood, the discussion will focus on this class of lipids.

The first step in the recycling of carbon stored as triacylglycerols is the removal (hydrolysis) of the three acyl (fatty acid) units from the glycerol molecule.[89] This is accomplished through the action of the enzyme lipase (acyl hydrolase) or more specifically triacylglycerol acyl hydrolase.[69] The sequence involves the removal of the fatty acid from the number 3 position yielding a 1,2 diacylglycerol, followed by the removal of a second fatty acid yielding either 1- or 2-monoacylglycerol. The final acyl group is then hydrolyzed leaving glycerol. The free acids can then be converted to acetate, a starting point for a number of synthetic reactions and a respiratory substrate. In addition, acetate can also be converted to sucrose, the primary transport form of carbon in plants and an essential step during the germination of many seeds.

Free fatty acids can be metabolized by several possible mechanisms in the plant.[89] The most prevalent mechanism for the degradation of fatty acids is β-oxidation (Figure 4.19). It results in the formation of acetyl-CoA, in which a major portion of the stored energy remains trapped in the thioester bond. This trapped energy can either be converted to ATP by the movement of acetyl-CoA through the tricarboxylic acid cycle or acetyl-CoA can move through the glyoxylate cycle and provide carbon skeletons for synthetic reactions. The second option (i.e., *via* the glyoxylate cycle) does not appear to be operative in most harvested products, however, it is extremely important in germinating seed that are high in lipids.

The β-oxidation reactions occur both in the cytosol and in the glyoxysomes in many oil containing seeds. In this scheme the two terminal carbons of the fatty acid are cleaved sequentially, moving down the chain. With each acetyl-COA cleaved, 5 ATP equivalents (1 $FADH_2$ and 1 NADH) are produced. In the initial step of the reaction (Figure 4.19), catalyzed

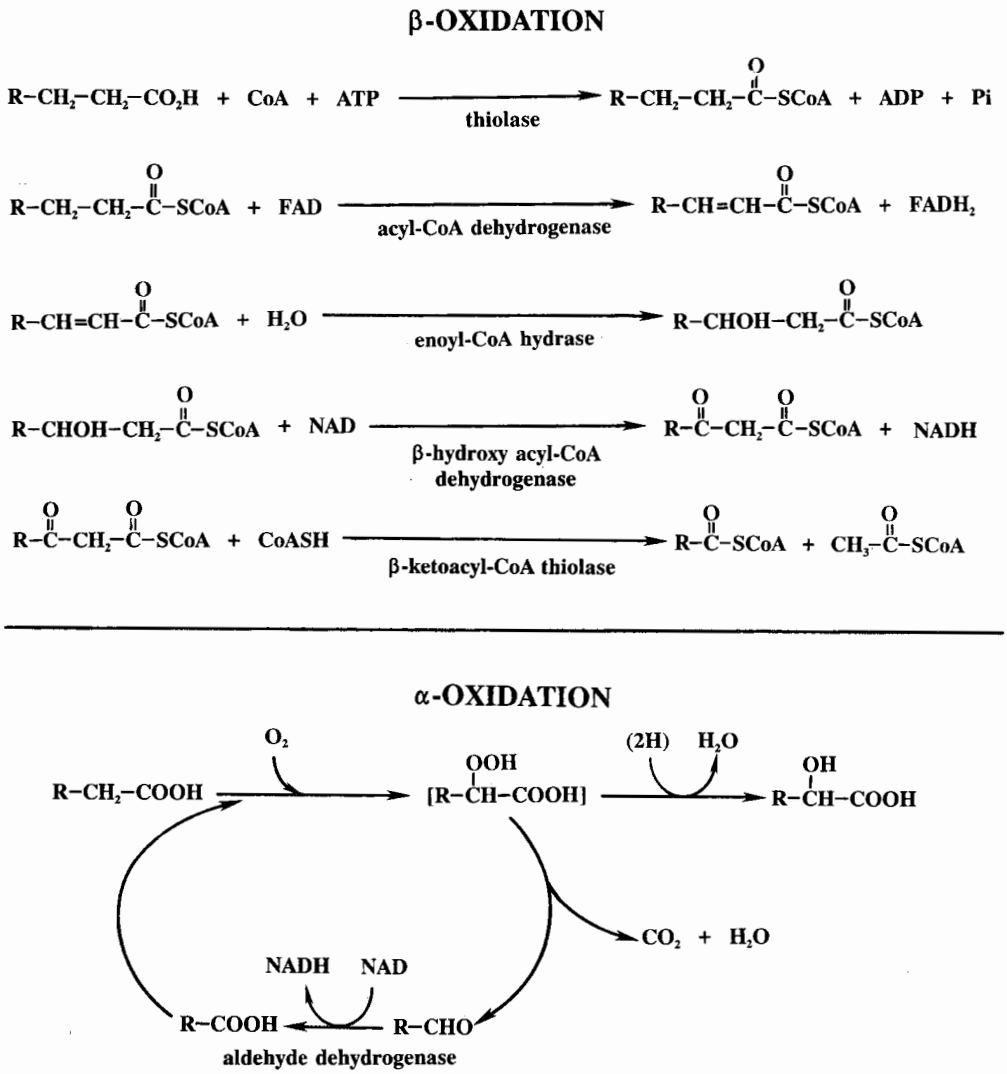


Figure 4.19. A comparison of β-oxidation and α-oxidation of free fatty acids (*redrawn from Shine and Stumpf*[216]).

by the enzyme thiolase, the free fatty acid combines with coenzyme A, a step requiring ATP to form acyl-CoA. Only one ATP is required to activate the fatty acid for complete degradation regardless of the number of carbon atoms in the chain. The next step is an oxidative reaction, catalyzed by acyl-CoA dehydrogenase that produces a double bond between the number 2 and 3 carbons and results in the formation of $FADH_2$. This is followed by the addition of water to the double bond (carbon 3) by enoyl-CoA hydrase and subsequent oxidation of the hydroxyl of the number 3 carbon, with the production of NADH. In the final step, acetyl-CoA (the two terminal carbons) is cleaved from the fatty acid molecule. This series of steps is then repeated, removing additional acetyl-CoA's.

Free fatty acids may also be degraded yielding CO_2, H_2O and energy by the α-oxidation pathway; however, its role appears to be only minor at least from the standpoint of energy production from stored lipids.[169] Unlike β-oxidation where the reactions involve an acyl thioester,

Table 4.12. Hydroperoxides and Aldehydes (with Single Oxygen Function) Possibly Formed in Autoxidation of Some Unsaturated Fatty Acids.*

Fatty acid	*Methylene Group Involved*	*Isomeric Hydroperoxides Formed From Structures Contributing to Intermediate Free Radical Resonance Hydrid*	*Aldehydes Formed by Decomposition of Hydroperoxides*
Oleic	11	11-Hydroperoxy-9-ene	Octanal
		9-Hydroperoxy-10-ene	2-Decenal
	8	8-Hydroperoxy-9-ene	2-Undecenal
		10-Hydroperoxy-8-ene	Nonanal
Linoleic	11	13-Hydroperoxy-9,11-diene	Hexanal
		11-Hydroperoxy-9,12-diene	2-Octenal
		9-Hydroperoxy-10,12-diene	2,4-Decadienal
Linolenic	14	16-Hydroperoxy-9,12,14-triene	Propanal
		14-Hydroperoxy-9,12,15-triene	2-Pentenal
		12-Hydroperoxy-9,13,15-triene	2,4-Heptadienal
	11	13-Hydroperoxy-9,11,15-triene	3-Hexenal
		11-Hydroperoxy-9,12,15-triene	2,5-Octadienal
		9-Hydroperoxy-10,12,15-triene	2,4,7-Decatrienal
Arachidonic	13	15-Hydroperoxy-5,8,11,13-tetraene	Hexanal
		13-Hydroperoxy-5,8,11,14-tetraene	2-Octenal
		11-Hydroperoxy-5,8,12,14-tetraene	2,4-Decadienal
	10	12-Hydroperoxy-5,8,10,14-tetraene	3-Nonenal
		10-Hydroperoxy-5,8,11,14-tetraene	2,5-Undecadienal
		8-Hydroperoxy-5,9,11,14-tetraene	2,4,7-Tridecatrienal
	7	9-Hydroperoxy-5,7,11,14-tetraene	3,6-Dodecadienal
		7-Hydroperoxy-5,8,11,14-tetraene	2,5,8-Tetradecatrienal
		5-Hydroperoxy-6,8,11,14-tetraene	2,4,7,10-Hexadecatetraenal

**Source:* Badings.[6]

Note: Only the most active methylene groups in each acid are considered.

α-oxidation acts directly on the free fatty acids. The proposed scheme for α-oxidation is presented in Figure 4.19.

In most cases, direct oxidation of fatty acids to CO_2 does not appear to be the primary physiological role of α-oxidation in plants. Under extreme conditions, for example, wounding of tissue slices, the initial rise in respiration appears to be largely due to α-oxidation. In normally metabolizing cells, α-oxidation is thought to function by creating odd-numbered fatty acids by the removal of a terminal carbon. It may also be used as an adjunct to β-oxidation for the removal of a carbon when the number 3 carbon of the fatty acid has a side group preventing the β-oxidation process.

4.7.1. Lipid Peroxidation

The oxidation of lipids in harvested plant products may occur either in biologically mediated reactions catalyzed by lipoxygenases or through direct chemical or photochemical reactions.[169] In enzymatically controlled oxygenation reactions, polyunsaturated fatty acids are attacked producing hydroperoxides that can be further degraded often forming characteristic tastes and odors, both desirable and undesirable (e.g., rancidity). For example, linolenic acid (18:3) can be oxidized forming a number of hydroperoxides that decompose into aldehydes

Lipid Peroxidation

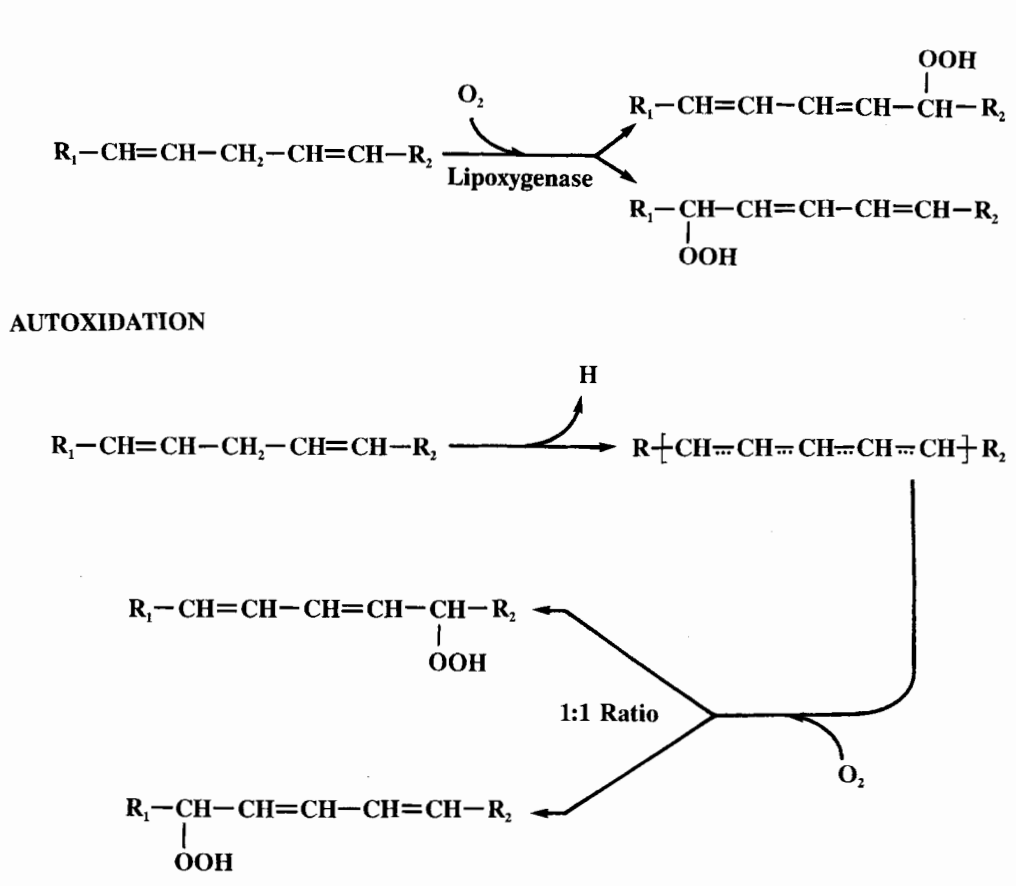


Figure 4.20. Enzymatic and non-enzymatic lipid peroxidation reactions occurring within the cell.

(Table 4.12). In cucumber fruit, linoleic acid is attacked by lipoxygenase forming both 9-hydroperoxide and 13-hydroperoxide which are cleaved to form the volatile flavor components *cis*-3-nonenal and hexenal, respectively.[70] While these enzymes occur widely in higher plants, the level of activity does not appear to follow any set botanical or morphological pattern. Very high activities have been recorded for bean seed, potato tubers, eggplant fruit and immature artichoke flowers.[185]

Peroxidation reactions also include autocatalytic oxidation, the direct reaction with oxygen, and a non-autocatalytic process mediated by light. As with enzymatic reactions, hydroperoxides are formed. The sequence of autoxidation is presented in Figure 4.20. In general, the rate of lipid oxidation is largely dependent upon the degree of unsaturation of the component fatty acids. Numerous other factors, both internal (e.g., antioxidants, pro-oxidants), and external (e.g., oxygen concentration, temperature and light intensity), exert a pronounced influence.[3]

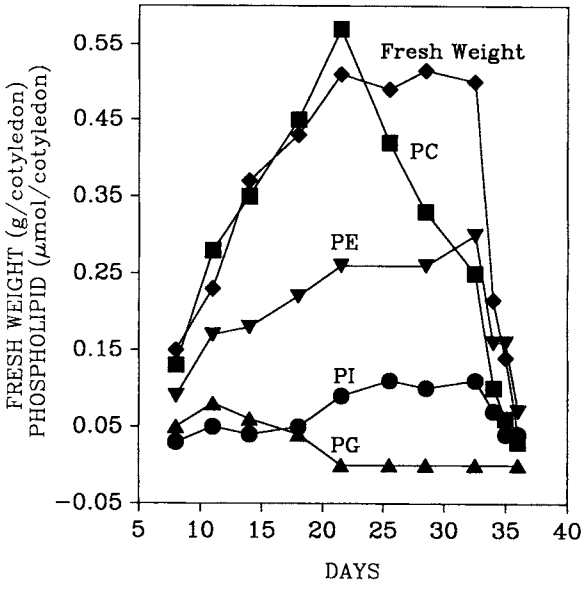


Figure 4.21. Changes in membrane phospholipids in cotyledons during the onset and development of senescence (PC = phosphatidyl choline; PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol; PI = phosphatidyl inositol) (*redrawn from Ferguson and Simon*[58]).

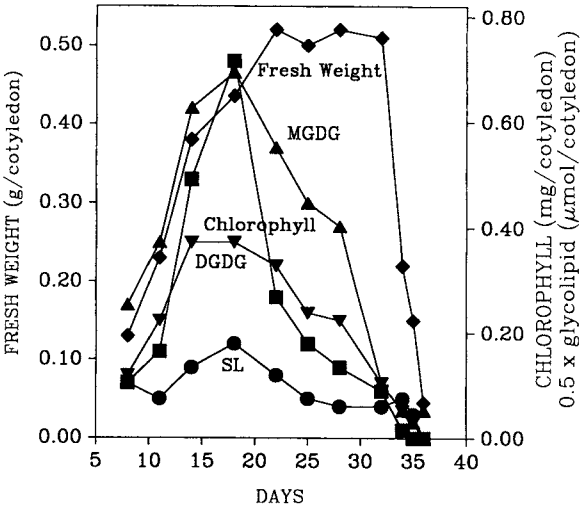


Figure 4.22. Changes in cotyledon chlorophyll and chloroplast glycolipids during the onset and development of senescence (MGDG = monogalactosyl diglyceride, DGDG = digalactosyl diglyceride; SL = sulpholipid) (*redrawn from Ferguson and Simon*[58]).

4.8. Galactolipases and Phospholipases

Plant cells contain enzymes capable of breaking down both glycolipids and phospholipids.[169] They function in the normal turnover of these molecules but also have additional roles. For example, galactolipases may be important in the breakdown of prolamellar bodies during the greening of etioplasts.[230] In addition, both types of enzyme appear to function during the onset of senescence of harvested products. During this period there is general breakdown of lipids with subsequent disorganization of the integrity of the cellular membranes.

Enzymes capable of attacking galactolipids, the predominant form of plant glycolipids, are found both in the cytosol and in the chloroplasts.[169] Lipolytic acyl hydrolase catalyzes the hydrolysis of the ester bonds yielding free fatty acids and the corresponding galactosylglycerols. The galactosylglycerols are subsequently attached by α- and β-galactosidases giving galactose and glycerol.

Phospholipids are degraded by four specific types of phospholipases, each of which attacks the parent molecule in a distinct manner. For example, the C type phospholipases cleave the ester bond of carbon 3 of glycerol and phosphoric acid. In the case of phosphatidycholine this yields a 1,2-diacylglycerol and phosphorylcholine.

4.9. Postharvest Alterations

Postharvest changes in the lipid fraction of high moisture crops have not been thoroughly studied. In oleaginous plant products such as pecan kernels (approximately 74% lipid), changes in lipids are largely qualitative rather than quantitative. Approximately 98% of the lipid fraction is triacylglycerols of which 90% are unsaturated.[209,210] Oxidation represents the primary qualitative alteration in lipids during the storage and marketing period. Large quantitative and qualitative changes in oil seed crops do occur during germination when stored lipids are recycled, however, this is not considered a postharvest alteration.

In the fruit of avocado, the composition of the oil does not change during maturation and storage. While there is a large increase in fruit respiration during the ripening climacteric, mesocarp lipids do not appear to represent the source of carbon utilized.[17]

Substantial changes in the lipids of non-oleaginous tissues occur during senescence. Here there are significant alterations in both glycolipids and phospholipids. In cucumber cotyledons, a model system used for studying lipid changes during the onset and development of senescence, phosphatidyl choline, the major phospholipid present, begins to disappear once the cotyledons reach their maximum fresh weight. As senescence progresses, rapid desiccation of the tissue begins. By this time, 56% of the phosphatidyl choline has been broken down and phosphatidyl ethanolamine begins to be metabolized (Figure 4.21). The glycolipids begin to be lost concurrently with chlorophyll, approximately two weeks prior to initial weight loss of the tissue (Figure 4.22).[58] These changes in lipid composition may mediate alterations in the structure of the membranes resulting in abnormal permeability and decreased activity of membrane associated enzymes, thus accelerating senescence.

5. PLANT PIGMENTS

Our lives are surrounded and in many ways dominated by plant colors. These colors are due to the presence of pigments within the plant and their interaction with light striking them. Sunlight is composed of a number of different wavelengths, the composite of which is called the spectrum (Figure 4.23). Within the visible portion of the spectrum, specific ranges in wavelength display individual colors (e.g., red, blue, yellow). Some wavelengths (colors) are absorbed by the pigments in a plant while others are either reflected or transmitted through the tissue. What is seen as a specific plant color, such as the blue of grape-hyacinth flowers, is due to the absorption by pigments of all of the other wavelengths in the visible spectrum except the blue region that is reflected from the flower tissue.

In biological tissues there are two types of reflected light, surface (also called regular or spectacular) and body reflectance influence our visual image of the object. With surface reflectance, the light striking the product is reflected from the surface without penetrating the tissue. This represents only about 4% of the light striking most biological samples. Much more important is body reflectance where the light actually penetrates into the tissue, becomes diffused (spread out in all directions) upon interacting with internal surfaces and molecules and is eventually either absorbed or reaches the surface and escapes from the tissue. Part of

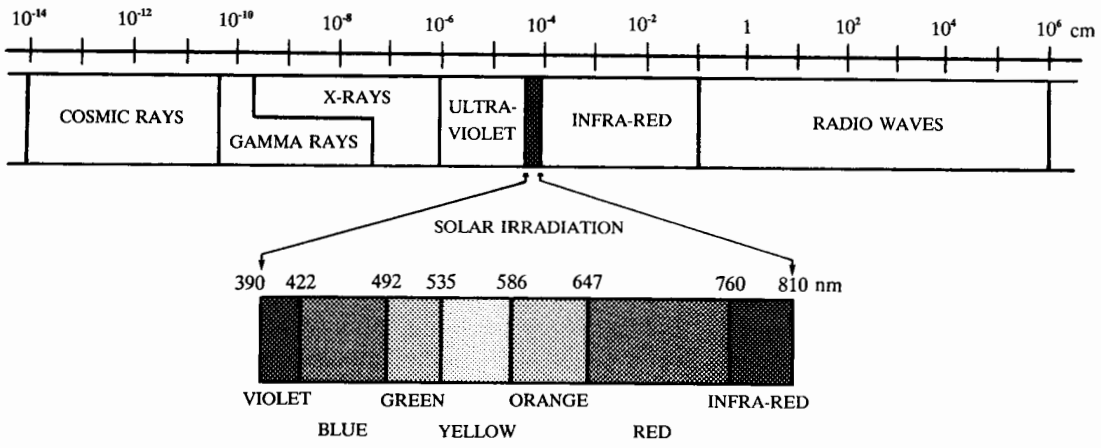


Figure 4.23. Visible light is a part of the spectrum of electromagnetic radiation from the sun. Radiation travels in waves; the length of each wave ranges from very short in gamma or cosmic rays (10^{-8} to 10^{-14}) to extremely long as in radio waves (1 to 10^{6} cm). What is perceived as the color of a harvested product is due to the absorption of part of the visible spectrum by pigments in the surface cells and reflection (body reflectance) of the remainder, part of which is detected by the human eye.

this light is absorbed by the component pigments, while the remainder moves back out of the tissue becoming the color we perceive.

Plant pigments can be separated into four primary classes based on their chemistry, the chlorophylls, carotenoids, flavonoids and betalains, the latter being fairly limited in distribution (Table 4.13). There are, of course, additional pigments (e.g., quinones, phenalones, phyrones and others), however, these typically make only a minor contribution to the color of plants.

In nature, pigments serve a number of functions. The chlorophylls and carotenoids trap light energy in photosynthesis. The carotenoids are integral components of light harvesting complexes that increase the overall efficiency of photosynthesis[62,99] and also act as protecting agents for chlorophyll molecules to prevent photooxidation. Pigments are of paramount importance in many plant species for their role in facilitating pollination.[189] Flower colors are known to attract certain insects, birds and in some cases, bats, which pollinate the flower. In the wild, the color of some fruits and seeds also plays an important role in dispersal. Dispersal helps to minimize the potential for competition between the parent and its progeny, enhancing the potential for colonization of new areas.

For man, color, form and freedom from defects are three of the primary parameters used

Table 4.13. Plant Pigments.

Pigment	*Color*	*Cellular Localization*	*Solubility*
Chlorophyll	Blue-green, yellow-green	Chloroplasts	Insoluble in water, soluble in acetone, ether, alcohols
Carotenoids	Yellow, orange- red	Chloroplasts, chromoplasts	Insoluble in water, soluble in acetone, ether, alcohols
Flavonoids	Yellow, orange, red, blue	Vacuole	Water soluble
Betalains	Yellow, orange, red, violet	Vacuole, cytosol	Water soluble

to ascertain quality in postharvest plant products. Pigmentation provides us with quality information, such as the degree of ripeness of fruits (e.g., banana) or the mineral nutrition of ornamentals. It is especially important with ornamentals since our visual impressions are almost exclusively relied upon for quality judgment.

5.1. Classes of Pigment Compounds

5.1.1. Chlorophyll

The plant world is dominated by the color green due to the presence of the chlorophyll pigments that absorb red and blue wavelengths. The chlorophylls are the primary light-accepting pigments found in plants that carry out photosynthesis through the fixation of carbon dioxide and the release of oxygen. In nature, there are two predominate forms, chlorophyll a and chlorophyll b, differing only slightly in structure (Figure 4.24). Both are normally found concurrently within the same plant and usually at a 2.5–3.5:1 ratio between a and b. Two other chlorophylls, c and d, are found in a relatively limited number of species. For example, chlorophyll c is found in several marine plants.

