

3

PHYSIOLOGY AND BIOCHEMISTRY

An important yet basic fact regarding the postharvest handling of horticultural produce is that they are 'living' structures. One readily accepts that produce is a living, biological entity when it is attached to the growing parent plant in its agricultural environment, but even after harvest the produce is still living as it continues to perform most of the metabolic reactions and maintain the physiological systems that were present when it was attached to the plant.

An important feature of plants — and therefore of fruit, vegetables and ornamentals — is that they respire by taking up oxygen and giving off carbon dioxide and heat. They also transpire (i.e. lose) water. While attached to the plant, the losses due to respiration and transpiration are replaced from the flow of sap, which contains water, photosynthates (principally sucrose and amino acids) and minerals. Respiration and transpiration continue after harvest, and since the produce is now removed from its normal source of water, photosynthates and minerals, the produce is dependent entirely on its own food reserves and moisture content. Therefore, losses of respirable substrates and moisture are not replenished and deterioration commences. In other words, harvested fruit and vegetables and ornamentals are perishable.

This chapter will consider the postharvest behaviour of horticultural commodities with particular reference to the physiological and biochemical changes that occur in ripening fruits. For this discussion, some understanding of the physiological development of fruit and vegetables and ornamentals is necessary.

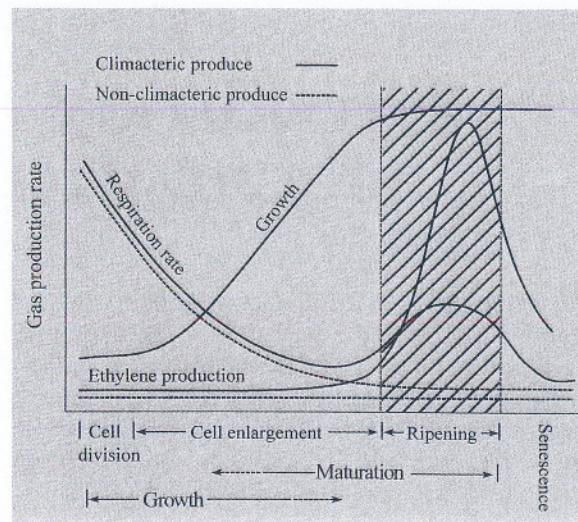
PHYSIOLOGICAL DEVELOPMENT

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The life of fruit and vegetables can be conveniently divided into three major physiological stages following germination. These are growth, maturation and senescence (Figure 3.1). However, clear distinction between the various stages is not easily made. Growth involves cell division and subsequent cell enlargement, which accounts for the final

Figure 3.1

Growth, respiration and ethylene production patterns of climacteric and non-climacteric plant organs.

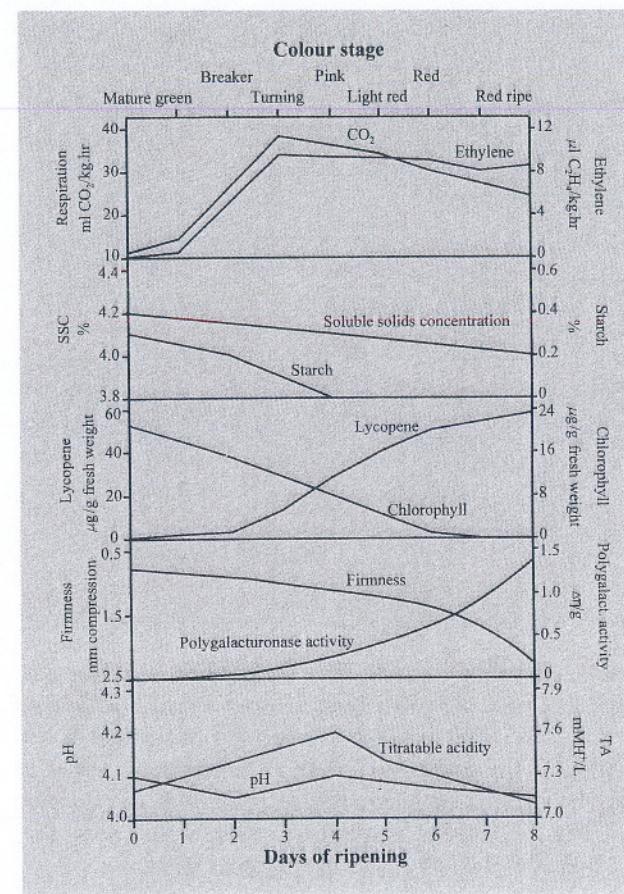


size of the produce. Maturation usually commences before growth ceases and includes different activities in different commodities. Growth and maturation are often collectively referred to as the development phase. Senescence is defined as the period when anabolic (synthetic) biochemical processes give way to catabolic (degradative) processes, leading to ageing and finally death of the tissue. Ripening — a term reserved for fruit — is generally considered to begin during the later stages of maturation and to be the first stage of senescence. The change from growth to senescence is relatively easy to delineate. Often the maturation phase is described as the time between these two stages, without any clear definition on a biochemical or physiological basis.