Each form of chlorophyll is a magnesium containing porphyrin formed from 4 pyrrole rings (Figure 4.24). Attached to the propionyl group of a pyrrole ring of chlorophylls a, b and d is a 20 carbon phytol chain ($C_{20}H_{39}OH$). Chlorophylls a and b differ structurally only in the replacement of a methyl group on chlorophyll a with an aldehyde (-CHO). Unlike the flavonoid pigments, the chlorophylls are hydrophobic and hence, not soluble in water. The primary function of chlorophyll is to absorb light energy and convert it to chemical energy; a process that occurs in the chloroplasts.

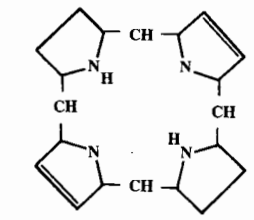
5.1.2. Carotenoids

The carotenoids are a large group of pigments associated with chlorophyll in the chloroplasts and are also found in other chromoplasts. Their colors range from red, orange, and yellow to brown and are responsible for much of the autumn leaf pigmentation.

Chemically carotenoids are terpenoids comprised of eight isoprenoid units (Figure 4.25). Nearly all carotenoids are composed of 40 carbon atoms. They are divided into two subgroups, the carotenes and their oxygenated derivatives, the xanthophylls. Both are insoluble in water, although the xanthophylls tend to be less hydrophobic than the carotenes.

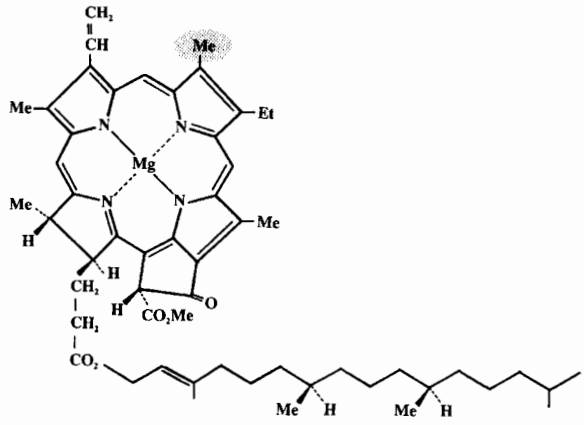
The nomenclature of the carotenoids is based on their 9 carbon end groups of which there are 7 primary types (Figure 4.25). These can be arranged in various combinations on the methylated straight chain portion of the molecule, for example α-carotene is β,ε-carotene while β-carotene is β,β-carotene. Lutein, a common xanthophyll, is structurally like α-carotene, differing only in the presence of a hydroxyl group on carbon 3 of both the β and ε end groups. Thus, a tremendous range in potential variation is possible and in some tissues, quite large assortments of specific compounds have been found. For example, in the juice of the 'Shamouti' oranges, 32 carotenoids have been identified (Table 4.14).

In photosynthetic tissue, carotenoids function both in the photosynthesis process *per se,* and as protectants, preventing the chlorophyll molecules from being oxidized (photooxidation) in the presence of light and oxygen. In flowers and fruits, carotenoids appear to act as attractants that aid in securing pollination or dispersal; however, in underground structures such as roots and tubers, their role is not understood.

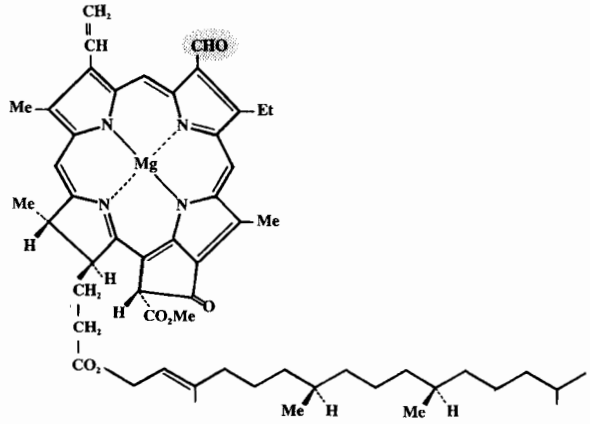


PORPHYRIN BASE STRUCTURE

PYRROLE RING



CHLOROPHYLL A



CHLOROPHYLL B

Figure 4.24. The chlorophylls are magnesium containing porphyrins derived from four pyrrole rings. The two prevalent chlorophylls, a and b, differ only in a single side group (shaded). Chlorophyll c, however, is structurally quite distinct from a, b, and d in that the phytol tail is not present.

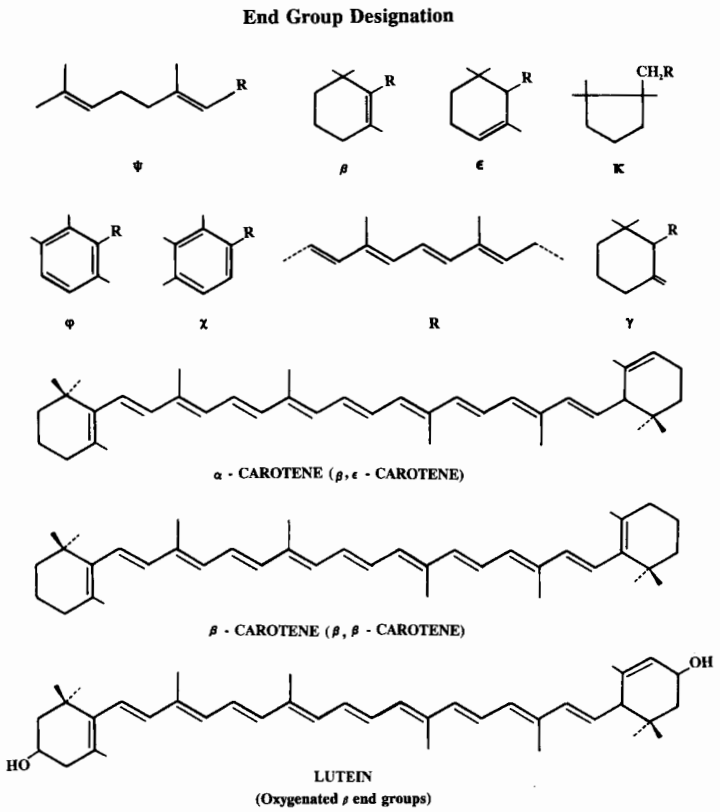


Figure 4.25. The base structures of various carotenoid end groups and the representative structures of two carotenes (α-carotene and β-carotene). The figure shows the end group designation in naming. Xanthophylls, carotenes oxygenated on one or both end groups, are represented by the pigment lutein.

Table 4.14. Quantitative Composition of Carotenoids in the Juice of 'Shamouti' Oranges.*

Carotenoid	*(% of total) Carotenoids*	*Carotenoid*	*(% of total) Carotenoids*
Mutatoxanthin	15.13	α-Carotene	1.24
Cryptoxanthin	12.88	poli-cis-Cryptoxanthin	1.05
Trollixanthin	9.64	Carbonyl 422	0.78
Luteoxanthin-like	6.92	Cryptoflavin	0.70
Antheraxanthin	6.20	Auroxanthin	0.58
Phytoene	5.70	OH-α-Carotene	0.50
Lutein	5.20	Pigment 426	0.39
Isolutein	4.00	α-Carotene	0.36
Luteoxanthin	3.98	Citraurin	0.11
Neoxanthin	3.97	Cryptoxanthin diepoxide	0.10
Violaxanthin	3.00	Chrysanthemaxanthin	0.07
OH-Sintaxanthin	2.48	Sintaxanthin	0.07
Phytofluene	2.40	Rubixanthin	0.05
cis-Cryptoxanthin	2.00	β-Apo-10'-carotenal	0.03
Trollichrome	1.46	Mutatochrome	0.02
β-Carotene	1.40		

**Source:* Gross et al.[76]

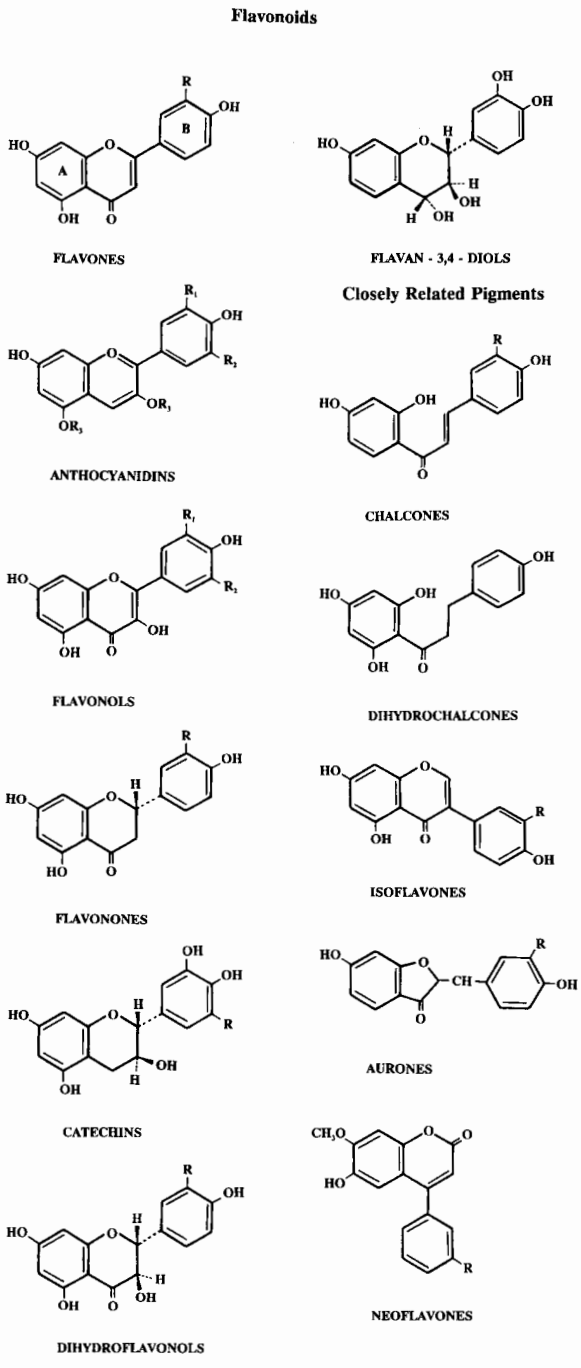
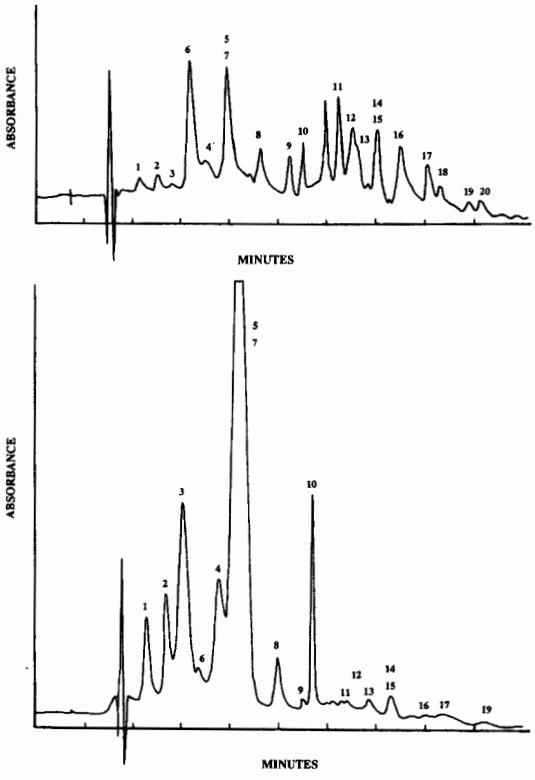


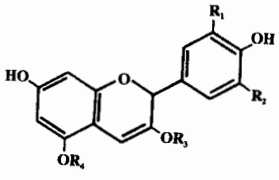
Figure 4.26. The basic structure of flavonoid pigments. Each consists of 2 benzene rings (A and B) joined by a 3 carbon link. The various classes of flavonoids differ only in the state of oxidation of the 3 carbon link. Within each class are a wide range of individual pigments varying in the number and position of groups (e.g., OH, CH_3, etc) attached to the 2 rings. Also illustrated are several closely related pigments (e.g., chalcones, dihydrochalcones, isoflavones, aurones and neoflavones).

5.1.3. Flavonoids

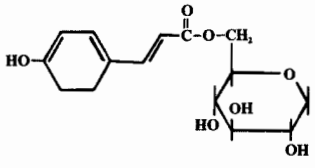
While green is the dominant color in plants, other colors have tremendous attraction both for man and other animals. Many of the intense colors of flowers, fruits, and some vegetables are the result of flavonoid pigments and closely related compounds. This large class of water-soluble compounds has a diverse range of colors. For example, there are yellows, reds, blues,



Order of Elution	Compound	Substitution R_1	R_2	R_3	R_4
	Diglucosides				
1	Delphinidin 3,5-diglucoside	OH	OH	Glu	Glu
2	Cyanidin 3,5-diglucoside	OH	H	Glu	Glu
3	Pentunidin 3,5-diglucoside	OCH_3	OH	Glu	Glu
4	Peonidin 3,5-diglucoside	OCH_3	H	Glu	Glu
5	Malvidin 3,5-diglucoside	OCH_3	OCH_3	Glu	Glu
	Monoglucosides				
6	Delphinidin 3-glucoside	OH	OH	Glu	H
7	Cyanidin 3-glucoside	OH	OH	Glu	H
8	Petunidin 3-glucoside	OCH_3	OH	Glu	H
9	Peonidin 3-glucoside	OCH_3	H	Glu	H
10	Malvidin 3-glucoside	OCH_3	OCH_3	Glu	H
	Acylated Diglucosides				
11	Delphinidin 3(6-0-p coumarylglucoside)-5 glucoside	OH	OH	PCG	Glu
12	Cyanidin 3(6-0-p-coumarylglucoside)-5 glucoside	OH	H	PCG	Glu
13	Petunidin 3(6-0-p-coumaryglucoside)-5 glucoside	OCH_3	OH	PCG	Glu
14	Peonidin 3(6-0-p-coumarylglucoside)-5 glucoside	OCH_3	H	PCG	Glu
15	Malvidin 3(6-0-p-coumarylglucoside)-5 glucoside	OCH_3	OCH_3	PCG	Glu
	Acylated Monoglucosides				
16	Delphinidin 3(6-0-p-coumaryl-glucoside)	OH	OH	PCG	H
17	Cyanidin 3(6-0-p-coumarylglucoside)	OH	H	PCG	H
18	Petunidin 3(6-0-p-coumarylglucoside)	OCH_3	OH	PCG	H
19	Peonidin 3(6-0-p-coumarylglucoside)	OCH_3	H	PCG	H
20	Malvidin 3(6-0-p-coumarylglucoside)	OCH_3	OCH_3	PCG	H



ANTHOCYANIN



6-O-p-COUMARYLGLUCOSIDE (PCG)

Figure 4.27. A comparison of the anthocyanins of two grape cultivars (*top,* Concord and *bottom,* DeChaunac) separated by high preformance liquid chromatography. Substitutions in the basic structure of the anthocyanin molecule occur at any of the four positions, denoted as R_1–R_4. These substitutions are given for 20 of the anthocyanins found in the two grape cultivars. Part of the anthocyanins have an organic acid esterified to the hydroxyl group of their attached sugar (termed acylated). In this case, p-coumaric (PCG), a derivative of cinnamic acid, is present (*redrawn from Williams and Hrazdina*[247]).

and oranges. Numerous variations in color are derived both from structural differences between compounds and the relative concentration of specific pigments within the cells. The flavonoids are generally found in the vacuole.

The basic structure of flavonoid pigments is presented in Figure 4.26. It consists of 2 benzene rings (A and B) joined by a 3-carbon link which forms a γ-pyrone ring through oxygen. Various classes of flavonoids differ only in the state of oxidation of the 3-carbon link, while individual compounds within these classes differ mainly by the number and orientation of the hydroxy,

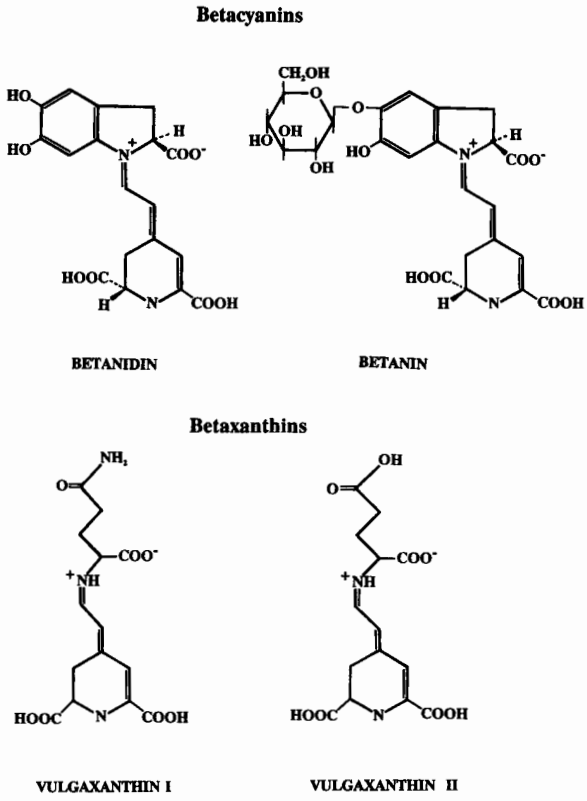


Figure 4.28. Examples of the two primary structural groups of betalains, the betacyanins and the betaxanthins. Betanidin, vulgaxanthin I, and vulgaxanthin II are found in the root of the common garden beet (*redrawn from Piattelli et al.*[183, 184]). Epimers of betacyanins are formed by alterations in the configuration, an example would be the epimer of betanin, isobetanin.

methoxy and other groups substituted on the 2 benzene rings. Individual classes of flavonoids include anthocyanidins, flavones, catechins, flavonols, flavanones, dihydroflavonols and the flavan-3,4-diols or proanthocyanidins (Figure 4.26). Most flavonoid pigments exist in live plant tissue as glycosides where one or more of their hydroxyl groups are joined to a sugar. In some anthocyanins, an organic acid may be esterified to one of the hydroxyls on the sugar, giving an acylated compound. This is the case with grapes where *p*-coumaric acid, a derivative of cinnamic acid, can be found attached to both the mono- and diglucoside anthocyanin pigments (Figure 4.27). The attached sugar confers higher solubility and stability (reduced reactivity).

Closely related to flavonoid compounds are the chalcones, dihydrochalcones, isoflavones, neoflavones and aurones (Figure 4.26). These do not have the 2-phenylchroman structure but are closely akin both chemically and in their biosynthesis.

5.1.4. Betalains

The betalains represent a fourth but substantially restricted group of plant pigments. They are found in the flower, fruits and in some cases in other plant parts, giving colors of yellow, orange, red and violet. Perhaps the best example is the red-violet pigment from the root of the beet, the first betalain isolated in crystalline form; hence the derivative name betalain.

As a group they are characterized by being water-soluble nitrogenous pigments that are found in the cytosol and in vacuoles. Chemically betalains are subdivided into two groups: the red-violet betacyanins illustrated by the structure of betanidin and betanin (Figure 4.28) and the yellow betaxanthins, characterized by vulgaranthin I and II. A number of the naturally oc-

curring betaxanthins have the tail portion of the molecule either partially or totally closed into a ring structure as in dopaxanthin from the flowers of *Glottiphyllum longum* (Haw.) N.E. Br.[108]

While the precise function of the betalains is not known, it is possible that they may function like the anthocyanins in flowers and fruits enhancing insect or bird pollination and seed dispersal. No role has been presently ascribed for their presence in plant parts such as roots, leaves and stems though they may serve as antioxidants in the human diet.[116]

5.2. Pigment Biosynthesis and Degradation

5.2.1. Chlorophylls

Chlorophyll synthesis is modulated by a number of external influences, two of the most important being light and mineral nutrition. The initial pyrrole ring, porphobilinogen, is formed from two molecules of δ-amino levulinic acid derived from glycine and succinate (Figure 4.29). Four molecules of porphobilinogen are polymerized producing a ring structure, uroporphyrinogen, which has acetyl and propinoyl groups attached to each of the component pyrroles. After a series of decarboxylation reactions, protoporphyrin is formed and in subsequent steps, magnesium is inserted followed by the addition of the phytol tail. Chlorophyll a, which is blue-green in color, differs from chlorophyll b (yellow-green) only in the presence of a single methyl group instead of a formyl group.

Decomposition of chlorophyll may, in many cases, be quite rapid and dramatic in effect as in the autumn coloration of deciduous trees in the northern temperate zones or the ripening of bitter melon. In many tissues this loss of chlorophyll is part of a transition of the chloroplasts into chromoplasts containing yellow and red carotenoid pigments. The loss of chlorophyll can be mediated through several processes, such as the action of the enzyme chlorophyllase, enzymatic oxidation or photodegradation.[157] While the precise sequence of biochemical steps are not known, the initial reactions appear to be much like the reverse of the final steps in the synthesis pathway. Phytol may be removed to yield chlorophyllide or both magnesium and phytol to give pheophoride. In subsequent degradative steps, the low molecular weight products that are formed are colorless.

5.2.2. Carotenoids

Carotenoids, typically 40 carbon compounds, are built up from 5 carbon isoprene subunits, the most important of which is isopentenyl pyrophosphate. These initial subunits are formed in a series of steps from acetyl-CoA and acetoacetyl-CoA in the terpenoid pathway.[20,36,38] Isoprene subunits are sequentially added, build up to a 20 carbon intermediate, geranylgeranyl pyrophosphate, two of which condense to give phytoene with the typical carotenoid skeleton (Figure 4.30). Subsequent steps involve ring closure and, in the case of the xanthophylls, the addition of one or more oxygens.[199] Some carotenoids (e.g., carotenols) may be esterified to long chain fatty acids (e.g., oleate or palmitate) or other compounds. This occurs with leaf coloration in the fall[231] and in the peel of apple fruit during ripening. While carotenol esters were once thought to represent breakdown products formed during senescence (i.e., esterified with fatty acids formed during membrane degradation), current evidence points toward a controlled synthesis of the pigments.[128]

In xanthophyll synthesis, oxygen is added to the cyclic portions of the molecule, from carbon 1 to carbon 6 and often as hydroxyls. For example, the most common structural feature

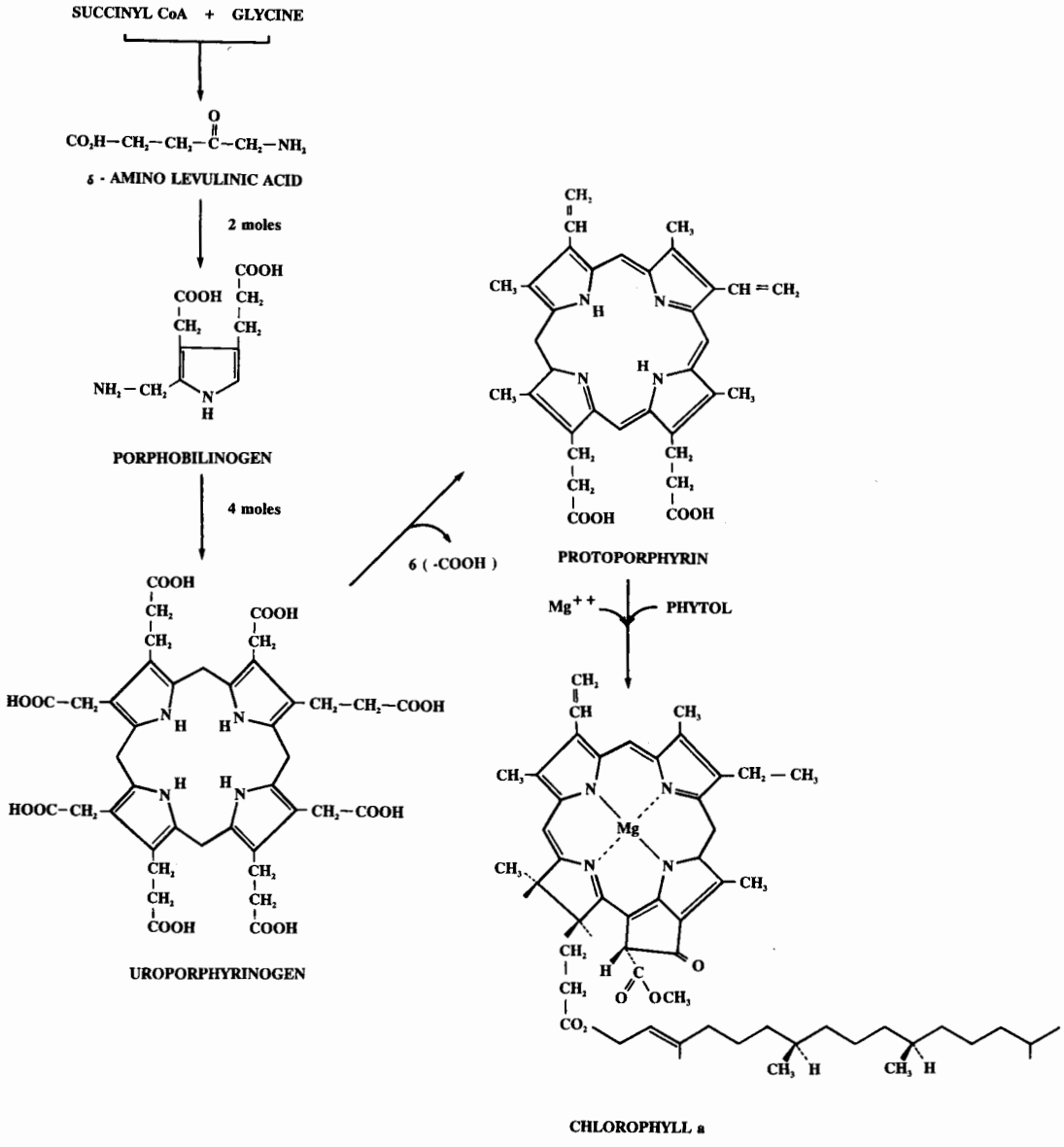


Figure 4.29. The general biosynthetic pathway for chlorophyll. Insertion of Mg^{++} and the addition of the phytol tail occur in the last series of steps.

of xanthophylls is the presence of a hydroxyl at the C-3 and C-3′ positions giving lutein (β,ε carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol). Because of the large number of potential combinations for the placement of one or more oxygens, there are numerous xanthophylls. Of the over 300 naturally occurring carotenoids that have been identified, approximately 87% are xanthophylls.

The stability of carotenoids is highly variable. In some cases, such as in narcissus flowers, degradation occurs in only a few days,[19] while with dried corn kernels over 50% of the carotenoids remain after 3 years of storage.[191] A number of factors affect the rate of loss of carotenoids. These include the specific type of pigment, storage temperature, product moisture

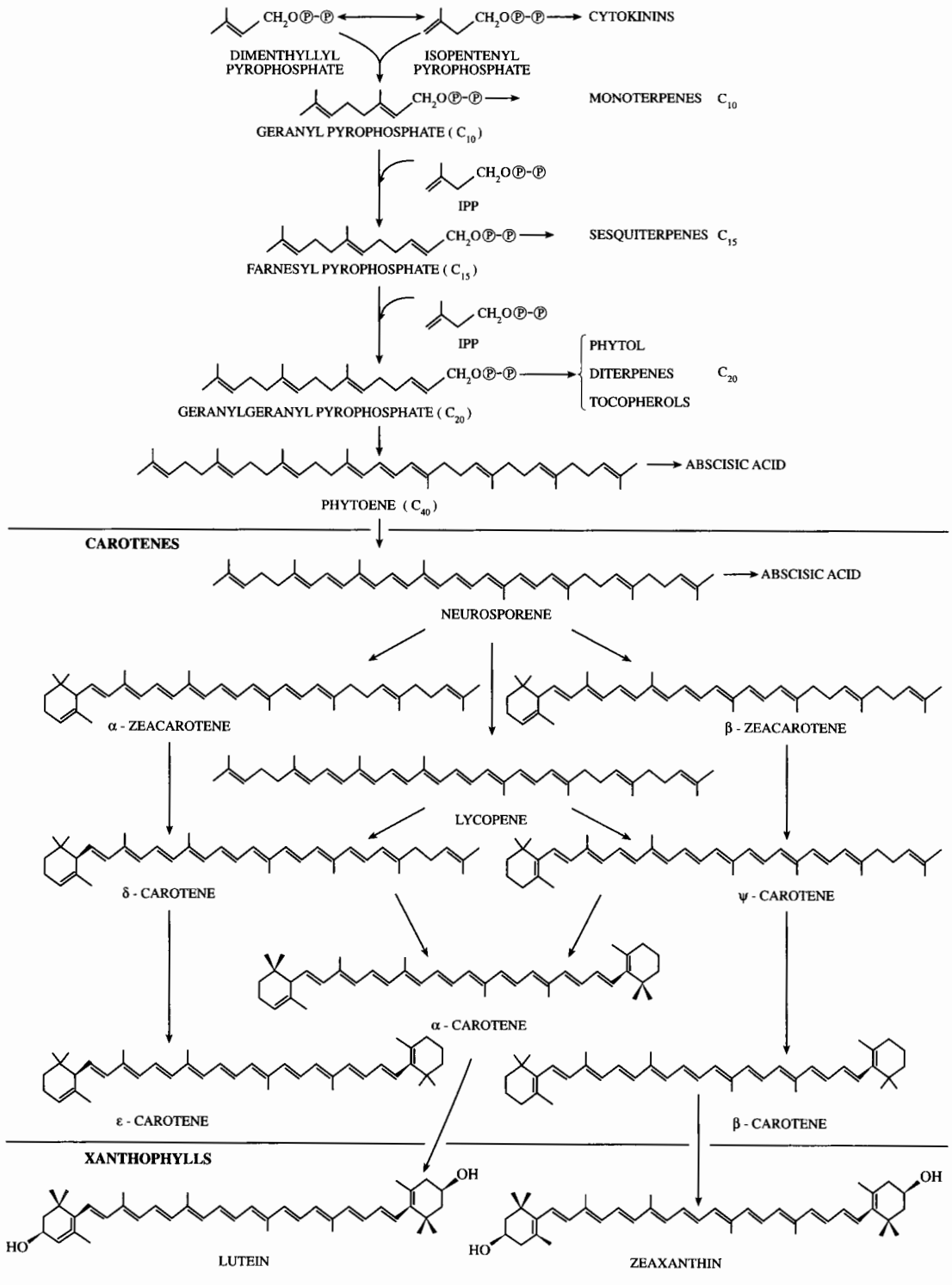


Figure 4.30. Generalized pathway for the biosynthesis of carotenes and their oxygenation to form xanthophylls.

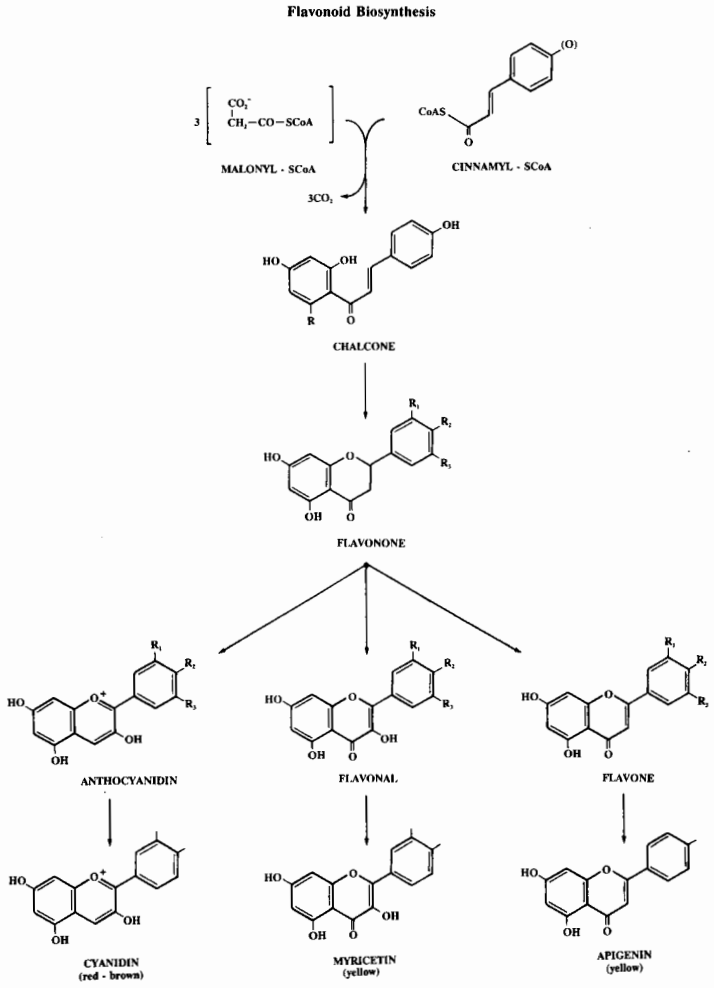


Figure 4.31. Flavonoid biosynthesis occurs in three stages starting with the combination of three malonyl-CoA molecules with one of cinnamyl-CoA. This is followed by ring closure forming a flavanone and conversion to the various classes of flavonoids. Final steps involve the formation of individual compounds with various additions to the two rings.

level, product type, prestorage treatments (e.g., drying of corn) and others. In edible products, the breakdown of β-carotene is of special concern due to its role as a precursor of vitamin A.

In the breakdown of carotenoids, the initial degradative steps involve oxygenation of the molecule. This differs from oxygenation reactions that occur largely on the ring structures during the formation of xanthophylls. Carotenoid degrading enzyme systems have been found in chloroplasts and mitochondria. The double bonds within the linear portion of the molecules are subject to attack by lipoxygenase[91] and activity is accelerated by the availability of oxygen, light and certain metals. A variety of shorter chain length terpenoids are formed, a number of which are volatile and in some cases, have distinct odors.[243]

As a group, xanthophylls are characteristically more stable than the carotenes. In leaf tissue in the autumn of the year, the xanthophylls are released into the cytoplasm upon the disruption of the chloroplasts. These molecules are subsequently esterified which appears to substantially enhance their stability, especially in contrast to the carotenes.

5.2.3. *Flavonoids*

Flavonoid biosynthesis begins with the formation of the basic $C_6C_3C_6$ skeleton through the combination of three malonyl-CoA molecules with one cinnamyl-CoA (Figure 4.31). This yields a chalcone and with ring closure, it is converted to a flavanone.[47] In the second series of steps, the flavanone may be converted to each of the different classes of flavonoids, e.g., flavone, flavonol, anthocyanidin. In the final stage these are converted to individual compounds, such as cyanidin, myricentin and apigenin.[61] The anthocyanins range from reds to blues in color. As the degree of methylation increases the individual compounds become increasingly red in color, while hydroxylation results in deeper blues. In addition, blue coloration can result from complexes formed by the chelation of Al^{+3} and Fe^{+3} to the hydroxyls of the A ring.

Of the flavonoids, the decomposition of anthocyanin has been studied in greatest detail.[47] The vulnerability of individual pigments to degradation tends to vary; substitutions at specific positions on the molecule can significantly affect its stability. For example, a hydroxyl at the 3' position enhances the pigments propensity for degradation.

Enzymes that have the potential to degrade anthocyanins have been isolated from a number of different tissues (e.g., flowers, fruits, and others). These tend to fall into two classes, glucosidases and polyphenol oxidases. Both have the ability to produce colorless products. Other possible mechanisms of pigment alteration and breakdown include pH alterations, which accompany ripening in some fruits, and attack of the charged portion of the molecule by naturally occurring nucleophiles (e.g., ascorbic acid).

5.2.4. *Betalains*

The betalains appear to be derived from 3,4-dihydroxyphenylalanine (L-DOPA). The betacyanins are formed from two of these molecules, one of which has an oxidative opening of the aromatic ring followed by subsequent closure to yield betalamic acid (Figure 4.32). The second forms cyclodopa and upon condensation with betalamic acid, yields betanidin, the base molecule for the formation of the various betacyanins. The betaxanthins are formed through the condensation of betalamic acid with an amino or imino group (other than cyclodopa).

Research on the breakdown of the betalains has centered to a large extent on color alterations that occur in beetroot, although these pigments may also be found in members of the Aizoaceae, Amaranthaceae, Basellaceae and Cactaceae families, which are of postharvest interest. This research was, in part, prompted by the elimination of the use of red dye number 2 in foods. Betalains were briefly considered as possible replacements. Nearly all of the studies to date have been done on either extracted pigments or processed tissue and, as a consequence, the extent to which these reactions can be implicated in color changes in stored fresh beetroot is not clear.

Beetroot tissue exposed to low pH (3.5–5.5) retains its color relatively well while at higher pH (7.5–8.5) discoloration occurs.[80] Action by β-glucosidase results in the removal of the sugar side group converting betanin and isobetanin to their aglucones, betanidin and isobetanidin. In addition, exposure to air and/or light results in the degradation of betalains, often causing a browning discoloration.

5.3. Postharvest Alteration in Pigmentation

During both the preharvest and postharvest period many products undergo significant changes in their pigment composition (Figures 4.33 and 4.34). These changes include both the

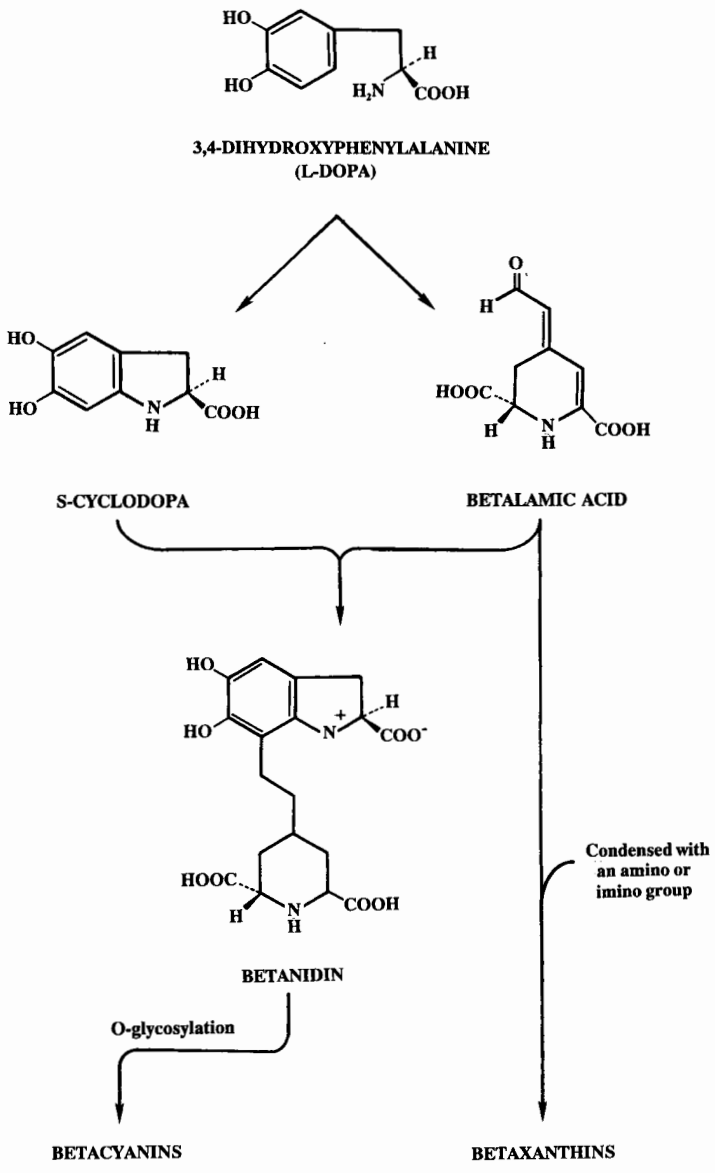


Figure 4.32. The proposed biosynthetic pathways for the betalains.

degradation of existing pigments and the synthesis of new pigments; in many cases, both processes may occur concurrently. Pigmentation changes are of paramount importance in many products in that they are used as a primary criterion for assessing quality.

The degradation of pigments can be subdivided into two general classes, pigments losses that are beneficial to quality and those that are detrimental. Many beneficial losses center around the degradation of chlorophyll with the concurrent synthesis of other pigments or the unmasking of pre-existing pigments already present with the tissue. Examples of this would be the degreening of oranges during which time carotenoids are being synthesized, and the loss of chlorophyll in banana, allowing the expression of the pigments that are already present. Detrimental losses of pigments after harvest can be seen in the color fading of flowers and in chlorophyll losses in broccoli florets or leaf crops. As with the degradation of pigments, the

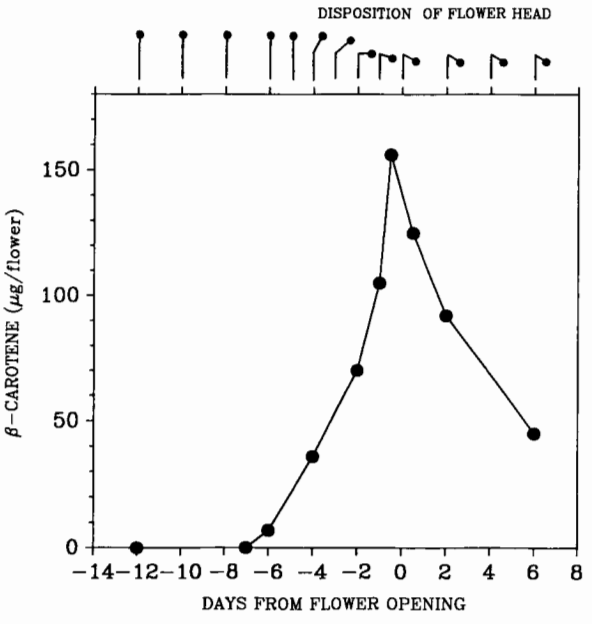


Figure 4.33. Time course of the synthesis and degradation of β-carotene in the corona of *Narcissus majalis* Curtis flowers. Day 0 represents anthesis (*redrawn from Booth*[19]).

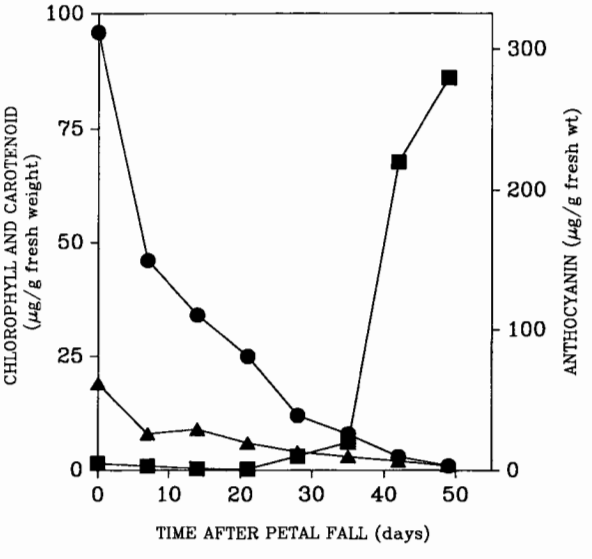


Figure 4.34. Changes in the pigment concentration in developing strawberry fruits (● chlorophyll; ▲ carotenoid; ■ anthocyanin (*redrawn from Woodward*[249]).

synthesis of pigments after harvest can be either beneficial or undesirable. The development of red coloration in the fruit of the tomato after harvest is highly desirable while the formation of chlorophyll in harvested potatoes or the synthesis of carotenoids in the bitter melon is undesirable.

Many postharvest factors affect the degree of change in pigmentation after harvest, the most important of which are light and temperature. Light is essential for the synthesis of chlorophyll and its presence delays the loss of these pigments in detached leaves. It also appears to be important in stimulating the synthesis of anthocyanins and lycopene in some products, however, not β-carotene in tomato fruits. Changes in the pigmentation of many tissues are temperature dependent. The effect of temperature, however, varies with the specific pig-

ment, the tissue of interest and whether synthetic or degradative processes are operative. For example, in pink grapefruit high temperatures (30–35°C) favor the accumulation of lycopene but not carotene; at low temperatures (5 to 15°C) the opposite is true.[165] However, in the tomato temperatures above 30°C suppress the biosynthesis of lycopene, but not β-carotene.[232]

Several plant growth regulators have also been shown to have a significant effect on the pigmentation in some harvested products. The use of ethylene for the degreening of citrus and banana fruits and stimulating the synthesis of carotenoids (tomato) has become a major commercial postharvest tool in many areas of the world. Ethylene is also known to stimulate the formation of anthocyanins in the grape when applied prior to harvest.[82] In floral crops, ethylene emanating from the harvested product or adjacent products dramatically accelerate pigment degradation, color fading and senescence in many flowers (e.g., *Vanda* orchids). To prevent pigment degradation, chemicals that either inhibit the biosynthesis of ethylene (e.g., amino oxyacetic acid, aminoethoxyvinylglycine) or impede its activity (silver ions, 1-methylcyclopropene) can be used.

Other growth regulators such as the cytokinins have pronounced effect on the retention of chlorophyll.[120] The potential use of synthetic cytokinins to retard chlorophyll loss has, as a consequence, been tested in several green vegetables (e.g., broccoli, Brussels sprouts, celery, endive and leaf lettuce). In broccoli stored at 13°C, the storage life can be doubled with a single application of zeatin and dihydrozeatin.[68] While many cases the results are positive, other postharvest techniques for color retention are commercially more acceptable.

6. VOLATILE COMPOUNDS

6.1. Classes of Volatile Compounds

Plants give off a wide range of volatile compounds, some of which are extremely important components of quality. Humans exhibit distinct patterns in the foods that they select to consume, and flavor is known to be a primary criterion in this selection. Most flavors are comprised of a combination of both taste and odor. Since taste is generally thought to be limited to four basic sensations—sweet, sour, salty and bitter—volatiles often play a very significant role in flavor. The four basic tastes can be contrasted with the potential perception of up to 10,000 distinct odors by the human nose olfactory epithelium. This difference in potential perception is also seen in the number of taste receptors that are in the thousands, while odor receptors are thought to be in the millions. Hence, aroma compounds are not only an extremely important part of flavor, they provide an almost unlimited potential for flavor diversity.

The importance of volatile compounds is not just limited to food products. The aroma of many ornamental and floral species complements our visual perception of these and often is an important part of the plant's aesthetic quality. The contribution of plant volatiles may impart either positive quality characteristics as in the fragrance of gardenia flowers or in the case of the carrion flowers a distinctly undesirable sensation, at least from the standpoint of humans. A primary requirement of plant volatiles is that they are present in a gaseous or vapor state as the molecules must be able to reach the olfactory epithelium in the roof of the nasal passages. In addition, some degree of water solubility is essential. Non-ionizable volatile compounds are typically only perceived by the olfactory system, while ionizable volatiles can contribute to the perception of both taste and aroma.

The volatiles emanating from living plant material after harvest represent a diverse array of chemical compounds. These include esters, lactones, alcohols, acids, aldehydes, ketones, acetals, hydrocarbons and some phenols, ethers and heterocyclic oxygen compounds. Over 300

volatile compounds have been identified from pear[193] and a comparable number from apple (Table 4.15). Superimposed on these raw product volatiles is a tremendous range to thermally generated volatile compounds formed during cooking.[119] The majority of edible plant material is consumed in a cooked form; for example, 370 of 390 commercially cultivated vegetables are routinely to intermittently cooked.[118] Exposure of foods to high temperatures can result in the synthesis of furans, furanones, pyranones, pyrroles, pyridines, pyrimidines, pyrazines, thiophenes, dihydrothiophenes, oxazoles, oxazolines, thiazoles, thiazolines, aldehydes, ketones, cyclopentanones, non-cyclic sulfur compounds and others that give rise to a tremendous range in flavors. Within each class of compounds is often a myriad of possible odors. For example, pyrazines range in aroma from roasted nut (2-methylpyrazine) to caramel-like (2,3-dimethyl-5-isopentylpyrazine) to bell pepper (2-propyl-3-methoxypyrazine). The specific volatile compounds formed vary with the chemistry of the plant material, cooking temperature, the length of cooking, and other factors.