It is difficult to assign specific biochemical or physiological parameters to delineate the various stages because the parameters for different commodities are not identical in their nature or timing. Figure 3.2 shows the major changes in certain biochemical and physiological parameters in the climacteric tomato as it ripens from mature green to ripe. Similar changes are found in many non-climacteric plant tissues such as the pineapple.

Development and maturation of fruit are completed only when it is attached to the plant, but ripening and senescence may proceed on or off the plant. Fruit are generally harvested either when mature or when ripe, although some fruits that are consumed as vegetables may be harvested even before maturation has commenced, such as zucchini.

Similar terminology may be applied to the vegetables or

**Figure 3.2**

Physicochemical changes that occur during ripening of harvested tomatoes at 20°C.

ornamentals, or to any determinant organ, except that the ripening stage does not occur. As a consequence it is more difficult to delineate the change from maturation to senescence in vegetables and ornamentals. Vegetables and ornamentals are harvested over a wide range of physiological ages, that is, from a time well before the commencement of maturation through to the commencement of senescence (see Figure 10.3 on page 169).

FRUIT RIPENING

Ripening fruit undergoes many physicochemical changes after harvest that determine the quality of the fruit purchased by the consumer.

TABLE 3.1 CHANGES THAT MAY OCCUR DURING THE RIPENING OF FLESHY FRUIT

- Seed maturation
- Colour changes
- Abscission (detachment from parent plant)
- Changes in respiration rate
- Changes in rate of ethylene production
- Changes in tissue permeability and cellular compartmentation
- Softening: changes in composition of pectic substances
- Changes in carbohydrate composition
- Organic acid changes
- Protein changes
- Production of flavour volatiles
- Development of wax on skin

SOURCE Adapted from H.K. Pratt (1975) *The role of ethylene in fruit ripening, Facteurs et régulation de la maturation des fruits*, Centre National de La Recherche Scientifique, Anatole, France, pp. 153–60.

Ripening is a dramatic event in the life of a fruit — it transforms a physiologically mature but inedible plant organ into a visually attractive olfactory and taste sensation. Ripening marks the completion of development of a fruit and the commencement of senescence, and it is normally an irreversible event. The following sections will discuss the general nature of fruit ripening, respiratory behaviour and the involvement of the gas ethylene (C_2H_4) with these processes.

Ripening is the result of a complex of changes, many of them probably occurring independently of one another. A list of the major changes that together make up fruit ripening is given in Table 3.1. The time course of some of these changes is shown in Figure 3.3 for the banana, which is a climacteric fruit. The principal difference between the climacteric tomato and banana and the non-climacteric pineapple is the presence of the respiratory peak that is characteristic of climacteric fruits. A sharp increase in respiration is shown by the increase in the production of carbon dioxide or decrease in the internal oxygen concentration. Two of the changes listed — namely respiration and ethylene production — have gained priority in attempts to develop an explanation of the mechanism of fruit ripening. Further characterisation of other changes occurring in climacteric and non-climacteric fruit is given later in this chapter (see ‘Chemical changes during maturation’ on page 54).