The volatile substances generally are present in very small amounts, often only a fraction of a part per million. Of the large number of volatiles given off by a plant or plant organ, typically only a very small number of compounds impart the characteristic aroma. Of the approximately 330 volatile compounds from apple, only approximately a dozen are critical components of the characteristic apple aroma. Although present in very small amounts, ethyl-2-methylbutyrate is responsible for some of the characteristic apple aroma.

These critical volatiles are called character impact compounds (CIC) and plant products can be divided into the following four general groups based in part on the presence or absence of a character impact compound.[175]

a. Those whose aroma is composed primarily of one character impact compound.
b. Those whose aroma is due to a mixture of a small number compounds, of which one may be a character impact compound.
c. Those whose aroma is due to a large number of compounds, none of which are character impact compounds, and with careful combination of these components the odor can be reproduced.
d. Those whose aroma is made up of a complex mixture of compounds that cannot be reproduced.

Examples of character impact compounds are 2-methyl-3-ethyl-pyrazine in raw white potato tubers and ethylvinylketone in soybeans. Examples of food whose aroma is made up of a small number of compounds are boiled white potatoes (2-methyl-3-ethyl pyrazine (CIC) and methional), apple (ethyl-2-methylbutyrate (CIC), butyl acetate, 2-methylbutyl acetate, hexyl acetate, hexanal and trans-2-hexenal) and boiled cabbage (dimethyl disulfide (CIC) and 2-propenylisothiocyanate). Important aroma volatiles for a cross-section of fruits and vegetables are given in Table 4.16.

6.2. Synthesis and Degradation

Due to the extremely wide range in types of chemical compounds that are important in the aroma of harvested products,[198] their biosynthesis will not be discussed in detail. There are, however, several generalizations that can be made regarding biosynthesis.

Volatile compounds that are important in the aroma of postharvest products are formed by one of three general means in intact tissue. The first group is formed naturally by enzymes found within intact tissues. These include nearly all of the odors from fresh fruits, vegetables, and flowers. While biosynthesis of many of these compounds has not been stud-

Table 4.15. Volatile Compounds from Apple Fruit.*

Alcohols
- Methanol
- Ethanol
- Isopropanol
- Butanol
- 2-Butanol
- Isobutanol
- Pentanol
- 2-Methyl butanol
- 2-Methyl-2-butanol
- 3-Methyl butanol
- 2-Pentanol
- 3-Pentanol
- 4-Pentanol
- 2-Methyl-2-pentanol
- 3-Methyl pentanol
- Hexanol
- *cis*-2-Hexenol
- *cis*-3-Hexenol
- *trans*-2-Hexenol
- *trans*-3-Hexenol
- 1-Hexen 3-ol
- 5 Hexenol
- Heptanol
- 2-Heptanol
- Octanol
- 2-Octanol
- 3-Octanol
- an Ethylhexanol
- Nonanol
- 2-Nonanol
- 6-Methyl-5-heptenol
- Decanol
- 3-Octenol
- Benzyl alcohol
- 2-Phenethanol
- Terpinen-4-ol
- α-Terpineol
- Isobareol
- Citronellol
- Geraniol

Esters

(Formates)
- Methyl formate
- Ethyl formate
- Propyl formate
- Butyl formate
- 2- or 3-Methyl butyl formate
- Pentyl formate
- i-Pentyl formate
- Hexyl formate

(Acetates)
- Methyl acetate
- Ethyl acetate
- Propyl acetate
- Butyl acetate
- Isobutyl acetate
- t-Butyl acetate
- Pentyl acetate
- 2-Methyl butyl acetate
- 3-Methyl butyl acetate
- Hexyl acetate
- Heptyl acetate
- Octyl acetate
- Benzyl acetate
- *cis*-3-Hexenyl acetate
- *trans*-2-Hexenyl acetate
- 2-Phenyl ethyl acetate
- Nonyl acetate
- Decyl acetate

(Propionates)
- Methyl n-propionate
- Ethyl propionate
- Ethyl 2-methyl propionate
- Propyl propionate
- Butyl propionate
- Isobutyl propionate
- 2- and/or 3-Methyl butyl propionate
- Hexyl propionate

(Butyrates)
- Methyl butyrate
- Ethyl butyrate
- Propyl butyrate
- Isopropyl butyrate
- Butyl butyrate
- Isobutyl butyrate
- Pentyl butyrate
- Isopentyl butyrate
- Hexyl butyrate
- Cinnamyl butyrate
- Ethyl crotonate

(Isobutyrates)
- Methyl isobutyrate
- Ethyl isobutyrate
- Butyl isobutyrate
- Isobutyl isobutyrate
- Pentyl isobutyrate
- Hexyl isobutyrate

(2 Methyl butyrates)
- Methyl-2-methyl butyrate
- Ethyl-2-methyl butyrate
- Propyl-2-methyl butyrate
- Butyl-2-methyl butyrate
- Isobutyl-2-methyl butyrate
- Pentyl-2-methyl butyrate
- Hexyl-2-methyl butyrate

(Pentanoates)
- Methyl pentanoate
- Ethyl pentanoate
- Propyl pentanoate
- Butyl pentanoate
- Amyl pentanoate
- Isoamyl pentanoate

(Isopentanoates)
- Methyl isopentanoate
- Ethyl isopentanoate
- Isopentyl isopentanoate

(Hexanoates)
- Methyl hexanoate
- Ethyl hexanoate
- Propyl hexanoate
- Butyl hexanoate
- Isobutyl hexanoate
- Pentyl hexanoate
- 2- and/or 3-Methyl butyl hexanoate

(Hexenoates)
- Butyl *trans*-2 hexenoate

(Heptanoates)
- Ethyl heptanoate
- Propyl heptanoate
- Butyl heptanoate

(Octanoates)
- Ethyl octanoate
- Propyl octanoate
- Butyl octanoate
- Isobutyl octanoate
- Pentyl octanoate
- Isopentyl octanoate
- Hexyl octanoate

(Nonanoates)
- Ethyl nonanoate

(Decanoates)
- Ethyl decanoate
- Butyl decanoate
- Isobutyl decanoate
- Pentyl decanoate
- Isopentyl decanoate
- Hexyl decanoate

Table 4.15. (*continued*)

(Dodecanoates)
Ethyl dodecanoate
Butyl dodecanoate
Hexyl dodecanoate
(Other)
Diethyl succinate
Ethyl-2-phenylacetate
Dimethylphthalate
Diethylphthalate
Dipropylphthalate

Aldehydes
Formaldehyde
Acetaldehyde
Propanal
2 Propenal
2-Oxopropanal
Butanal
Isobutanal
2-Methyl butanal
trans-2-Butenal
Pentanal
Isopentanal
Hexanal
trans-2-Hexenal
cis-3-Hexenal
trans-3-Hexenal
Heptanal
trans-2-Heptenal
Octanal
Nonanal
Decanal
Undecanal
Dodecanal
Benzaldehyde
Phenyacetaldehyde

Ketones
2 Propanone
2 Butanone
3 Hydroxybutan-2-one
2,3-Butanedione
2-Pentanone
3-Pentanone
4-Methylpentan-2-one
2-Hexanone
2-Heptanone
3-Heptanone
4-Heptanone
2-Octanone
7-Methyloctan-4-one
Acetophenone

Ethers
Diethyl ether
Methyl propyl ether
Dibutyl ether
2- and/or 3-Methyl butyl ether
Dihexyl ether
Methylphenyl ether
4 Methoxyallybenzene
cis-Linalool oxide
trans-Linalool oxide

Acids
Formic
Acetic
Propanoic
Butanoic
Isobutanoic
2-Methyl butanoic
3-Methyl butanoic
Pentanoic
Pentenoic
4-Methyl pentanoic
Hexanoic
trans-2-Hexenoic
Heptanoic
cis-3-Heptenoic
Octanoic
cis-3-Octenoic
Nonanoic
cis-3-Nonenoic
Decanoic
Decenoic
Undecanoic
Undecenoic
Dodecanoic
Dodecenoic
Tridecanoic
Tridecenoic
Tetradecanoic
Tetradecenoic
Pentadecanoic
Pentadecenoic
Hexadecanoic
Hexadecenoic
Heptadecanoic
Heptadecenoic
Octadecanoic
9-Octadecenoic
9,12-Octadecadienoic
9,12,15-Octadecatrienoic
Nonadecanoic
Nonadecenoic
Eicosanoic
Benzoic

Bases
Ethylamine
Butylamine
Isoamylamine
Hexylamine

Acetals
Diethyoxymethane
Dibutoxymethane
Dihexoxymethane
1-Ethoxy-1-propoxyethane
1-Butoxy-1-ethoxyethane
1-Ethoxy-1-hexoxyethane
1-Ethoxy-1-octoxyethane
1,1-Diethoxyethane
1,1-Dibutoxyethane
1-Butoxy-1-2-methyl butoxy ethane
1-Butoxy-1-hexoxyethane
1,1-Di-2-methyl butoxy ethane
1,2-Methyl butoxy-1-hexoxy ethane
1,1-Di-hexoxyethane
1,1-Diethoxypropane
1,1-Dipthoxypentane
4-Methoxyally benzene
Furan
Furfural
5-Hydroxymethylfurfural
2,4,5-Trimethyl-1,3-dioxolane

Hydrocarbons
Ethane
Ethylene
α-Farnesene
β-Farnesene
Benzene
Ethyl benzene
1-Methylnaphthalene
2-Methylnaphthalene
Damascenone
α-Pinene

*Source: Dimick and Hoskin[46]

Table 4.16. Volatile Compounds Responsible for the Characteristic Aroma of Selected Fruits, Vegetables and Nuts.*

Crop	*Compounds*
Vegetables	
Asparagus	Methyl-1,2-dithiolane-4-carboxylate; 1,2-diothiolane- 4-carboxylic acid
Beans	Oct-1-ene-3-ol, hex-*cis*-3-enol
Beet	2-*sec*-Butyl-3-methoxypyrazine, geosmin
Bell pepper	2-(2-methylpropyl)-3-methoxypyrazine, (*E,Z*)-2,6- nonadienal, (*E,E*)-2,4-decadienal
Brussel sprouts	2-Propenyl isothiocyanate, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide
Cabbage	2-Phenylethyl, 3-(methylthio) propyl, 4-(methylthio)-butyl isothiocyanates
Carrot	2-*sec*-Butyl-3-methoxy-pyrazine, sabinene, terpinolene, myrcene, octanal, 2-decenal
Celery	3-Butyl-phtalide, sedanolide (3-butyl-3*a*,4,5,6-tetra- hydrophthalide, β-selinene
Cucumber	(*E,Z*)-2,6-Nonadienal
Endive	Hexanal, (*Z*)-3-hexenol, (*Z*)-3-hexenal, β-ionone
Garden cress	4-Pentenyl isothiocyanate
Garlic	(*E*)- and (*Z*)-4,5,9-trithiadodeca-1,6,1-triene-9-oxide
Leek	(*E*)- and (*Z*)-3-Hexenol, dipropyl, trisulfide, propanethiol, methyl propyl disulfide, 2-propenyl propyl disulfide, 1-propenyl propyl disulfide, methyl propyl trisulfide, dipropyl trisulfide
Lettuce	2-Isopropyl-, 2-*sec*-butyl-, 2-(2-methyl-propyl)-3- methoxypyrazine
Mushrooms	1-Octen-3-one, 1-octen-3-ol, lenthionine
Onions	Thiopropanal S-oxide, 3,4-dimethyl-2, 5-dioxo-dihydro-thiophene, propyl methane-thiosulfonate
Parsley	β-Phellandrene, terpinolene, 1-methyl-4-isopropenyl benzene, *p*-mentha-1,3,8-triene, apiole
Parsnip	Myristicin
Peas	2-Alkenals, 2,4-alkadienals, 2,6-nonadienal, 3,5-octadien-2-ones, 2-alkyl-3-methoxypyrazines, hexanol
Radish	5-(Methylthio) pentyl isothiocyanate
Shallot	Methyl propyl trisulfide, dimethyl trisulfide, dipropyl trisulfide, (*E*)- and (*Z*)-1-propenyl propyl disulfide
Soybean	1-Penten-3-one, (*Z*)-3-hexenol, 2-pentylfuran, 2-pentylfuran, ethyl vinyl ketone
Sweetpotato	Maltol, phenylacetaldehyde, methyl geranate, 2-acetyl furan, 2-pentyl furan, 2-acetyl pyrrole, geraniol, β-ionone
Tomato	3-Methylbutanal, β-ionone, 1-penten-3-one, hexanal, (*Z*)-3-hexenol, 2- and 3-methylbutanol, 2- (2-methylpropyl) thiazole, eugenol, 6-methyl-5-hepten- 2-one, dimethyl trisulfide
Truffle	Dimethyl sulfide, methylenebis (methyl sulfide)
Watercress	2-Phenylethyl isothiocyanate
Fruits	
Apple	Ethyl-2-methylbutyrate, hexenal, butanol, (*E*)- 2-hexenol, hexyl acetate, (*E*)-2-hexenyl acetate
Apricot	Linalool, isobutyric acid, alkanolides
Banana	3-Methylbutyl acetate, butanoate, 3-methylbutanoate, 2-methylpropanol, 3-methylbutanol
Beli	3-Methylbutyl acetate, 3-methyl-2-buten-1-ol, α-phellandrene
Black currant	4-Methoxy-2-methyl-2-mercaptobutane, linalool, α-terpineol, 1-terpinen-4-ol, citronellol, *p*-cymene-8-ol, 2,3-butanedione
Blackberry	3, 4-Dimethoxyallybenzene, 2-heptanol, *p*-cymen-8-ol, (3,4,5-trimethoxyallybenzene), eugenol, isoeugenol, 4-hexanolide, 4-decanolide
Blueberry	Hydroxycitronellol, farnesyl acetate, farnesol, myristicine, linalool
Cashew apple	Hexanal, car-3-ene, limonene, (*E*)-2-hexenal, benzaldehyde

Table 4.16. (*continued*)

Crop	*Compounds*
Cherimoya	1-Butanol, 3-methyl-1-butanol, 1-hexanol, linalool, hexanoic acid, octanoic acid
Cherry	Benzaldehyde, (*E,Z*)-2, 6-nonadienal, linalool, hexanal, (*E*)-2-hexenol, benzaldehyde, phenylacetaldehyde, linalool, (*E,Z*)-2, 6-nonadienal, eugenol
Durian	Propanethiol, ethyl-2-methylbutanoate
Grape	Linalool, geraniol, nerol, linalool oxides
Grapefruit	Nootkatone, 1-*p*-menthene-8-thiol, limonene, acetaldehyde, decanal, ethyl acetate, methyl butanoate, ethyl butanoate
Guava	Myrcene, β-caryophyllene, α-humulene, α-selinene, α-copaene, benzaldehyde, 2-methylpropyl acetate, hexyl acetate, ethyl decanoate
Kiwi Fruit	Ethyl butanoate, (*E*)-2-hexenal
Lemon	Neral, geranial, geraniol, geranyl acetate, neryl acetate, bergamotene, caryophyllene, β-pinene, γ-terpinene, α-bisabolol
Litchi	2-phenylethanol, esters of cyclohexyl, hexyl, benzyl, citronellyl, and neryl alcohol, limonene, nonanal, decanal, citronellol, geraniol
Loquat	Phenylethanol, phenylacetaldehyde, hexenols, methyl cinnamate, β-ionone
Mango	β-carophyllene, limonene, myrcene, α-terpinolene, β-selinene
Mangosteen	Hexyl acetate, (*Z*)-3-hexenyl acetate, α-copaene
Melon	β-Ionone, benzaldehyde, (*Z*)-6-nonenal, (*Z,Z*)-3, 6- nonadienol
Orange	*d*-Limonene, ethyl butanoate, ethyl 2-methylbutanoate, ethyl propionate, methyl butanoate
Passion Fruit	2-Heptyl, 2-nonyl, (*Z*)-3-hexenyl esters, (*Z*)-3-octenyl acetate, geranyl, citronellyl esters, 3-methylthiohexanol, 2-methyl-4-propyl-1, 3-oxathianes
Papaya	Linalool, benzyl isothiocyanate
Peach	4-Decanolide, 3-methyl-butyl acetate, carvomenthenal, α-terpineol, linalool
Pear	Hexyl acetate, methyl and ethyl decadienoates, ethyl (*E*)- 2-octenoate, (*Z*)-4-decenoate, butyl acetate, ethyl butanoate
Pineapple	2-Methylbutanoates, hexanoates, methyl and ethyl 3-methylthiopropanoate
Plum	Benzaldehyde, linalool, ethyl nonanoate, methyl cinnamate, 4-decanolide
Pummelo	*d*-Limonene, myrcene, linalool, citronellal
Raspberry	1-(4-Hydroxyphenyl)-3-butanone, α- and β-ionones, linalool, gerniol
Rose-apple	Linalool, phenyl-1-propanol
Sapodilla fruit	Methyl benzoate, methyl salicylate, ethyl benzoate, phenylpropanone
Strawberry	Ethyl hexanoate, ethyl butanoate, (*E*)-2-hexenal, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, linalool
Nuts	
Brazil nut	Hexanal, methylbenzylfuran
Coconut	δ-Octalactone, δ-decalactone
Peanut	Methanol, acetaldehyde, ethanol, pentane, 2-propanol, propanal, acetone
Walnut	Hexanal, pentanal, 2, 3-pentanedione, and 2-methyl- 2-pentenal

* *Source:* Askar et al.;[5] Baldry et al.;[11] Bellina-Agostinone et al.;[12] Berger;[15] Berger et al.;[13] Berger et al.;[14] Boelens et al.;[18] Buttery et al.;[23,24,25,26] Carson;[31] Chitwood et al.;[32] Chung et al.;[33] Cronin;[37] Dürr and Röthlin;[48] Engel and Tressl;[51,52] Etievant et al.;[54] Flath and Forrey;[59] Flora and Wiley;[60] Fröhlich and Schreier;[67] Georgilopoulos and Gallois;[72] Guichard and Souty;[77] Haro and Faas;[85] Hayase et al.;[87] Hirvi et al.;[92] Hirvi;[93] Hirvi and Honkanen;[94,95] Horvat and Senter;[100] Idstein et al.;[104,106,107,109] Idstein and Schreier;[105] Jennings and Tressl;[112] Jennings;[113] Johnson and Vora;[114] Johnston et al.;[115] Kasting et al.;[117] Kemp et al.;[121,122] Kerslake et al.;[125] Kjaer et al.;[126] Kolor;[133] Koyasako and Bernhard;[134] Lee et al.;[135, 136] MacLeod and Islam;[139,140] MacLeod and Pikk;[141] MacLeod and Pieris;[142,143,147,149] MacLeod and de Troconis;[144, 145, 146,148] MacLeod et al.;[150] Marriott;[154] Mazza;[163] Morales and Duque;[170] Moshonas and Shaw;[171,172] Murray et al.;[173] Nyssen and Maarse;[176] Pyysalo;[190] Russell et al.;[197] Sawamura and Kuriyama;[202] Schreier et al.;[204] Schreier;[205] Schreyen et al.;[206,207] Shaw;[215] Shaw et al.;[213] Shaw and Wilson;[214] Spencer et al.;[219] Swords et al.;[223] Takeoka et al.;[226,227] Talou et al.;[228] Tressl and Drawert;[233] Tressl et al.;[234] Wallbank and Wheatley;[239] Wang and Kays;[241] Whitfield and Last;[245] Whitaker;[244] Winterhalter;[248] Wu et al.;[250] Yabumoto and Jennings;[252] Young and Paterson.[254]

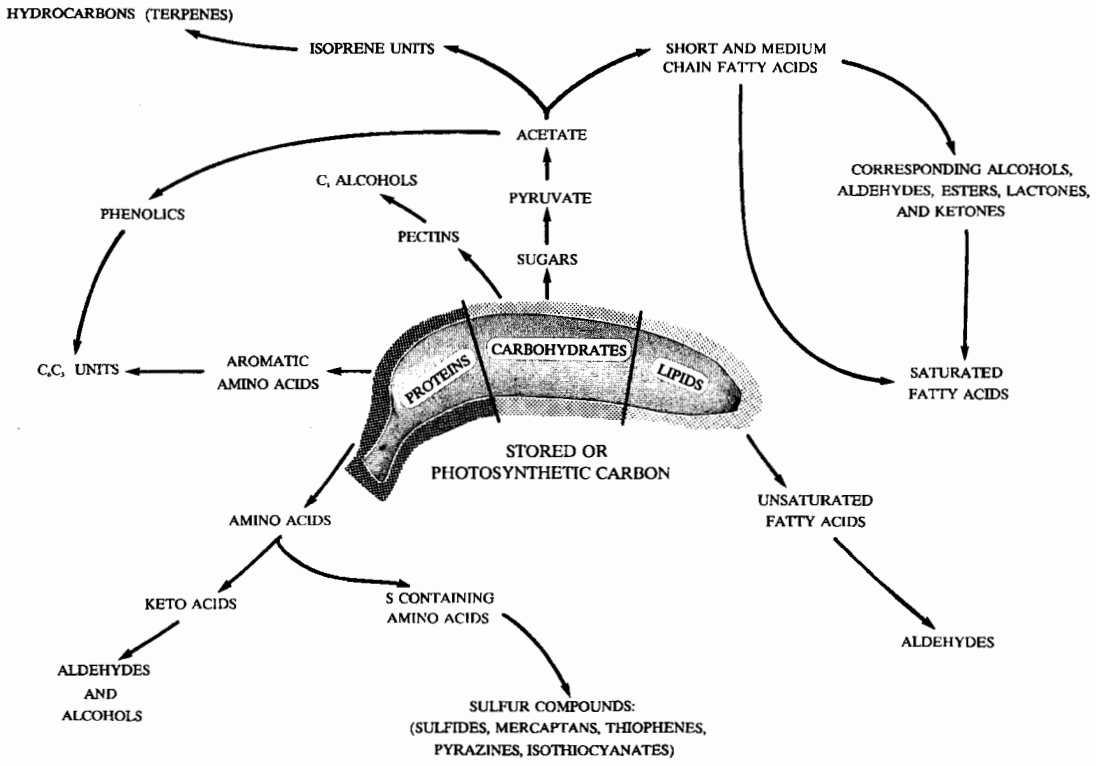


Figure 4.35. A general schematic of the biosynthetic pathways for volatile aromas of plant products.

ied in detail, three major pathways are known to be important (Figure 4.35). These are the isoprenoid pathway, the shikimic acid pathway and β-oxidation. The isoprenoid pathway contributes many of the terpenes (e.g., limonene in lemon aroma) that are found as multiples of 5 carbon isoprene subunits.[212] Over 200 monoterpenes (2 isoprene units, C_{10}) and 1,000 sesquiterpenes (5 isoprene units, C_{25}) have been identified.[229] The shikimic pathway provides benzyl alcohol, benzylaldehyde and many of the volatile phenolic compounds.[90] Beta-oxidation represents an important pathway for the production of volatiles throughout the oxidation of fatty acids. In Table 4.17, a list of potential oxidation products for three fatty acids, oleic, linoleic and linolenic, is given. Esters, an important class of fruit aromas, are formed by the enzyme acyl alcohol transferase that condenses acyl CoA with primary alcohols.[200]

A second group of volatiles are produced enzymatically after damage to the tissue. Examples would be part of the aroma of cucumbers (*cis*-3-nonenal and hexanal) formed during disruption of the intact cells[70] or the formation of methyl and propyl disulfides in onions. Cellular disruption allows enzymes and substrates, previously sequestered separately within the cells, to interact. The production of the aroma of onion is perhaps the most thoroughly studied example of this type of volatile flavor production.[192] Here the amino acids s-methyl-L-cysteine sulfoxide and s-*n*-propyl-L-cysteine sulfoxide are enzymatically degraded forming the characteristic onion volatiles. Other amino acids may also represent precursors for volatiles and as mentioned in the volatile production of undisturbed tissue, β-oxidation of fatty acids is also important in disrupted cells.

The third general means of flavor synthesis is through direct chemical reaction. This normally occurs with heating during processing or cooking. Since this alters the live product to a processed state, these volatiles are of less of a direct interest to postharvest biologists. They can

Table 4.17. Oxidation Products of Three Unsaturated Fatty Acids.*

Oleic	Propanal	Linoleic	2-Decenal
	Pentanal		Non-2,4-dienal
	Hexanal		Dec-2,4-dienal
	Heptanal		Undec-2,4-dienal
	Nonanal		Oct-1-en-3-ol
	2-Octenal		2-Heptenal
	2-Nonenal	Linolenic	Acetaldehyde
	2-Decenal		Propanal
Linoleic	Acetaldehyde		Butanal
	Propanal		2-Butenal
	Pentenal		2-Pentenal
	Hexanal		2-Hexenal
	2-Propenal		2-Heptenal
	2-Pentenal		2-Nonenal
	2-Hexenal		Hex-1,6-dienal
	2-Heptenal		Hept-2,4-dienal
	2-Octanal		Non-2,4-dienal
	2-Nonenal		Methyl ethyl ketone

**Source:* Data from Hoffman.[98]

be important, however, when a particular postharvest handling practice alters the eventual flavor of a processed product.

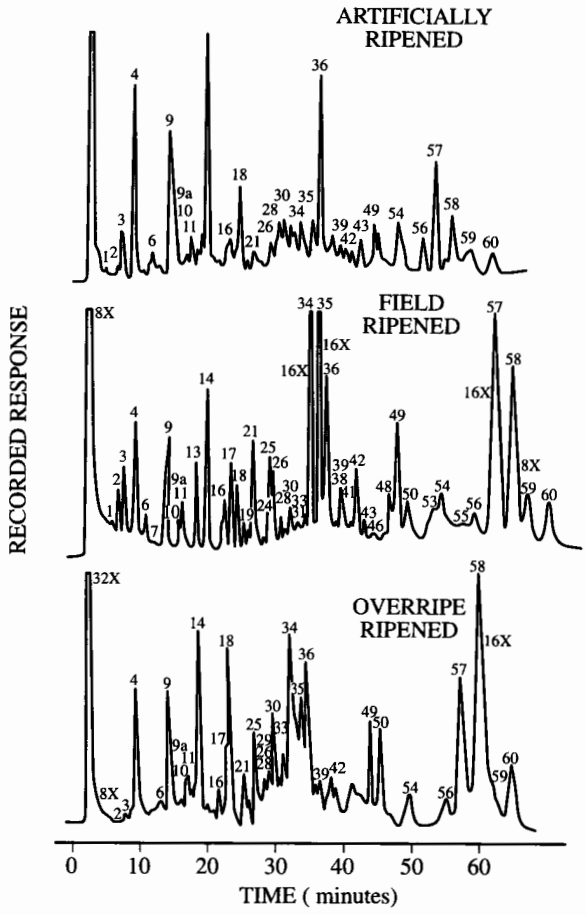
In contrast to the biosynthesis of volatile compounds by plants, there has been much less interest in their degradation, due largely to the fate of the molecules once formed. Being volatile, most of these compounds simply dissipate into the atmosphere, eventually being degraded by biological, chemical or photochemical reactions.

6.3. Postharvest Alterations

The volatiles produced by harvested products can be altered by a wide range of preharvest and postharvest factors.[7] These include cultivar, maturity, season, production practices (e.g., nutrition), handling, storage, artificial ripening and eventual method of preparation.[95] Due to the importance of volatiles in the flavor quality of food crops and aesthetic appeal of many ornamentals, care must be taken during the postharvest period to minimize undesirable changes.

Early harvest is known to have detrimental effect on the synthesis of the volatile constituents of many fruits.[96] In the tomato, the production of volatiles increases with the development of the fruit and early harvest (breaker stage) with forced ripening (22–20°C) does not yield the same volatile profile as vine-ripened fruits (Figure 4.36). The concentrations of nonanal, decanal, dodecanal, neral, benzylaldehyde, citronellyl propionate, citronellyl butyrate, geranyl acetate and geranyl butyrate are higher in field ripened than artificially ripened fruits. As tomatoes develop from a ripe to an overripe state, the concentration of 2,3-butanedione, isopentyl butyrate, citronellyl butyrate 2,3-butanedione and geranyl butyrate increase while in general the concentration of alcohols, aldehydes, acetates and propionates tend to decrease. In non-climacteric fruits (e.g., oranges) that do not ripen normally if picked at a pre-ripe stage of development, the undesirable effect of early harvest on the flavor volatiles is even more pronounced.

Storage conditions and duration may also have a significant effect on the synthesis of volatiles after removal from storage.[218] Apple volatiles can be significantly influenced by genotype, cultural practices, ripening and storage atmospheres.[56] For example, apples stored under



Peak[1]	Compound	Concentration (ppm in the fruit) Compound	Field Ripened	Overripe Red
2	2-Propanol	0.45	0.93	0.24
4	3-Methyl butanal	7.16	2.22	2.38
6	Propyl acetate	1.57	0.58	0.79
9	1-Hexanal	5.93	2.51	2.68
10	1-Butanol	1.12	0.35	0.76
11	2-Methyl-1-butanol	1.57	0.67	0.91
13	3-Pentanol	1.57	1.17	0.61
14	2-Methyl-3-hexol	7.94	2.51	2.68
17	3-Hexen-1-ol	0.78	1.28	0.79
18	Isopentyl tyrate	2.80	0.82	3.17
19	Isopentyl isovalerate	0.11	0.23	0.18
21	1-Nonanal	0.45	1.46	0.92
25	Benzaldehyde	0.22	1.17	1.40
26	1-Decanal	0.67	1.05	0.67
30	2-3-Butanedione	1.68	0.46	2.56
34	Citral b	1.56	5.95	3.66
35	1-Dodecanal	2.24	7.71	2.68
56	Linalyl acetate	2.80	2.28	1.22
57	Citronellyl propionate	5.82	17.87	7.14
58	Citronellyl butyrate	3.80	8.76	19.03
59	Geranyl acetate	2.78	2.92	2.38
60	Geranyl butyrate	1.68	2.80	4.02

[1]Peak numbers correspond to chromatogram

Figure 4.36. Effect of stage of maturity and artificial ripening on tomato fruit volatiles (*redrawn from Shah et al.*[211]).

controlled atmosphere conditions (2% oxygen, 3.5°C) have abnormal production of the critical flavor esters, butyl acetate and hexyl acetate upon removal from storage. Similar findings have been reported for hypobaric storage of fruits and the exposure of some fruits to chilling injury. Ozone (strawberry),[182] 1-methylcyclopropene (banana),[74] curing (sweetpotato)[240] and other treatments are known to alter the profile of volatiles after harvest.

Presently there is a very limited amount of information on the relationship between postharvest conditions and the beneficial or detrimental changes in the aroma of plant products. Much of the research to date has focused on determining the volatiles present and identifying specific character impact compounds. The relationship between aroma and flavor with regard to consumer preference is complex;[40,160] however, as more is learned about this area of postharvest biology, it will be possible to better control or perhaps even improve the aroma of many products.

7. PHENOLICS

Plant phenolics encompass a wide range of substances that have an aromatic ring and at least one hydroxyl group (Table 4.18). Included are derivatives of these aromatic hydroxyl compounds due to substitutions, for example, the presence of O-methylation instead of hydroxyls

Table 4.18. The Major Classes of Phenolics in Plants.*

Number of Carbon Atoms	*Basic Skeleton*	*Class*	*Examples*
6	C_6	Simple phenols	2,6-Dimethoxybenzoquinone
		Benzoquinones	Catechol, hydroquinone,
7	C_6-C_1	Phenolic acids	p-Hydroxybenzoic, salicylic
8	C_6-C_2	Acetophenones	3-Acetyl-6-methoxybenzaldehyde
		Phenylacetic acids	p-Hydroxyphenylacetic
9	C_6-C_3	Hydroxycinnamic acids	Caffeic, ferulic
		Phenylpropenes	Myristicin, eugenol
		Coumarins	Umbelliferone, aesculetin
		Isocoumarins	Bergenin
		Chromones	Eugenin
10	C_6-C_4	Naphthoquinones	Juglone, plumbagin
13	C_6-C_1-C_6	Xanthones	Mangiferin
14	C_6-C_2-C_6	Stilbenes	Lunularic acid
		Anthraquinones	Emodin
15	C_6-C_3-C_6	Flavonoids	Quercetin, cyanidin
		Isoflavonoids	Genistein
18	$(C_6$-$C_3)_2$	Lignans	Pinoresinol
		Neolignans	Eusiderin
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids	Amentoflavone
n	$(C_6$-$C_3)_n$	Lignins	
	$(C_6)_n$	Catechol melanins	
	$(C_6$-C_3-$C_6)_n$	Flavolans (Condensed tannins)	

**Source:* After Harborne.[84]

on methyleugenol. In this group, common phenolics are the flavonoids, lignin, the hormone abscisic acid, the amino acids tyrosine and dihydroxyphenylalanine, coenzyme Q and numerous end products of metabolism. Phenolics represent one of the most abundant groups of compounds found in nature and are of particular interest in postharvest physiology because of their role in color and flavor. The concentration of phenolics varies widely in postharvest products. For example, in ripe fruits it ranges from very slight to up to 8.5% (persimmon) of the dry weight (Table 4.19).

The general biological role of some phenolics in plants is readily apparent (e.g., pigments, abscisic acid, lignin, coenzyme Q), while others are involved in host plant defense, feeding deterrents, wood and bark characteristics, flower and fruit color and taste and aroma. However, for the majority of plant phenolics, their biological role has not been ascertained.

Plant phenolics are generally reactive acidic substances that rapidly form hydrogen bonds with other molecules. Often they will interact with the peptide bonds of proteins and when the protein is an enzyme this generally results in inactivation, a problem commonly encountered in the study of plant enzymes. The protein binding capacity of persimmon phenolics is so great that it is used to remove the protein from Japanese rice wine (sake), clarifying it. As a group, the phenols are susceptible to oxidation by the phenolases that convert monophenols to diphenols and subsequently to quinones. In addition, some phenols are capable of chelating metals.

Phenolic compounds rarely occur in a free state within the cell; rather they are commonly conjugated with other molecules. Many exist as glycosides linked to monosaccharides or disaccharides. This situation is especially true of the flavonoids, which are normally glycosylated. In addition, phenols may be conjugated to a number of other types of compounds. For example, hydroxycinnamic acid may be found esterified to organic acids, amino groups, lipids,

Table 4.19. Total Phenolic Content in Ripe Fruits*

Fruit	*Phenolic Content ($g \cdot kg^{-1}$ fresh weight)*
Apple	
Various cultivars	1–10
'Cox's Orange Pippin'	0–55
'Baldwin'	2.5
Cider apple 'Launette'	11
Cider apple 'Waldhofler'	4.5
Banana	5.3
Date	5.0
Cherry	
'Montmorency'	5.0
Grape	
'Riesling', cluster	9.5
'Tokay', cluster	4.8
'Muscat', skin	3.5
'Muscat', pulp	1.0
'Muscat', seed	4.5
Passion fruit	0.014
Peach	
Mixed cultivars	0.3–1.4
'Elberta', flesh	0.7–1.8
'Elberta', skin	2.4
Pear	
'Muscachet'	4.0
Persimmon	85
Plum	
'Victoria', flesh	21
'Victoria', skin	57

*Source: van Buren[235]

terpenoids, phenolics and other groups, in addition to sugars. Within the cell, this serves to render mono- and diphenols less phytotoxic than when in the free state.

Phenolics are commonly divided into three classes based on the number of phenol rings present. The simplest class includes the monocyclic phenols composed of a single phenolic ring. Common examples found in plants are phenol, catechol, hydroquinone and *p*-hydroxycinnamic acid. Dicyclic phenols such as the flavonoids have two phenol rings while the remainder tend to be lumped into the polycyclic or polyphenol class. The structures of several common phenols of each class are illustrated in Figure 4.37. These general classes can be further divided into subclasses based upon the number of carbon atoms and the pattern of the basic carbon skeleton of the molecule (Table 4.18).

7.1. Biosynthesis of Phenols

Nearly all of the phenols are formed, initially from phosphoenolpyruvate and erythrose 4-phosphate through shikimate in the shikimic acid pathway (Figure 4.38). The aromatic amino acid phenylalanine is a central intermediate that is deaminated and hydroxylated in the para position on the phenol ring yielding *p*-hydroxycinnamic acid. As mentioned earlier in the section on pigments, malonate is essential in formation of the flavonoids. Three molecules of mal-

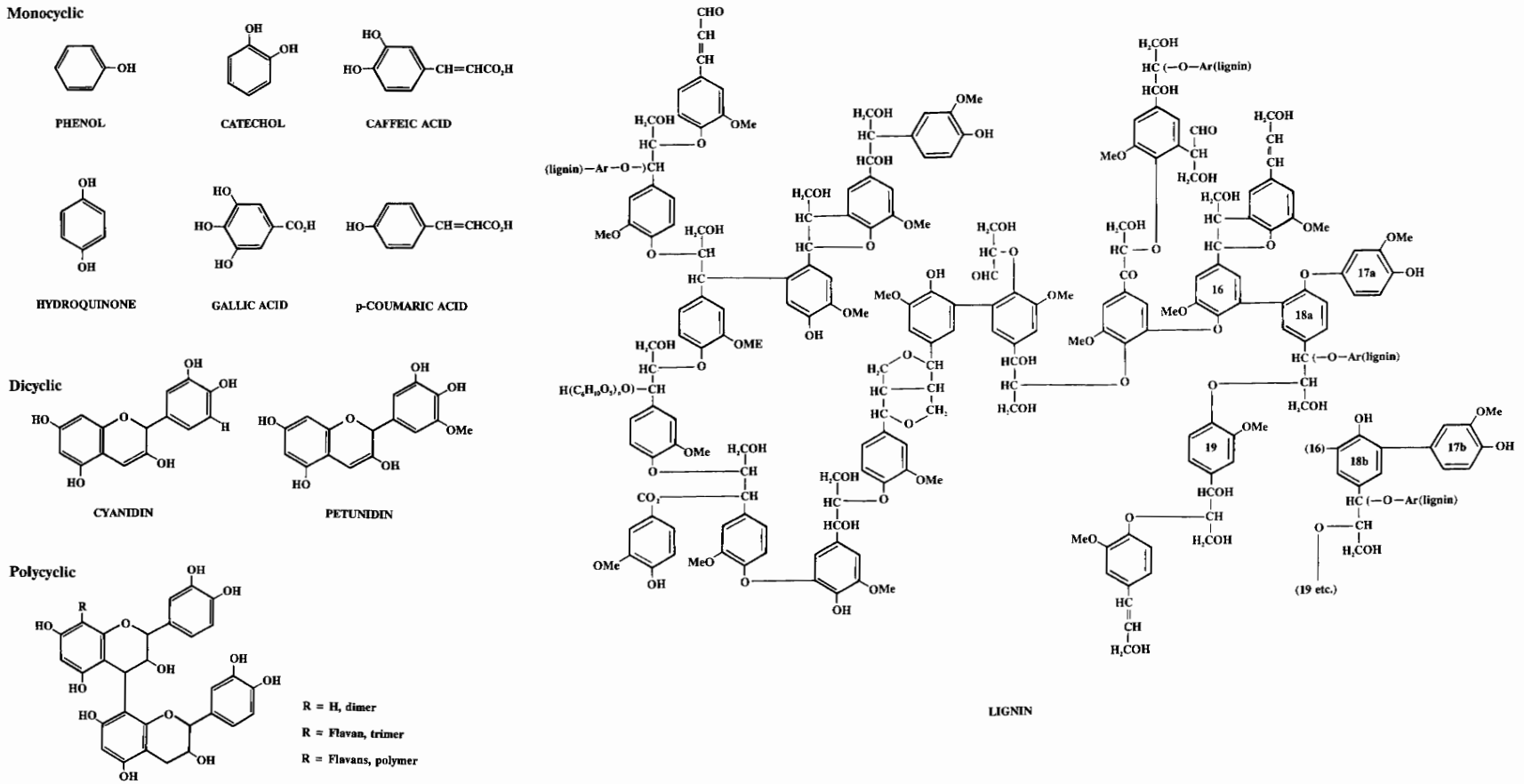


Figure 4.37. Structure of several common plant phenolics and the proposed structure of lignin (*the latter redrawn from Adler et al.*[1]).

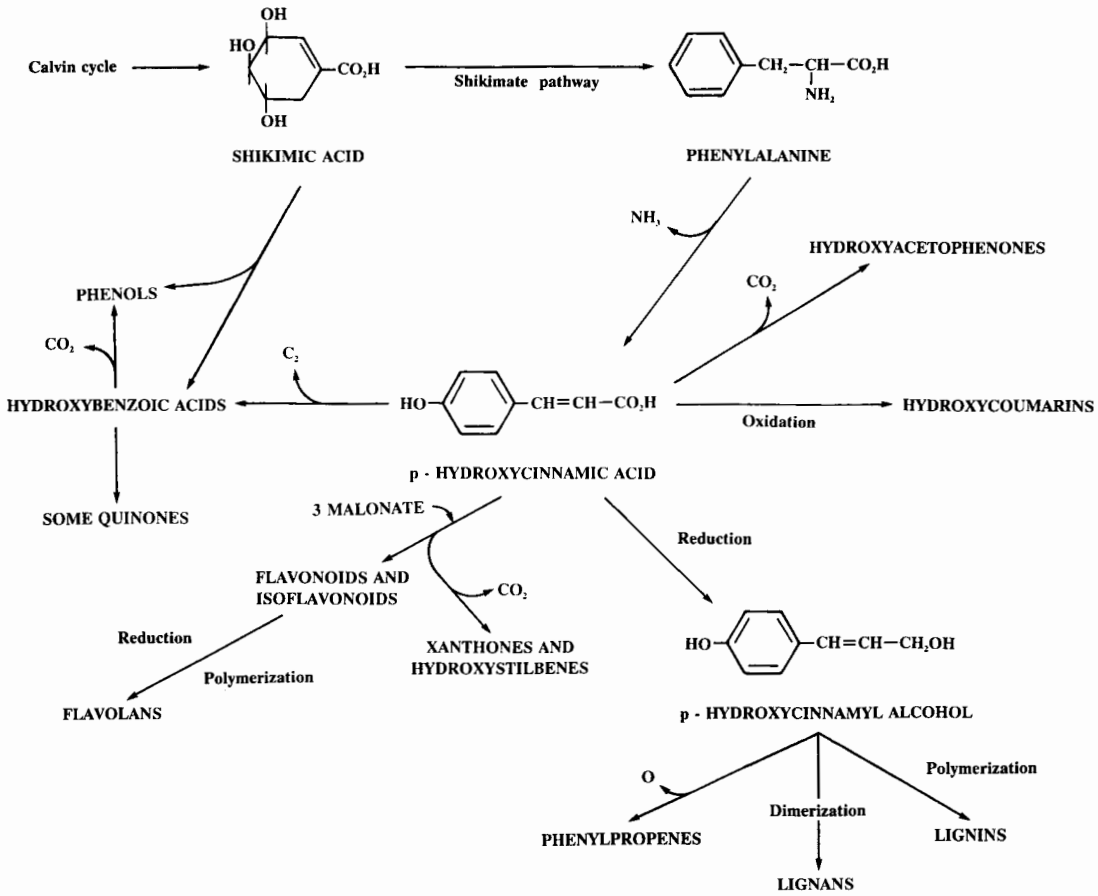


Figure 4.38. The biosynthesis of plant phenolics from shikimate and phenylalanine (*redrawn from Harborne*[84]).

onate in the form of malonyl-CoA combine with cinnamic acid (cinnamyl-CoA) to form a chalcone, which with ring closure gives the base structure for the flavonoids.

A number of physiological factors affect phenolic synthesis. These include light, temperature and cellular carbohydrate, mineral and water status. The response varies with the specific phenol and species in question. For example, at low temperature the synthesis of anthocyanin is favored while the synthesis of proanthocyanidin is repressed.

7.2. Decomposition of Phenols

For years many of the phenols were erroneously considered end products of metabolism, due in part to our inability to ascribe specific roles for these compounds within the cell. Some are now known to function as feeding deterrents to animals and insects and in disease resistance. Isotope labeling studies have shown that phenols do not simply accumulate unchanged in the plant, but in fact are in a constant state of flux, undergoing turnover *via* synthesis and degradation. The rate of turnover varies widely, from hours in some floral pigments to, in some cases, weeks. The actual sequence of catabolism varies with the diverse number of types of phenolic compounds. One common feature, however, is eventual ring cleavage and opening.

Higher plants have been shown to possess active aromatic ring-cleaving enzymes. With the simpler phenolics, β-oxidation appears to play a major role.

During the postharvest period of some plant products, changes in specific phenols are extremely important. This often entails the polymerization of the molecule into large, more complex substances.

7.3. Postharvest Alterations

Plant phenolics are of particular importance during the postharvest period due to their role in both flavor and color. While the majority of phenolics at the concentrations in which they are found in food products have no significant taste, several do substantially alter the flavor of certain products. The phenolic acids, when present in a sufficiently high concentration are distinctly sour and some of the flavonoids in citrus (e.g., naringin) are bitter. In addition, phenolics in the immature fruits of several species are highly astringent. For example, immature banana fruit contain 0.6% water soluble tannins (phenolics) on a fresh weight basis, the fruit of Chinese quince—0.4%, carob beans—1.7%, and persimmon fruit—2.0%.[159] These compounds are also found in other plant parts such as leaves, bark and seed coats of some species. In the persimmon, the water soluble tannins are composed of cathechin, catechin-3-gallate, gallocatechin, gallocatechin-3-gallate and an unknown terminal residue.[158]

During persimmon maturation and fruit ripening, the level of astringency and the concentration of water-soluble tannins diminish, eventually giving fruit of excellent flavor. This decrease in astringency is due to polymerization of the existing tannins, forming larger, water-insoluble molecules no longer capable of reacting with the taste receptors in the mouth. It has been known since the early part of this century that the astringency removal process could be substantially shortened by exposing the fruit to a high carbon dioxide atmosphere.[75] The rate of the induction response is temperature dependent; at 40°C the exposure may be as short as 6 hours. This initial induction process is followed by a period of astringency removal that occurs during a three-day air storage period (30°C). It is thought that the anaerobic conditions result in a small but significant build-up in acetaldehyde. Aldehydes react readily with phenols resulting in cross-linking between neighboring molecules, hence producing the insoluble non-astringent form. This same basic aldehyde-phenol reaction was used in the production of Bakelite plastic and when discovered in the early nineteen hundreds ushered in the plastics revolution.

Another important postharvest phenolic response is the discoloration (browning) of many products upon injury to the tissue. Injury may occur during harvest, handling or storage, resulting in the breakdown of organizational resistance between substrates and enzymes within the cell. Common examples would be bruising of fruits or broken-end discoloration of snap beans. When browning occurs, constituent phenols are oxidized to produce a quinone or quinone-like compound that polymerizes, forming brown pigments. These unsaturated brown polymers are sometimes referred to as melanins or melaninodins. Among the compounds believed to be important substrates are chlorogenic acid, neochlorogenic acid, catechol, tyrosine, caffeic acid, phenylalanine, protocatechin and dopamine.

Research on phenolic discoloration has focused on the cellular constituents responsible for browning and the handling and storage techniques that can be used to prevent browning. The success of attempts to correlate the concentration of total and specific phenols or the level of activity of the phenolase enzymes to tissue discoloration varies widely, depending on the tissue in question and in some cases, even with the cultivar studied. Several postharvest techniques, however, have been successful for inhibiting the browning of some products. For example, during the harvest of snap beans it is not uncommon for the ends of many of the bean

pods to be broken. These brown fairly readily and upon processing yield a distinctly substandard product. The reactions involved in discoloration can be inhibited by exposing the beans to 7500 to 10,000 $\mu L \cdot L^{-1}$ sulfur dioxide for 30 seconds.[89] The use of controlled atmosphere storage and antioxidants has been successful with other products.

8. VITAMINS

Vitamins represent a group of organic compounds required in the human diet in relatively small amounts for normal metabolism and growth. Plant products provide a major source for many of the vitamins required by man. Exceptions to this would be vitamin B_{12} that appears to be synthesized only by microorganisms and vitamin D, obtained mainly from the exposure of our skin to ultraviolet light. In plants, many of the vitamins perform the same biochemical function as they do in animal cells. As a consequence, most have a vital role in plant metabolism, in addition to being a source of vitamins for animals.

Although vitamins are required in only small amounts in the diet, deficiencies have been a serious problem throughout the history of man. Even today deficiencies of certain vitamins result in major nutritional diseases in many areas of the world. Insufficient vitamin A in the diet is perhaps the most common, especially in parts of Asia. Prolonged deficiency, particularly in young children, results in blindness.

Typically, vitamins are separated into two classes based on their solubility: the water-soluble vitamins (thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid and ascorbic acid) and those that are lipid-soluble (vitamins A, E and K). Normally the lipid-soluble vitamins are stored in the body in moderate amounts; as a consequence, a consistent daily intake is not essential. The water-soluble vitamins, however, tend not to be stored and a fairly constant day-to-day supply is required.

Vitamins function in a catalytic capacity as coenzymes—organic compounds that participate in the function of an enzyme. The role of the water-soluble vitamins (principally coenzymes) is for the most part fairly well understood, while the precise metabolic function of the lipid-soluble vitamins is not yet clear.

8.1. Water-Soluble Vitamins

8.1.1. Thiamine

Structurally thiamine or Vitamin B_1 is a substituted pyrimidine joined to a substituted thiazole group through a methylene bridge (Figure 4.39). It is water-soluble and is relatively stable at pH's below 7.0. The vitamin is found widely in both plants and animals, in several forms. In plant tissues it is found most abundantly as free thiamine; however, it may also be found as mono-, di- and triphosphoric esters and as mono- and disulfides in various biological materials. Thiamine functions in plants as the coenzyme thiamine pyrophosphate, which plays a major role in the glycolytic pathway (the decarboxylation of pyruvic acid), the tricarboxylic acid cycle (the decarboxylation of α-ketoglutaric acid) and in the pentose phosphate pathway (as a carboxyl group transferring enzyme).

In contrast to some vitamins such as ascorbic acid, the concentration of thiamine in various plants and plant parts is fairly uniform, generally varying only within a 20 to 40-fold range. Typically, dried beans and peas contain approximately 700 to 600 $\mu g \cdot 100\ g^{-1}$; nuts 500 to 600 μg; whole grain cereals, 300 to 400 μg; and fruits and vegetables 20 to 90 μg. In addition to variation among species, the absolute concentration of thiamine in edible plant products varies somewhat with cultivar and growing conditions. After harvest, the vitamin is relatively stable

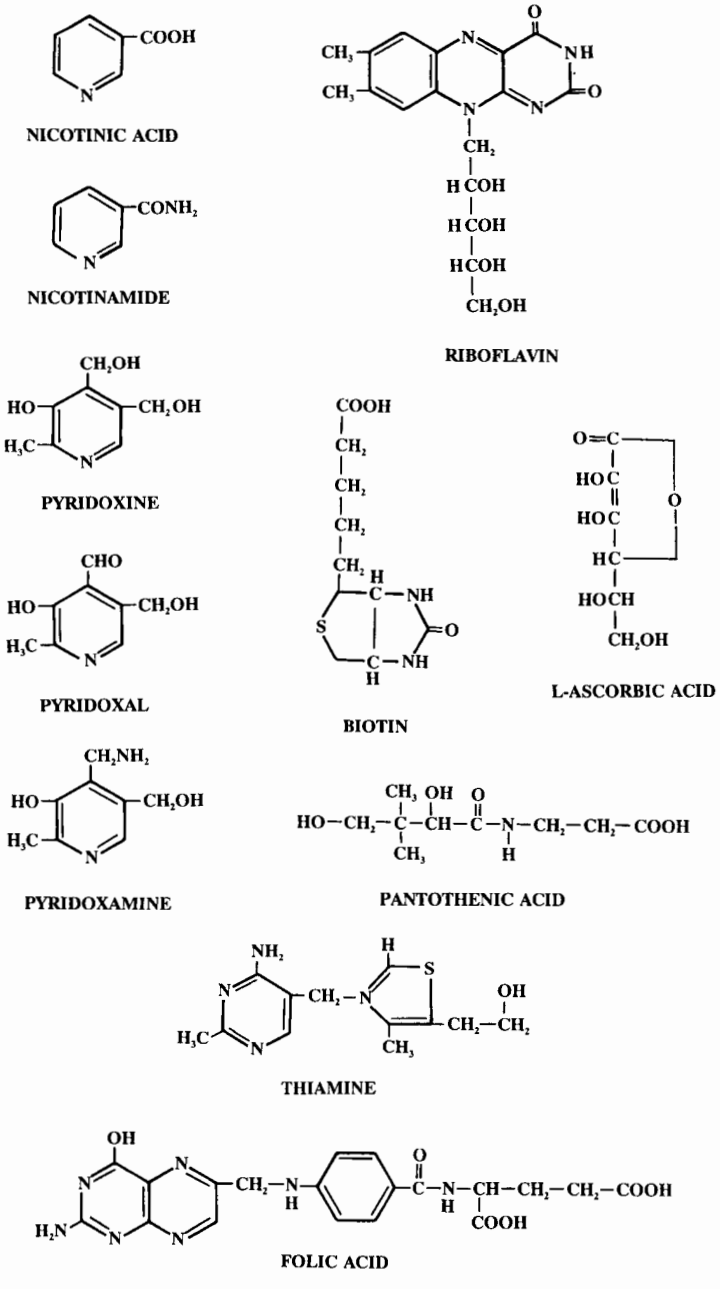


Figure 4.39. Structures of water soluble vitamins.

during storage. Primary losses incurred are most commonly the result of cooking, due largely to the molecules high water solubility.

8.1.2. Riboflavin

Riboflavin is a water-soluble derivative of D-ribose which contains an isoalloxazine ring [6,7-dimethyl-9-(D-1′-ribityl) isoalloxazine] (Figure 4.39). It is also know as vitamin B_2 and lactoflavin. Riboflavin or derivatives of riboflavin are found in all plants and many microorgan-

isms; however, it is not synthesized by higher animals. Within plants it is found combined with other groups, largely as either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). This is also the case in most parts of the body of animals although in the retina of the eye it is found in its free form. Flavin nucleotides act as prothestic groups in oxidation-reduction enzymes. The isoalloxazine ring portion of the flavin nucleotide undergoes a reversible reduction to yield the reduced nucleotides $FMNH_2$ and $FADH_2$. Enzymes containing flavin nucleotides are essential for the oxidation of pyruvate and fatty acids and function in the electron transport system. Typically, the oxidized form of the molecule is colored yellow, red or green, while the reduced form is colorless.

Leafy vegetables represent a relatively good source of riboflavin, although vegetables that are high in riboflavin (e.g., pimento pepper, 0.46 mg · 100 g^{-1} fresh wt., mushrooms, 0.30 mg; lotus root, 0.22 mg; salsify, 0.22 mg) are consumed in only small amounts in most diets.

8.1.3. Niacin

Niacin or nicotinic acid is found widely in both plants and animals, either as the acid or amide (Figure 4.39). The name nicotinic acid comes from the molecules role as a component of the toxic alkaloid, nicotine from tobacco. Nicotinic acid is synthesized by animals, if their diet contains sufficient protein which is high in the amino acid tryptophan, the primary precursor of the vitamin. The coenzymes contain nicotinamide as an essential component. These are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), known also as pyridine coenzymes. Both function as coenzymes in a large number of oxidation-reduction reactions catalyzed by what are known as pyridine-linked dehydrogenases. Most of the dehydrogenase enzymes are specific for either NAD or NADP, although several can utilize either form. In general, these reactions are reversible and are extremely important in many pathways within the cell.

8.1.4. Pyridoxine

Pyridoxine or vitamin B_6 is found in three forms, pyridoxine, pyridoxal and pyridoxamine (Figure 4.39)—pyridoxine typically being converted to the latter two forms, which are more efficacious. The active coenzyme forms of the vitamin are the phosphate derivatives: pyridoxal phosphate and pyridoxamine phosphate. Pyridoxine coenzymes function in a wide range of important reactions in amino acid metabolism such as transamination, decarboxylation and racemization reactions. Pyridoxal phosphate is also involved in the biosynthesis of ethylene, acting at the point of conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC). Ethylene synthesis is blocked at this step by 2-amino-4-aminoethoxy-*trans*-3-butenoic acid (AVG), a potent inhibitor of pyrixodal phosphate mediated enzyme reactions.

The three forms of the vitamin are found widely distributed in both the plant and animal kingdom; the predominant form, however, varies between sources. In vegetables, pyridoxial is the predominant form. Cereals (0.2 to 0.4 mg · 100 g^{-1}) and vegetables (e.g., Brussels sprouts, 0.28 mg · 100 g^{-1}; cauliflower, 0.2 mg; lima beans, 0.17 mg; spinach, 0.22 mg) represent good sources of the vitamin while many of the fruits are quite low (e.g., apple, 0.045 mg · 100 g^{-1}; orange, 0.05 mg). Glycosyl forms are also found.[224]

8.1.5. Pantothenic Acid

Pantothenic acid, formed from pantoic acid and the amino acid β-alanine, is found in limited quantities in most fruits and vegetables (Figure 4.39). The active form of the vitamin, co-

enzyme A (CoA), is synthesized from pantothenic acid in a series of steps. Coenzyme A functions as a carrier of acyl groups in enzymatic reactions during the synthesis and oxidation of fatty acids, pyruvate oxidation and a number of other acetylation reactions within the cell.

Deficiencies of the vitamin in animals are rare; a limited amount of storage of the molecule does occur in the heart, liver and kidneys. In diets with sufficient animal protein, most of the pantothenic acid is derived from this source. Dried peas and peanuts are considered good sources of the vitamin; walnuts, broccoli, peas, spinach, and rice, intermediate sources (0.5 to 2.0 mg · 100 g^{-1}); and onions, cabbage, lettuce, white potatoes, sweetpotatoes and most fruits, poor sources (0.1 to 0.5 mg · 100 g^{-1}).

8.1.6. Biotin

The vitamin biotin consists of fused imidazole and thiophene rings with an aliphatic side chain (Figure 4.39). Its structure, established in 1942, suggested the possible role of pimelic acid as the natural precursor of the molecule, which was later proven to be correct. Biotin is found widely in nature, usually in combined forms bound covalently to a protein through a peptide bond. When bound to a specific enzyme, it functions in carboxylation reactions. Here it acts as an intermediate in the transfer of a carboxyl group from either a donor molecule or carbon dioxide to an acceptor molecule. Examples of enzymes in which biotin acts as a carboxyl carrier are propionyl-CoA carboxylase and acetyl-CoA carboxylase.

Biotin is found widely distributed in foods and is also synthesized by bacteria in the intestine. As a consequence, deficiencies are extremely rare. When present they are normally associated with high intake of avidin, a protein found in raw egg whites that binds to the vitamin making it unavailable. Legumes, especially soybeans, represent an excellent plant source of biotin (61 μg · 100 g^{-1} edible product). In addition, nuts such as peanuts (34 μg), pecans (27 μg) and walnuts (37 μg) and a number of vegetables are good sources (e.g., southern peas, 21 μg; cauliflower, 17 μg; mushrooms, 16 μg), while most fruits and processed grains are consistently low in biotin.

8.1.7. Folic Acid

Folic acid is found widely distributed in plants, its name being derived from the Latin word *folium* for "leaf" from which it was first isolated. Structurally the molecule is composed of three basic subunits: 1) a substituted pteridine, 2) *p*-aminobenzoic acid, and 3) glutamic acid (Figure 4.39). The active coenzyme form of the vitamin is tetrahydrofolic acid, formed in a two-step reduction of the molecule. It functions as a carrier of one-carbon units (e.g., hydroxymethyl-CH_2OH, methyl-CH_2 and formyl-CHO groups) when these groups are transferred from one molecule to another. These reactions are critical steps in the synthesis of purines, pyrimidines and amino acids.

Folic acid is found widely in the plant kingdom and is also synthesized by microorganisms including intestinal bacteria. It is needed by humans and other animals in very small amounts (e.g., 0.4 mg · day^{-1} for humans) but is rapidly excreted from the body. Asparagus, spinach and dried beans are excellent sources of the vitamin; corn, snap beans, kale and many nuts, moderate sources (30 to 90 μg · 100 g^{-1}); and cabbage, carrots, rice, cucumbers, white potatoes, sweetpotatoes and most fruits, poor sources (0–30 μg · 100 g^{-1}).

8.1.8. Ascorbic Acid

Ascorbic acid is structurally one of the least complex vitamins found in plants.[217] It is a lactone of a sugar acid (Figure 4.39), synthesized in plants from glucose or another simple carbohy-

drate. It was first isolated in crystalline form in 1923. In spite of years of research, the precise physiological function of ascorbic acid in plant and animal cells remains unclear.[217] It is known to act as a cofactor in the hydroxylation of proline to hydroxyproline, however, other reducing agents can replace it.

Ascorbic acid is required in the diet of man and only a small number of other vertebrates and is supplied primarily by fruits and vegetables, although a small amount is found in animal products such as milk, liver and kidneys. In comparison with the other water-soluble vitamins in plants, ascorbic acid is found in relatively high concentrations. Guava (300 mg $\cdot 100\ g^{-1}$ fresh weight), black currants (210 mg), sweet peppers (125 mg) and several greens (kale, collards, turnips—120 mg) are excellent sources. The West Indian cherry (acerola) contains approximately 1300 mg $\cdot$ 100 g^{-1} fresh weight.[179] Staples such as rice, wheat, corn and many of the starchy tubers tend to be extremely low. Fruits have a distinct advantage in the diet in that they are often served raw. During cooking a significant portion of the ascorbic acid of many vegetables is lost. This is due primarily to leaching of the water-soluble vitamin out of the tissue and to oxidation of the molecule. Losses from leaching tend to be greater in leafy vegetables due to the surface area in contrast to bulkier products.

The concentration of ascorbic acid often varies with location within a specific plant part and between different parts on the same plant. For example, in many fruits, the concentration in the skin is higher than in the pulp. The concentration of ascorbic acid declines fairly rapidly in many of the more perishable fruit and vegetables after harvest. Losses are greater with increasing storage temperature and duration.

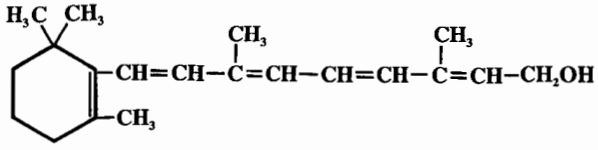
8.2. Lipid-Soluble Vitamins

8.2.1. Vitamin A

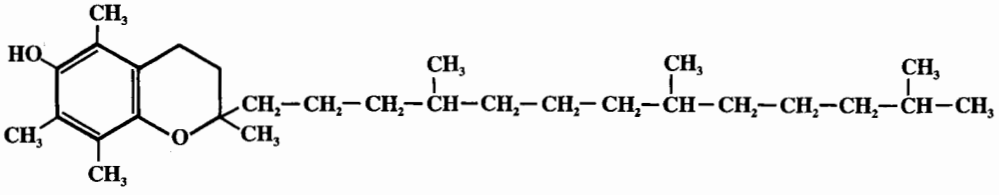
Vitamin A or retinol is an isoprenoid compound with a 6 carbon cyclic ring and an 11 carbon side chain (Figure 4.40). It is formed in the intestinal mucosa by cleavage of carotene. Of the numerous naturally occurring carotenoids, only 10 have the potential to be converted into vitamin A and of these, β-, α- and γ-carotene are the most important. The presence of a β end group is essential for the formation of the molecule. Beta-carotene with two β end groups has twice the potential vitamin A as α-carotene, which is composed of a β and ε end group. Cleavage appears to be due to the presence of the enzyme β-carotene-15,15′-dioxygenase that oxidizes the central double bond; however, it is possible that other conversion mechanisms may occur.

Vitamin A is extremely important in human nutrition in that its synthesis is dependent upon carotene ultimately from plant sources. In contrast to ascorbic acid, only a small amount of vitamin A is needed in the diet. This ranges from 0.4 to 1.2 mg $\cdot$ day^{-1} depending on age and sex. Although it appears to be required in all of the tissues of the body, its general function in metabolism is not known, aside from its role in eyesight. A deficiency in young children results in permanent blindness, a common problem in many tropical areas of the world.

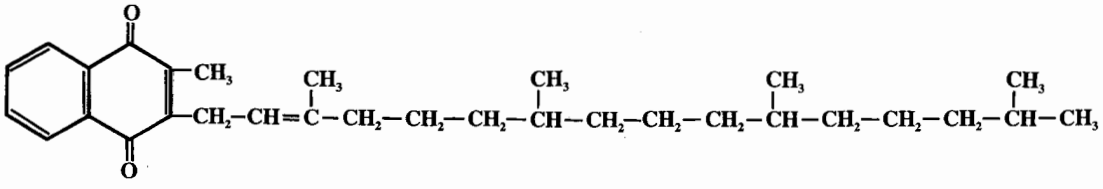
Since vitamin A per se is not present in plants, its potential concentration is measured in "international units" (IU), based on the concentration of α- and β-carotene in the tissue. One international unit of vitamin A is equal to 0.6 μg of β-carotene or 1.2 μg of α-carotene. Leafy vegetables average approximately 5000 IU $\cdot$ 100 g^{-1} fresh weight; and fruits 100–500 IU, although the mango (3000 IU) and papaya (2500 IU) are distinctly higher; while staple crops such as rice, peanuts and cassava have virtually none. An exception to this would be orange fleshed sweetpotatoes in which some of the high carotene cultivars contain up to 14,000 IU of vitamin A $\cdot$ 100 g^{-1} fresh weight.



VITAMIN A



VITAMIN E



VITAMIN K

Figure 4.40. Structures of lipid soluble vitamins.

The concentration of carotene is known to vary widely among species and cultivars and less so due to production environmental conditions and cultural practices, although temperature and light intensity are known to have significant effects.[57, 212]

8.2.2. Vitamin E

Vitamin E or α-tocopherol is a molecule composed of a chromanol ring and a side chain formed from a phytol residue (Figure 4.40). In addition to α-tocopherol, β-, γ- and δ-tocopherol are also found in photosynthetic plants, although α-tocopherol is the most active form as a vitamin.

The biological role of α-tocopherol in animals, as in plants, is unclear. Vitamin E deficiency results in a number of symptoms in test animals, one of which is infant mortality, hence the derivation of the name tocopherol from the Greek word *tokos* meaning "childbirth." Tocopherols are known to have antioxidant activity, which prevents the autoxidation of unsaturated lipids. As a consequence, one function may be the protection of membrane lipids.

In plants, α-tocopherol is found associated with the chloroplast membrane and is thought to also be present in mitochondria. It also appears to be located in various plastids. Plant oils are an excellent source of tocopherols. Significant concentrations are found in

wheat germ, corn and pecan oils. Pecans contain up to 600μg of tocopherol · g^{-1} of oil approximately 6 weeks prior to maturity. This amount declines to 100 to 200 μg · g^{-1} by maturation. In contrast to many other nuts (e.g., filberts, walnuts, Brazil nuts, almonds, chestnuts and peanuts), the γ-tocopherol isomer is found almost exclusively (>95%) in lieu of the α-tocopherol isomer.

The role of tocopherols in plants is thought to be related to their antioxidant properties.[39] This is supported by the correlation between the concentration of tocopherols in pecans, which tend to deteriorate in storage due to the oxidation of their component lipids, and the length of time they can be successfully stored. In pecan oils with a constant linolate concentration, keeping time increased in a linear fashion up to 800 μg of tocopherol · g^{-1} of oil.[196] The germination of wheat seeds is also correlated with tocopherol content.[45] Tocopherols are also thought to function as a structural component of chloroplast membranes and may in some way function in the initiation of flowering of certain species.

8.2.3. Vitamin K

Vitamin K or phylloquinone is a lipid soluble quinone, which is in many ways structurally very similar to α-tocopherol. Both are cyclic compounds with a phytol residue side chain composed of isoprene units (Figure 4.40). Two forms of the vitamin, K_1 and K_2, are known. A deficiency of vitamin K limits proper blood clotting in animals through the repressed formation of fibrin, the fibrous protein portion of blood clots. This in turn results in a tendency to hemorrhage. Aside from this specific function, its widespread occurrence in plants and microorganisms suggests a more general, but presently undefined biological role. As a quinone, it could possibly function as an electron carrier.

In plants, phylloquinone is present in most photosynthetic cells, hence leafy green tissues represent an excellent dietary source of the vitamin. Plant parts that normally do not contain chlorophyll have little vitamin K. Likewise, mineral deficiencies that repress chlorophyll synthesis (e.g., Fe) also appear to decrease the concentration of vitamin K.

9. PHYTOHORMONES

Five major groups of naturally occurring compounds, the phytohormones, are currently known to exist each of which exhibits strong plant growth regulating properties.[124] Included are ethylene, auxin, gibberellins, cytokinins and abscisic acid (Figure 4.41); each is structurally distinct and active in very low concentrations within plants.[41] Several new compounds have been identified (salicylic acid, jasmonate, and polyamines) which are involved in the plant's response to environmental stress and disease and insect resistance. In that their importance during the postharvest period is only beginning to be deciphered, the following critique will focus on the five "classical" plant hormones.

While each of the phytohormones has been implicated in a relatively diverse array of physiological roles in plants and detached plant parts, the precise mechanism in which they function is not yet completely understood.[42] During the postharvest period, ethylene is of major importance in that it is closely associated with the regulation of senescence in some products and the ripening of many fruits.[4] This section focuses primarily on the synthesis and deactivation of these molecules and changes in their concentration occurring during the postharvest period.

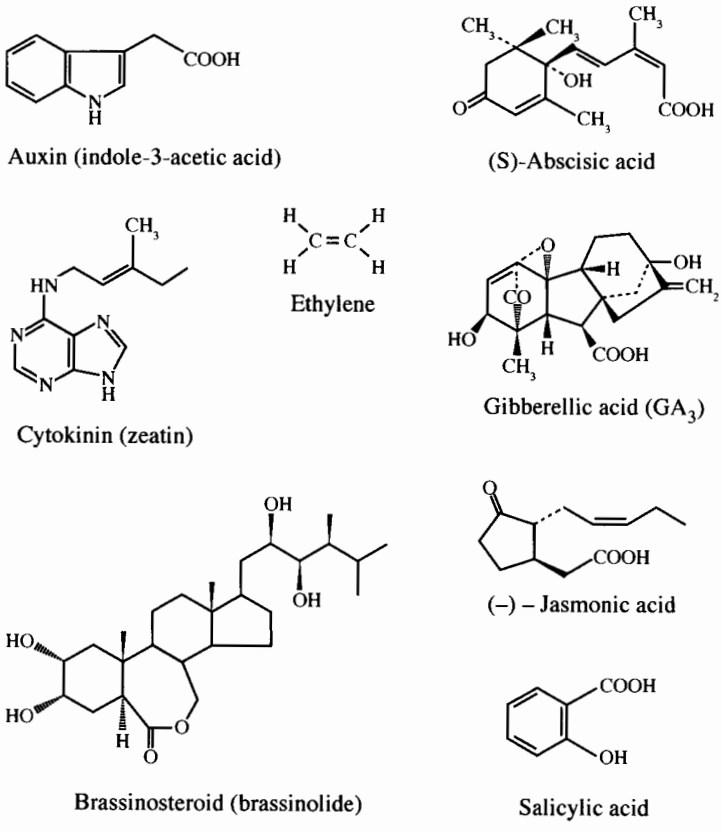


Figure 4.41. Structures of the phytohormones auxin, abscisic acid, ethylene, cytokinin, gibberellic acid, jasmonic acid, salicylic acid, brassinosteroid, and polyamines, represented by spermidine.

9.1. Classes, Synthesis and Degradation of Phytohormones

9.1.1. Ethylene

Ethylene, being a gaseous hydrocarbon, is unlike the other naturally occurring plant hormones. Although ethylene was known to elicit such responses as gravitropism and abscission early in the last century, it was not until the 1960's that it began to be accepted as a plant hormone.[4]

The effect of ethylene on plants and plant parts is known to vary widely. It has been implicated in ripening, abscission, senescence, dormancy, flowering and other responses. Ethylene appears to be produced by essentially all parts of higher plants, the rate of which varies with specific organ and tissue and their stage of growth and development. Rates of synthesis range from very low (0.04–0.05 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) as in blueberries to extremely high (3400 $\mu L \cdot kg^{-1} \cdot hr^{-1}$) in fading blossoms of *Vanda* orchids. Alterations in the rate of synthesis of ethylene

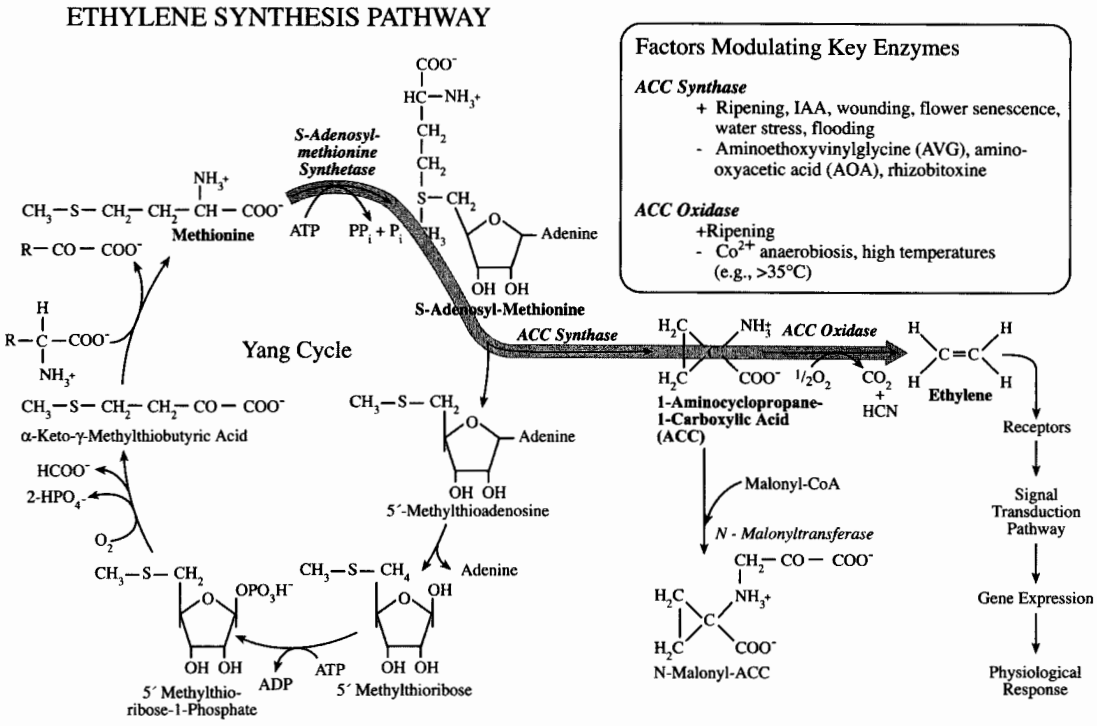


Figure 4.42. The ethylene synthesis pathway and Yang cycle. The initial precursor is the amino acid methionine and key regulatory enzymes in the pathway are ACC synthase and ACC oxidase which are positively and negatively modulated by a number of factors (see box). Hydrogen cyanide, formed in the last step of ethylene synthesis, is detoxified by cysteine. The ACC pool can be diminished *via* conversion to *N*-malonyl ACC. After synthesis ethylene may bind to receptors and through a series of steps instigate a physiological response or simply diffuse out of the tissue.

have been found, in some cases, to be closely correlated with the development of certain physiological responses in plants and plant organs, for example the ripening of climacteric fruits and the senescence of flowers.

Ethylene is synthesized from the sulfur-containing amino acid methionine which is first converted to s-adenosyl methionine (SAM) and then to the 4 carbon compound, 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (Figure 4.42). During conversion to ACC, the sulfur-containing portion of the molecule, 5-methylthioadenosine, is cycled back to methionine *via* the formation of ribose and condensation with homoserine.[123,253] The final step in the synthesis pathway is the conversion of ACC to ethylene by ACC oxidase (ACO) which requires oxygen (previously called the "ethylene forming enzyme"). Stress (water, mechanical and others) is known to stimulate ethylene synthesis and, under some conditions, markedly so.

While ethylene appears to be synthesized in all cells, ACO is possibly associated with the tonoplast, vacuoles isolated from protoplasts were able to convert ACC to ethylene.[79,166] Likewise, protoplasts that had their vacuoles removed (evacuolated) lose their capacity to produce ethylene from ACC; when the vacuoles were allowed to reform, synthesis is reinstated.[53]

Several potent inhibitors of ethylene synthesis have been found (rhizobitoxine and AVG, Figure 4.42) and were integral components in elucidating the pathway. Lieberman first showed that fungal metabolites from *Rhizobium japonicum, Streptomyces* sp. and *Pseudomonas aerug-*

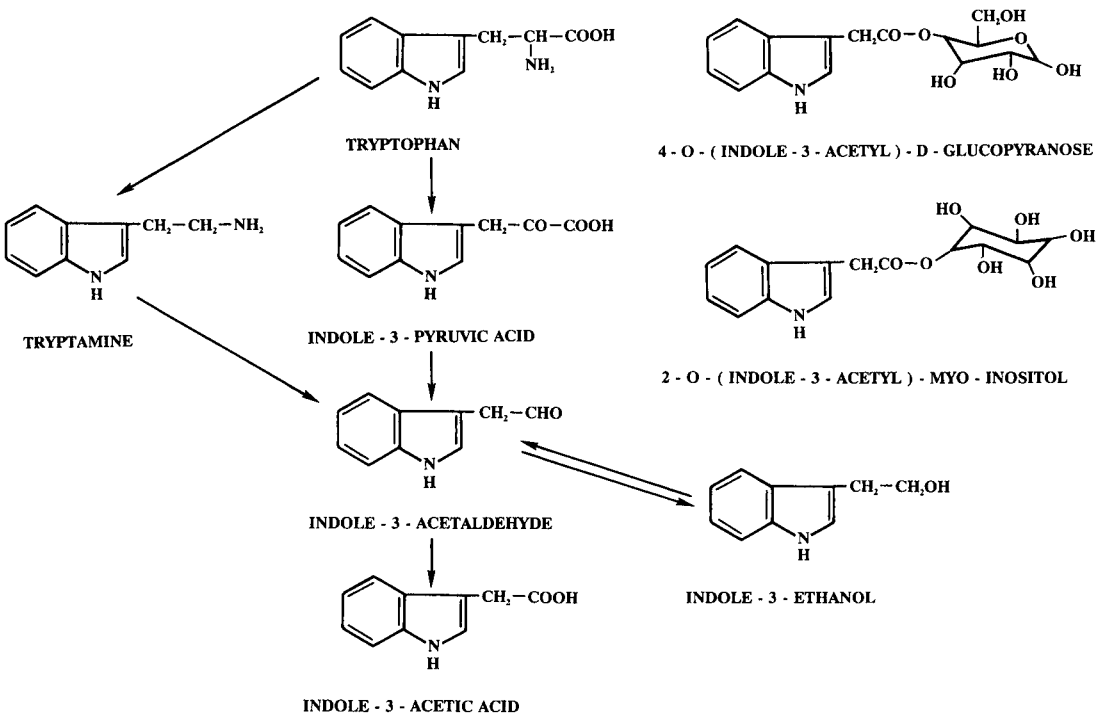


Figure 4.43. Biosynthesis of indoleacetic acid from tryptophan *via* both the indole-3-pyruvic acid and tryptamine pathways.

inosa inhibit the conversion of SAM to ACC. These unfortunately also inhibit other pyridoxal phosphate requiring enzymes in plants and animals and as a consequence are of little commercial value for postharvest products that are to be consumed.[4] Silver and 1-methylcyclopropene (1-MCP) are effective inhibitors of ethylene action *via* their interference with the binding site for ethylene. 1-Methylcyclopropene provides a more transitory (i.e., 5 to 7 days) inhibition than silver ions which exhibit a longer-term effect.

Since ethylene is continuously being produced by plant cells, some mechanism is essential to prevent the build-up of the hormone within the tissue. Unlike other hormones, gaseous ethylene diffuses readily out of the plant. This passive emanation of ethylene from the plant appears to be the primary means of eliminating the hormone. During the postharvest period, techniques such as ventilation and hypobaric conditions help to facilitate this phenomenon by maintaining a high diffusion gradient between the interior of the product and the surrounding environment. A passive elimination system of this nature would imply that the internal concentration of ethylene is controlled largely by the rate of synthesis rather than the rate of removal of the hormone.

Ethylene may also be metabolized within the cell, decreasing the internal concentration. Products such as ethylene oxide and ethylene glycol have been found, however, their importance in regulating the internal concentration of ethylene in most species appears to be very minimal.

9.1.2. Auxin

The name auxin, from the Greek word "*auxin*" meaning to increase, is given to a group of compounds that stimulate elongation. Indoleacetic acid (IAA) (Figure 4.43) is the prevalent

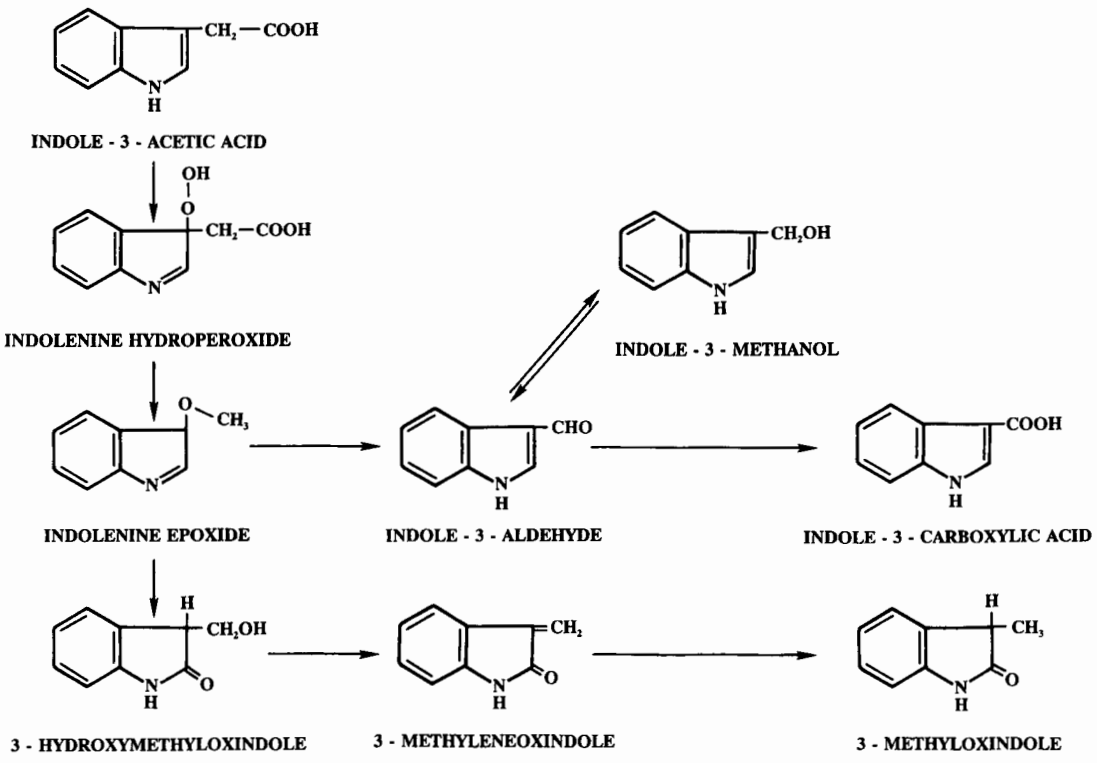


Figure 4.44. Proposed pathway for oxidative degradation of indoleacetic acid.

form, however, evidence suggests that there are other indolic auxins naturally occurring in plants.[9] Although auxin (indoleacetic acid) is found throughout the plant, the highest concentrations are localized in actively growing meristematic regions. It is found as both the free molecule and as inactive conjugated forms. When conjugated, auxin is metabolically bound to other low molecular weight compounds. This process appears to be reversible. The concentration of free auxin in plants ranges from 1 to 100 mg · kg^{-1} fresh weight. In contrast, the concentration of conjugated auxin can be substantially higher.

One striking characteristic of auxin is the strong polarity exhibited in its transport throughout the plant.[138] Auxin is transported *via* an energy dependent mechanism, basipetally-away from the apical tip of the plant toward the base. This flow of auxin represses the development of axillary lateral buds along the stem thus maintaining apical dominance. Movement of auxin out of the leaf blade toward the base of the petiole also appears to prevent leaf abscission.

Auxin has been implicated in the regulation of a number of physiological processes. For example, evidence for its role in cell growth and differentiation, fruit ripening, flowering, senescence, gravitropism, abscission, apical dominance and other responses has been given. The actual binding of the molecule, the signaling sequence and the means by which it instigates this diverse array of physiological events has not been fully elucidated. During auxin-induced cell elongation, it is thought to act both through a rapid direct effect on an ATPase proton pump mechanism in the plasma membrane and a secondary effect mediated through enzyme synthesis.

The obvious similarity between the amino acid tryptophan and indoleacetic acid (Figure 4.43) lead to the initial proposal that tryptophan represented the precursor of the hormone.

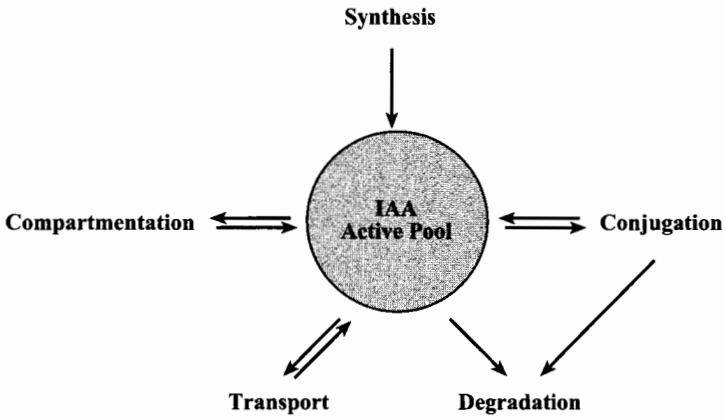


Figure 4.45. The concentration of IAA in the active pool within the cell is controlled by the rates of synthesis, conjugation, degradation, transport, and compartmentation.

Subsequent tests with labeled tryptophan substantiated its role as precursor and helped to elucidate the specific steps involved in the degradation of the side chain of the amino acid. This involves deamination, decarboxylation and two oxidation steps, by at least two general pathways, one through indole-3-pyruvic acid and a second by way of tryptamine (Figure 4.43). In addition to these two primary means of auxin synthesis, the identification of several chlorinated auxins, suggested the potential for alternate pathways.[9] Here the chlorine atom is found on the benzene ring, and appears to be added prior to alteration of the tryptophan side chain.

Auxin is active at very low levels in plant cells and, as a consequence, precise control over the internal concentration of the molecule is essential. As environmental conditions to which the plant is exposed change, relatively rapid and significant alterations in auxin concentration may be necessary. The concentration of auxin within a group of cells can be altered by: a) the rate of synthesis of the molecule; b) its rate of transport into or out of those cells; c) the rate of breakdown of the molecule; and d) the formation of conjugates or conversely auxin liberation from existing conjugates. Interconversion and catabolism of indoleacetic acid can be mediated by both enzymatic ("IAA oxidase," most probably a peroxidase) and non-enzymatic (e.g., H_2O_2 direct oxidation, light, UV radiation, and others) means. The proposed pathway for indoleacetic acid oxidation is presented in Figure 4.44. Peroxidases are found throughout the plant kingdom and some, in addition to exhibiting peroxidase activity, also appear to have the ability to oxidize auxin. Thus the endogenous concentration of auxin can be decreased by the action of these enzymes. Enzymatic control of the concentration of auxin, therefore, could represent a method of regulating certain physiological processes in which auxin is involved. In fact, the level of activity of auxin degrading enzymes has been correlated with the development of specific responses (e.g., fruit ripening). Another way of regulating response is to vary the tissues' sensitivity to auxin.

Conjugation of auxin to other low molecular weight compounds also represents a means of modulating the concentration of the hormones within a cell (Figure 4.45). This process does not exclude the potential for reversibility; thus the reaction can be reversed to yield the free active forms. At present, there are three major groups to which auxin has been found to be bound, in each case through the carboxyl group of the hormone. These include peptidyl IAA conjugated where auxin is linked to an amino acid through a peptide bond, glycosyl IAA conjugates where auxin is linked to a sugar through a glycosidic or an ester bond, and a myo-inositol conjugate where auxin is linked to myo-inositol through an ester bond.

9.1.3. *Gibberellins*

The gibberellins represent a group of acidic diterpenoids found in angiosperms, gymnosperms, ferns, algae and fungi; they do not however, appear to be present in bacteria. Over 70 different free and 16 conjugated gibberellins have been isolated, many of which represent intermediates in the synthesis pathway and lack hormonal activity. Typically the different gibberellins are designated with a number (e.g., GA_3, GA_4, GA_5, . . .) based on their chronological order of isolation and identification. While gibberellins have been shown to induce stem elongation and other responses [e.g., increase radial diameter in stems (conifers), induce flowering, etc.] their precise role in plants remains unknown. Often several gibberellins are found in the same plant.

The base molecule for the various forms is gibberellin, a 20 carbon diterpeniod (Figure 4.46).[225] Some, however, are minus the carbon 20 methyl group and therefore only have 19 carbons (referred to as the C_{19}-GAs). Individual gibberellins differ from each other in the oxidation state of the ring structure and the carbon and hydroxyl groups present. In plants and plant

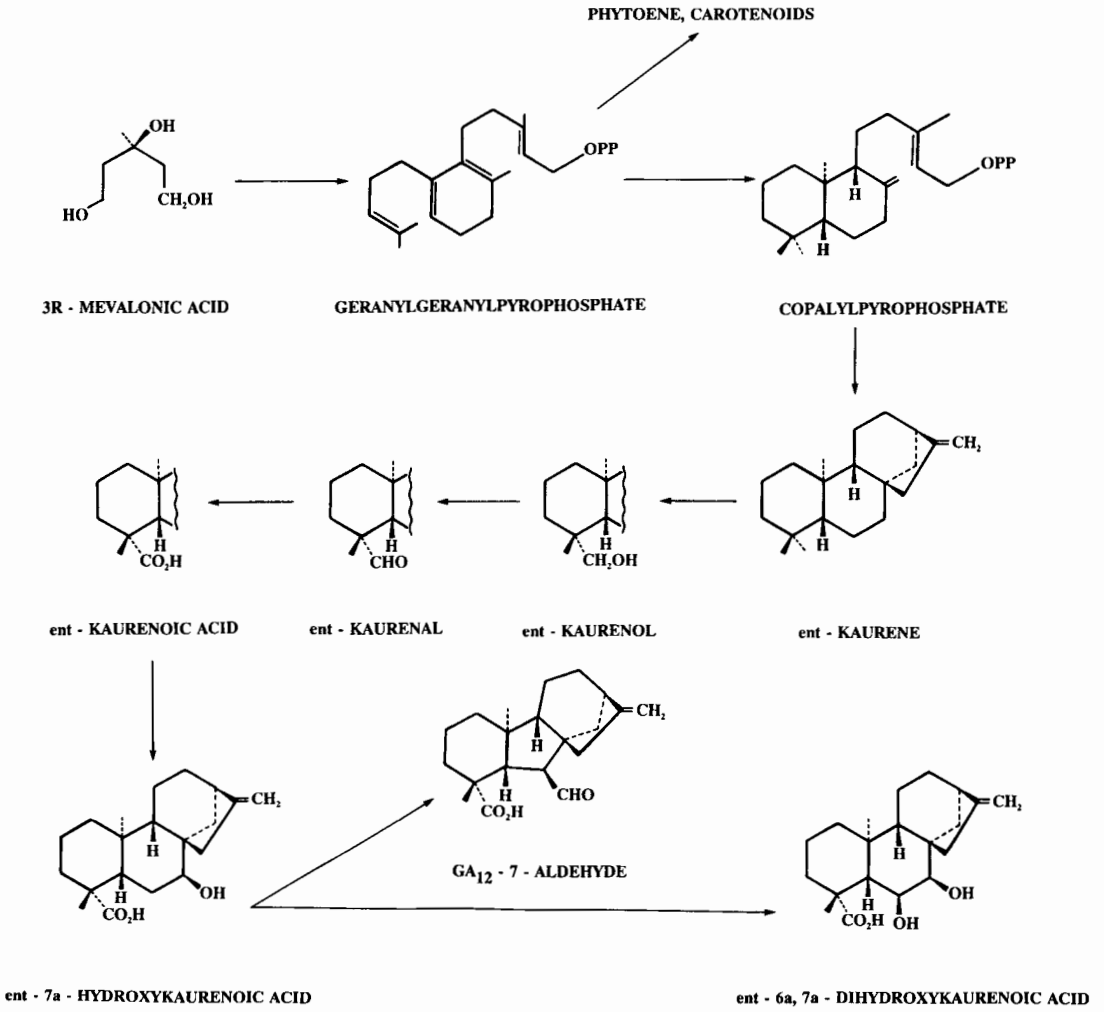


Figure 4.46. Pathway for gibberellic acid synthesis.

parts, gibberellins are also found as glycosides (typically of glucose) and other bound inactive forms. Several of these can be readily converted back to the free molecule upon hydrolysis.

Much of the data on the synthesis of gibberellins has come from studies on the fungus *Gibberella fujikuroi.* It appears that the same general pathway, at least until GA_{12}, is operative in higher plants. Since gibberellins are diterpenoids, labeled precursors of the terpenoid biosynthesis pathway (e.g., acetyl CoA and mevalonate) are monitored to establish the proposed pathway (Figure 4.46). The first complete cyclic compound in the pathway is kaurene. Kaurene is then, through a series of not clearly understood reactions, converted to various gibberellins.[86,88] Some interconversion between specific gibberellins occurs within plants. For example GA_1 can be converted to GA_3 or GA_5 and subsequently to GA_8.

Conjugates may represent an important means of modulating the internal concentrations of gibberellins within the plant. When gibberellins are applied to plant tissue there is often a rapid conversion to the inactive glucoside form. In addition, although more stable than auxins, gibberellins can be degraded to inactive compounds and some evidence for compartmentalization has been found.

Gibberellins are synthesized in the apical leaf primordia, root tips and in developing seeds. The hormone does not exhibit the same strongly polar transport as seen with auxin, although in some species there is basipetal movement in the stem. In addition to being found in the phloem, gibberellins have also been isolated from xylem exudate that suggests a more general, bi-directional movement of the molecule in the plant.

Several synthetic compounds have been shown to inhibit elongation of some plant species suggesting anti-gibberellin activity, a few of which are widely used in ornamental horticulture for producing more compact plants. They inhibit the normal synthesis of gibberellin within the plants, many of which through the inhibition of the enzyme kaurene synthase that catalyzes the synthesis step between geranylgeranyl pyrophosphate and copalyl pyrophosphate. Their use is of interest here in that inhibitors of GA synthesis appear to also significantly modify the postharvest response of many plants.

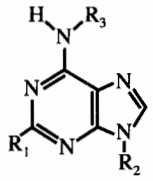
9.1.4. Cytokinins

Cytokinins are naturally occurring plant hormones that stimulate cell division. Initially they were called kinin, however, due to prior use of the name for a group of compounds in animal physiology, cytokinin (cyto kinesis or cell division) was adapted.

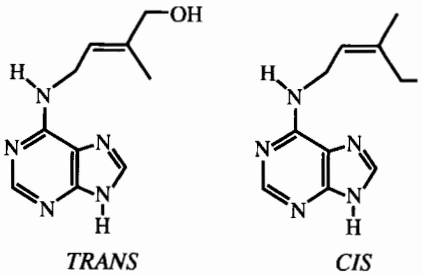
All of the naturally occurring cytokinins contain a N^6-substituted adenine moiety (Figure 4.47). Zeatin was the first naturally occurring cytokinin isolated and identified, however, since that time a number of others have been identified. They are found as the base molecule, a riboside (presence of ribosyl group at the R_3 position) or a ribotide.

The highest concentrations of cytokinins are found in embryos and young developing fruits, both of which are undergoing rapid cell division. The presence of high levels of cytokinins may facilitate their ability to act as a strong sink for nutrients. Cytokinins are also formed in the roots and are translocated *via* the xylem to the shoot. When in the leaf, however, the compounds are relatively immobile.

The precise mode of action of cytokinins is not known. While they do stimulate cell division, exogenous application is also known to cause several significant responses. When applied to detached leaves, cytokinins delay senescence, thus the rate at which degradative processes occur significantly decreases.[164] This is due in part to a facilitated movement of amino acids and other nutrients into the treated area. The site of response is localized to where the hormone is placed on the leaf, indicating little movement of cytokinin in the leaf. Considerable interest has been shown in this anti-senescence ability of cytokinins. Synthetic cytokinins

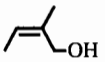
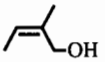
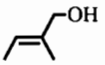
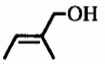
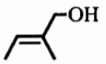
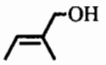
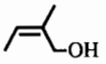
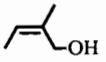
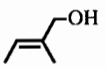
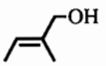


BASE STRUCTURE OF CYTOKININ



TRANS *CIS*

STERIO ISOMERS OF ZEATIN

SUBSTITUENTS			TRIVIAL NAME	SYSTEMATIC NAME	ABBREVIATION
R_1	R_2	R_3*			
H	H		N^6-(Δ^2-ISOPENTENYL) ADENOSINE	6-(3-METHYLBUT-2-ENYLAMINO)-PURINE	i^6Ade
H	RIBOSYL		N^6-(Δ^2-ISOPENTENYL) ADENOSINE	6-(3 -METHYLBUT-2-ENYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	i^6A
H	H		*CIS*-ZEATIN	6-(4-HYDROXY-3-METHYL-*CIS*-BUT-2-ENYLAMINO)-PURINE	*c*-io^6Ade
H	RIBOSYL		*CIS*-ZEATIN RIBOSIDE	6-(4-HYDROXY-3-METHYL-*CIS*-BUT-2-ENYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	*c*-io^6A
H	H		*TRANS*-ZEATIN	6-(4-HYDROXY-3-METHYL-*TRANS*-BUT-2-ENYLAMINO)-PURINE	*t*-io^6A
H	RIBOSYL		*TRANS*-ZEATIN RIBOSIDE	6-(4-HYDROXY-3-METHYL-*TRANS*-BUT-2 -ENYLAMINO)-9-β-RIBOFURANOSYLPURINE	*t*-io^6A
H	H		DIHYDROZEATIN	6-(4-HYDROXY-3-METHYLBUTYLAMINO) PURINE	H_2-io^6Ade
H	RIBOSYL		DIHYDROZEATIN RIBOSIDE	6-(4-HYDROXY-3-METHYLBUTYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	H_2-io^6A
CH_3-S	H			2-METHYLTHIO-6-(3-METHYLBUT-2-ENYLAMINO)-PURINE	ms^2-i^6Ade
CH_3-S	RIBOSYL			2-METHYLTHIO-6-(3-METHYLBUT-2-ENYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	ms^2-i^6A
CH_3-S	H			2-METHYLTHIO-6-(4-HYDROXY-3-METHYL-*CIS*-BUT-2-ENYLAMINO)-PURINE	ms^2-*c*-io^6Ade
CH_3-S	RIBOSYL			2-METHYLTHIO-6-(4-HYDROXY-3-METHYL-*CIS*-BUT-2-ENYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	ms^2-*c*-io^6A
CH_3-S	H			2-METHYLTHIO-6-(4-HYDROXY-3-METHYL-*TRANS*-BUT-2-ENYLAMINO)-PURINE	ms^2-*t*-io^6Ade
CH_3-S	RIBOSYL			2-METHYLTHIO-6-(4-HYDROXY-3-METHYL-*TRANS*-BUT-2-ENYLAMINO)-9-β-p-RIBOFURANOSYLPURINE	ms^2-*t*-io^6A

* In all cases, N^6 is linked to the C - 1 of the isoprenoid side chain

Figure 4.47. Naturally occurring cytokinins in plants (*redrawn from Sembdner et al.*[208]).

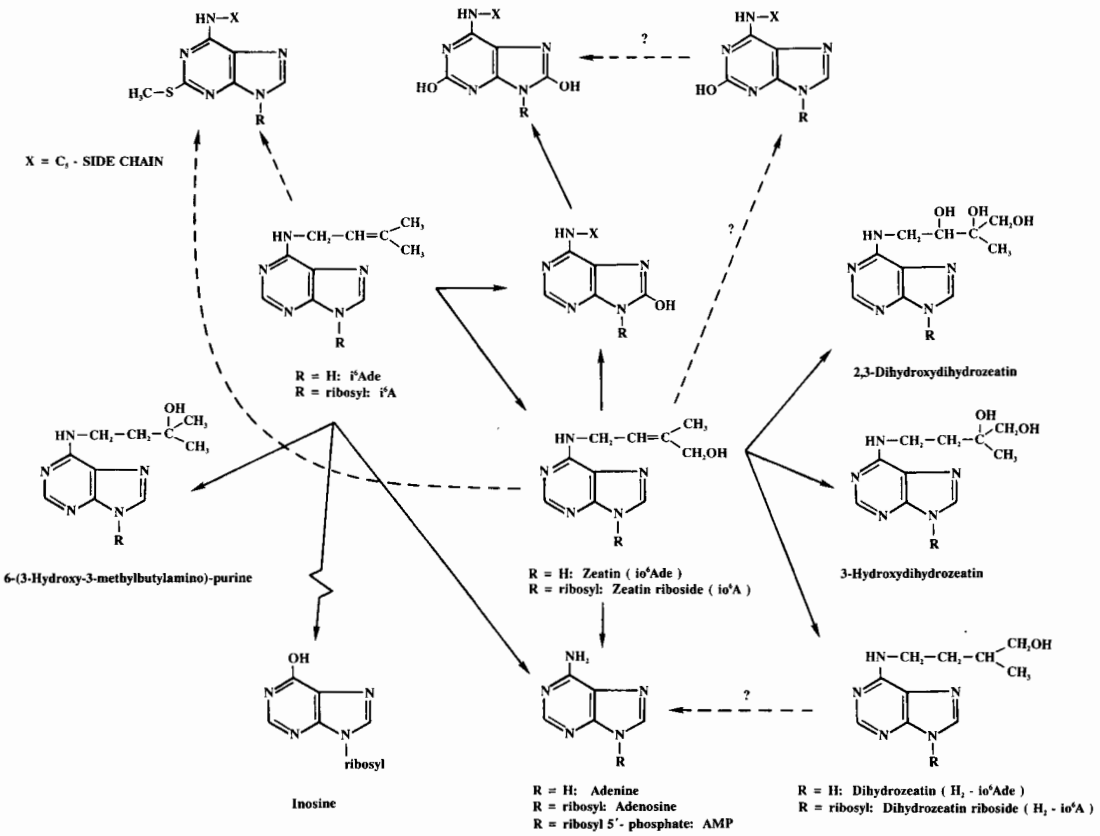


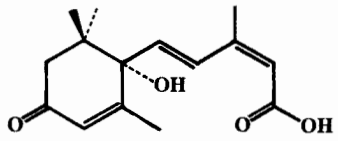
Figure 4.48. Proposed scheme for cytokinin interconversion (*redrawn from Sembdner et al.*[208]).

such as N^6-benzyladenine have been applied to a number of postharvest products with varying degrees of success.

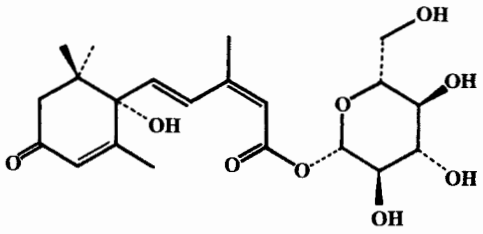
Other general effects of cytokinins on plants have been reported. These include: a) stimulation of seed germination; b) stimulation of the formation of seedless fruit; c) breaking seed dormancy; d) inducing bud formation; e) enhancing flowering; f) altering fruit growth; and g) breaking apical dominance. These responses tend to only be found in certain species and in some cases, cultivars, and are not widespread. It would appear, therefore, that they represent pharmacological responses, rather than precise physiological roles of the molecule in the plant. While these types of responses may not greatly expand our understanding of how cytokinins function in plants, some are of considerable interest in the commercial production and handling of agricultural plant products.

The biosynthesis of cytokinins is closely related to the metabolism of the purine adenine.[164] The isopentenyl side chain is added by the enzyme isopentenyl transferase. A general scheme for the interconversion of cytokinins, forming the various derivatives has been proposed (Figure 4.48). The level of activity is affected by the structure of these derivatives. The length of the side chain, degree of side chain unsaturation, and the stereochemistry of the double bond are important. Dihydroxyzeatin, without a double bond in the side chain, is only one-tenth as active as zeatin. Both the *cis* and *trans* stero-isomers of the side chain's double bond are found (Figure 4.47); however, the *trans* forms appear to be much more active.

Deactivation of cytokinins can occur through the conjugation of the molecule with a glycoside giving an inactive molecule. Cytokinins may also be degraded by the action of cytokinin



(+) - ABSCISIC ACID



(+) - ABSCISYL - β - D - GLUCOPYRANOSIDE

Figure 4.49. Structures of abscisic acid and its inactive glucose conjugate.

oxidase that cleaves the side chain. The adenine portion of the molecule is then metabolized as a substrate or oxidized.

9.1.5. Abscisic Acid

Abscisic acid, previously known as dormin and abscisin, is a naturally occurring growth inhibitor in plants. Chemically, it is a terpenoid that is structurally very similar to the terminal portion of many carotenoids (Figure 4.49). Both *cis* and *trans* isomers are possible, however, only the *cis* form, designated (+)-ABA is active and is found almost exclusively in plants.

Abscisic acid is a potent growth inhibitor that has been proposed to play a regulatory role in such diverse physiological responses as dormancy, leaf and fruit abscission and water stress. Typically the concentration within plants is between 0.01 and 1 ppm, however, in wilted plants the concentration may increase as much as 40×. Abscisic acid is found in all parts of the plant; however, the highest concentrations appear to be localized in seeds and young fruits.

Two schemes for the synthesis of abscisic acid have been proposed.[137] The first or direct method involves the formation of the C_{15} carbon skeleton of abscisic acid from 3 isoprene units derived from mevalonic acid (Figure 4.50). The precise series of steps have yet to be fully elucidated. A second or indirect method was initially suggested based on the close similarity between the terminal ends of certain carotenoids, for example violaxanthin and abscisic acid. A lipoxygenase was subsequently isolated which would cleave carotenoids giving a range of compounds that were structurally similar to abscisic acid (e.g., xanthoxin). Exogenously applied xanthoxin was then converted to abscisic acid. In some cases this indirect scheme of synthesis may occur, however, its importance appears to be minimal. The major pathway for abscisic acid synthesis is the direct scheme from mevalonic acid.

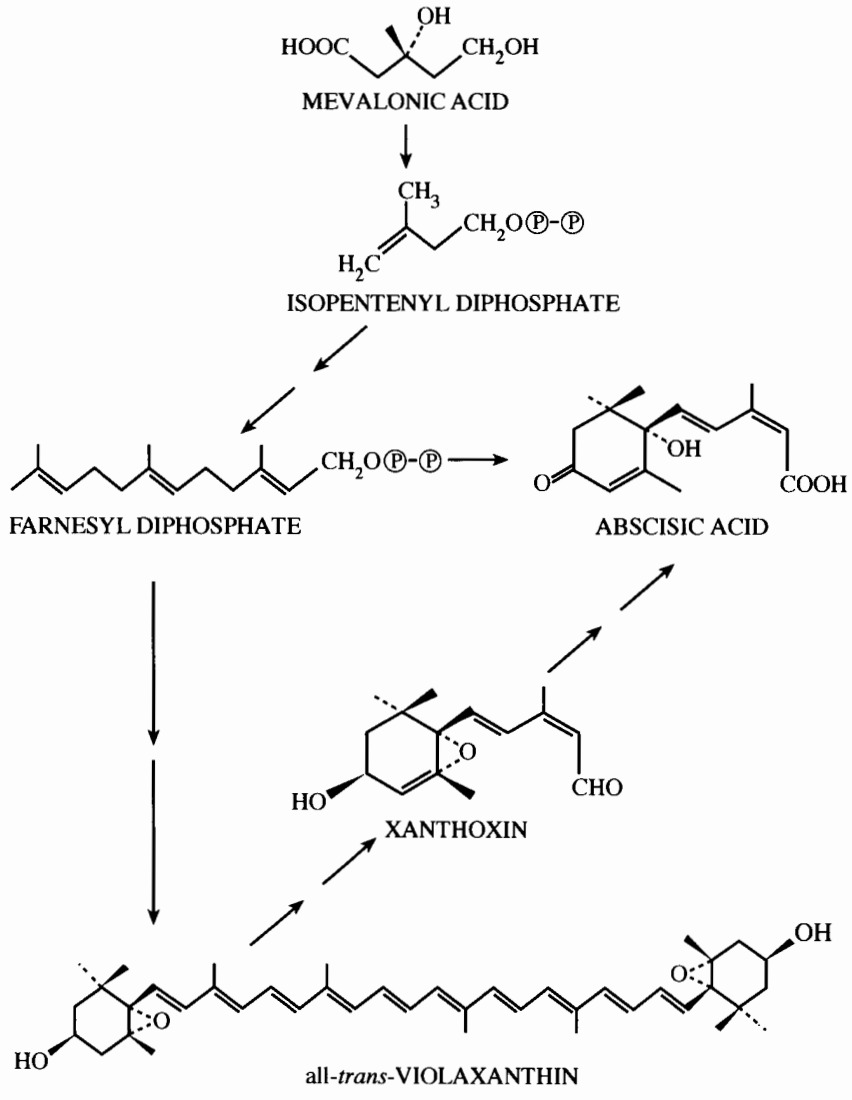


Figure 4.50. General pathway for the synthesis of abscisic acid from mevalonic acid (top) and a possible indirect scheme for the synthesis of abscisic acid from carotenoids (bottom). Violaxanthin is attacked by lipoxygenase yielding xanthoxin and other products. Xanthoxin is then converted to abscisic acid. This does not appear to represent a significant pathway *in vitro*.

Degradation of abscisic acid or loss of activity occurs through two primary mechanisms,[39] conjugation and metabolism (Figure 4.51). Abscisic acid rapidly forms an inactive conjugated with glucose. Glucosyl abscisate has been identified in a number of plants. Abscisic acid presumably may also form conjugates with other carbohydrates and types of compounds (i.e., proteins or lipids). It has been proposed that conjugation represents a means of interconversion between active and inactive forms of the molecule, thus controlling the internal concentration within the cell. Abscisic acid is rapidly metabolized in the plant. This results in a much less active derivative, (e.g., phaseic acid) or inactive compounds.

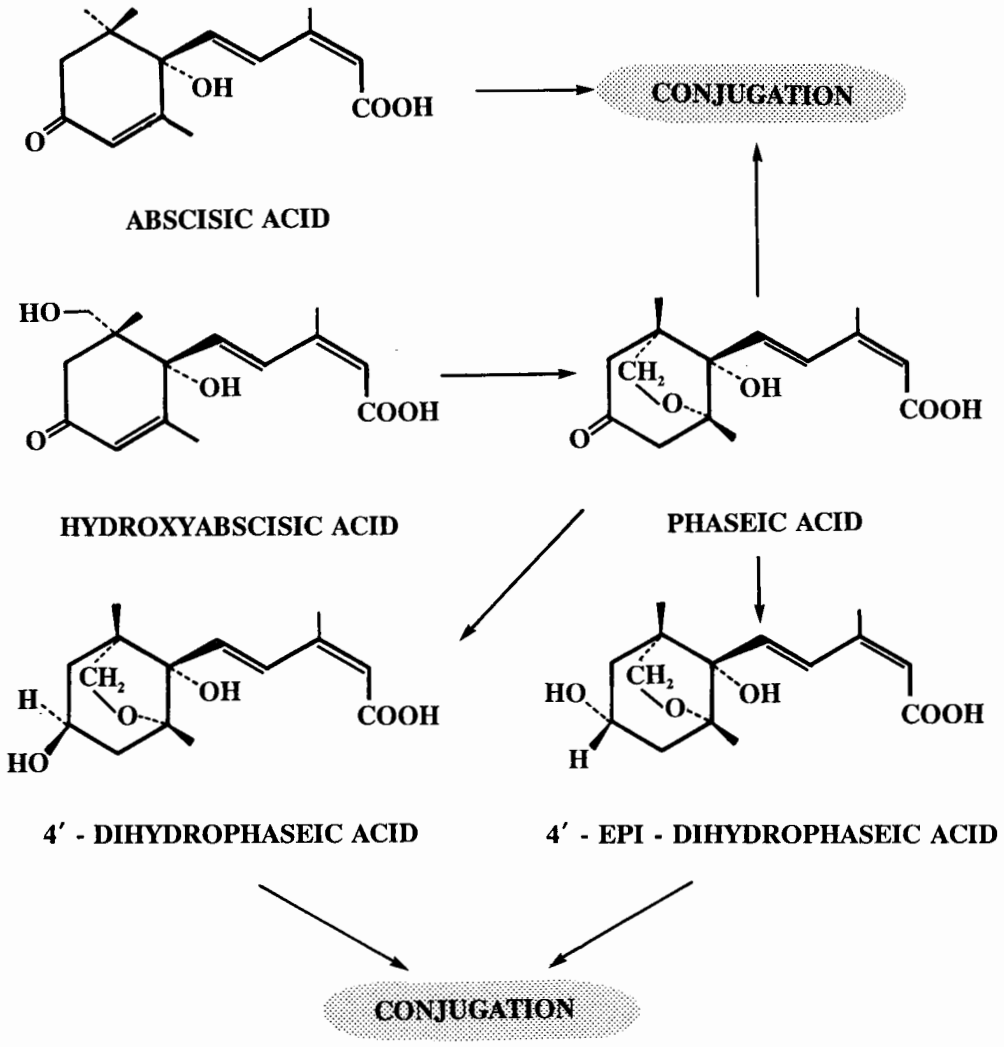


Figure 4.51. Conversion of abscisic acid to metabolites of low hormonal activity or devoid of activity.

9.2. Postharvest Alteration in the Concentration of Phytohormones

Due to the apparent effect of phytohormones on an array of physiological responses within the plant, the fate of these compounds after harvest is of considerable interest. Unfortunately, techniques for the measurement of phytohormones, especially auxin, gibberellins, cytokinins and abscisic acid are extremely complicated. In addition, few studies have monitored concurrently all five phytohormones and, as a consequence, a clear picture of gross postharvest alterations is not yet available. Typically, only one or occasionally two phytohormones are assessed in a single study. More importantly, the interpretation of the results of most studies is complicated by isolation and/or quantification procedures used. Often internal standards are not utilized during isolation and bioassays are relied upon to measure the relative activity of the impure isolates. Even when essential isolation and quantification prerequisites are met,

one must assume that the hormone is not sequestered in a concentrated pool(s) somewhere within each cell (hence disruption during isolation would dilute the concentration) and that all the cell types making up an organ such as a fruit, have equal amounts of the hormone. Both are rather dubious assumptions. Thus, even when a close correlation is found between the gross concentration and a specific physiological event, the precise meaning remains in question. Interpretation of the response is further complicated by changes in tissue sensitivity during development.

The phytohormone ethylene, being a gas which diffuses readily out of a tissue, has the distinct advantage that tissue disruption is not required for isolation, and it can be analytically quantified relatively easily using gas chromatography at concentrations as low as several $nL \cdot L^{-1}$ of air (parts per billion). Because of this and the apparent relative importance of this phytohormone in senescence and fruit ripening, two major postharvest phenomena of both physiological and commercial interest, the following discussion will focus to a large extent on ethylene.

9.2.1. Flower Senescence

Distinctive alterations in the concentration of phytohormones are found during the senescence of flowers whether attached or detached from the parent plant. The synthesis of ethylene rises sharply as the result of pollination and senescence in the carnation and other flowers. Exogenous application of ethylene accelerates senescence while factors that inhibit its synthesis or action delay senescence. The rise in ethylene production occurs first in the carnation stigma followed by the ovary, receptacle and, lastly, the petal tissue. The chronological timing of the ethylene increase by the stigma would suggest that it is mediated by pollen tube penetration of the stigma rather than fertilization of the ovules. Thus, the initial response may be due partly to wounding.

With the increase in ethylene production upon pollination, there is a stimulation of ovary development with movement of carbohydrates out of the petals into the gynoecium. This transport effect can be stimulated without the occurrence of pollination by exposure of the flower to an appropriate concentration of ethylene. The close correlation between ethylene synthesis, ovary development and perianth senescence provides a strong argument for a natural role for ethylene in the flowering response.

Abscisic acid concentration also increases during carnation flower and rose petal senescence; its exogenous application can accelerate the process. The relationship between increases in abscisic acid concentration and senescence are not clear. In rose petals, the increase in abscisic acid occurs several days after an increase in ethylene synthesis, while the opposite appears to be the case with the carnation flower. Thus the nature of the interaction between these phytohormones is obscure.

Exogenous application of a synthetic cytokinin will extend the longevity of cut carnations[50] and anthuriums.[181] In addition, the endogenous concentration of cytokinin increases as rose flowers begin to open but declines substantially thereafter.[161] Based on this, it has been suggested that cytokinins are important during the opening and maturation response of the flower.

9.2.2. Fruit Ripening

Climacteric fruits exhibit an exponential increase in the rate of ethylene emanation near the onset of ripening.[2] During ripening of these fruits there is a large increase in the rate of respi-

ration and carbon dioxide production. Since exogenous application of ethylene to preclimacteric fruits will induce ripening, the natural role of ethylene in inducing the ripening response has been a subject of considerable interest.

Many fruits exhibit a respiratory climacteric where the increase in ethylene synthesis precedes the increase in respiration; however, this sequence of events is not universal. In some species (e.g., mango and apple) the respiratory climacteric appears more or less simultaneously with, but not preceded by an increase in ethylene synthesis. In feijoa, cherimoya and avocado fruits, the respiratory climacteric significantly precedes the increase in ethylene synthesis. In addition, the fruit's sensitivity to the internal concentration of ethylene may change as ripening approaches. Thus, while ethylene appears to be present in all ripening fruits, its concentration does not necessarily need to rise in order to initiate ripening. In these cases, tissue sensitivity to ethylene could be changing.

Other phytohormones also appear to be important in the ripening response. Many avocado cultivars do not ripen while attached to the tree, suggesting that some inhibitor produced by the parent plant is operative. Likewise, when apple fruit are detached from the tree, the rate of ethylene synthesis increases.

Changes in auxin concentration have been proposed as part of a multi-hormonal scheme controlling ripening. This is supported by the retardation of ripening with the application of auxin and acceleration with an anti-auxin compound.[64,65] A decline in auxin concentration within some fruits as they approach the onset of ripening appears to be due to the action of "IAA oxidase."[63] However, a decline in IAA does not appear to be a universal phenomenon (e.g., apple). Thus there is insufficient evidence at this time to support IAA having a direct role in controlling the onset of ripening.

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