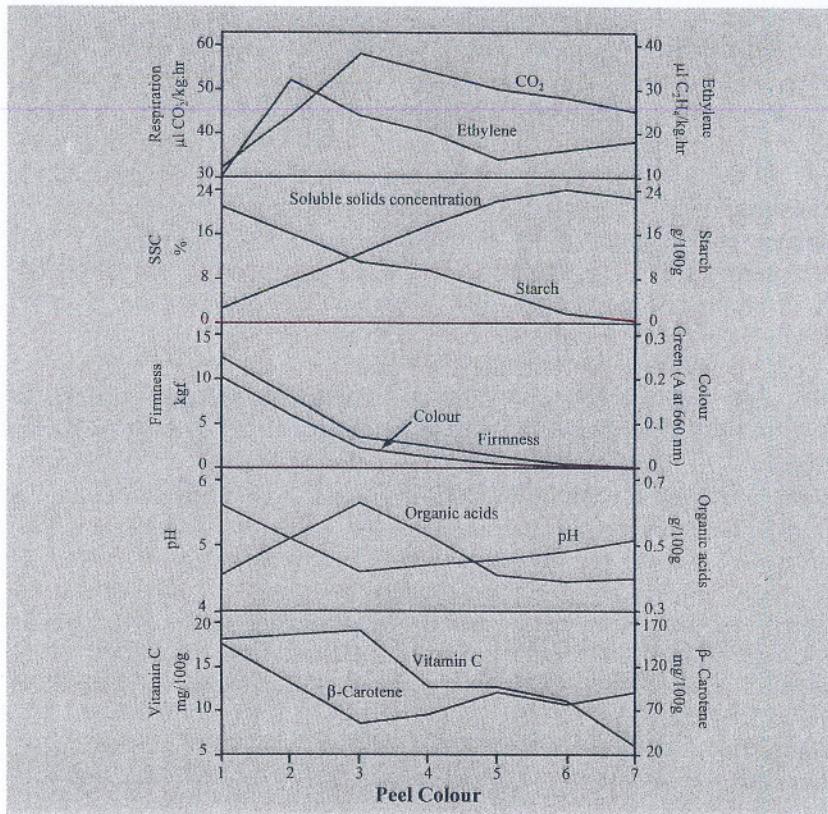


Figure 3.3

Physiochemical changes that occur during ripening of the Cavendish banana (variety Williams). The peel colour stages indicate the change from green (stage 1) to full yellow (stage 7), and finally to a stage when skin spotting occurs (stage 7). Williams bananas take about 8 days to progress from stage 1 to stage 7 at 20°C.

SOURCE Adapted from R.B.H. Wills, J.S.K. Lim and H. Greenfield (1984) Changes in chemical composition of “Cavendish” banana (*Musa acuminata*) during ripening. *J. Food Biochem.* 8: 69–77.

Physiology of respiration

A major metabolic process taking place in harvested produce or in any living plant product is respiration. Respiration can be described as the oxidative breakdown of the more complex materials normally present in cells, such as starch, sugars and organic acids, into simpler molecules, such as carbon dioxide and water, with the concurrent production of energy and other

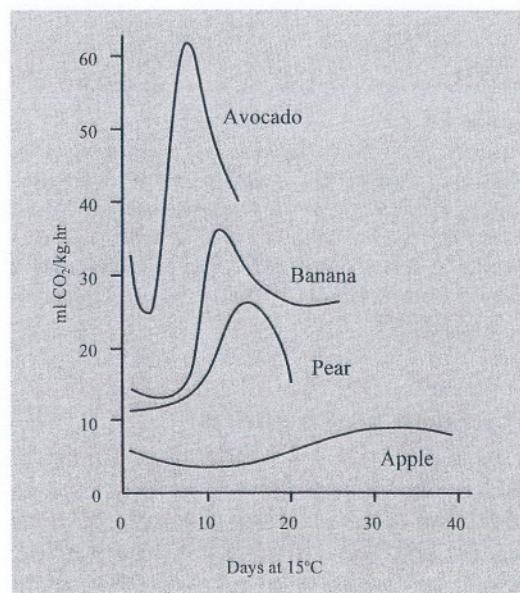
molecules that can be used by the cell for synthetic reactions. Respiration can occur in the presence of oxygen (aerobic respiration) or in the absence of oxygen (anaerobic respiration, which is sometimes called fermentation).

The respiration rate of produce is an excellent indicator of metabolic activity of the tissue and thus is a useful guide to the potential storage life of the produce. If the respiration rate of a fruit or vegetable is measured — as either oxygen consumed or carbon dioxide evolved — during the course of its development, maturation, ripening and senescent periods, a characteristic respiratory pattern is obtained. Respiration rate per unit weight is highest for the immature fruit or vegetable and then steadily declines with age (Figure 3.1).

A significant group of fruits that includes the tomato, mango, banana and apple shows a variation from the described respiratory pattern in that a pronounced increase in respiration coincides with ripening (Figure 3.1). Such an increase in respiration is known as a respiratory climacteric, and this group of fruits is known as the climacteric class of fruits. The intensity and duration of the respiratory climacteric, first described in 1925 for the apple, varies widely among fruit species, as depicted in Figure 3.4. The commencement of the respiratory climacteric coincides approximately with the attainment of maximum fruit size (Figure 3.1), and it is during the climacteric that all the other changes characteristic of ripening occur. The respiratory climacteric, as

Figure 3.4

Respiratory patterns of some harvested climacteric fruits.
SOURCE J.B. Biale (1950)
Postharvest physiology and
biochemistry of fruits, *Ann.*
Rev. Plant Physiol. 1: 183–206.
(With permission.)



well as the complete ripening process, may proceed while the fruit is either attached to or detached from the plant (except for avocado, which will only ripen when detached from the plant).

Those fruits that do not exhibit a respiratory climacteric, such as citrus, pineapple and strawberry, are known as the non-climacteric class of fruit. Non-climacteric fruit exhibit most of the ripening changes, although these usually occur more slowly than those of the climacteric fruits. Table 3.2 lists some common climacteric and non-climacteric fruits. All vegetables can also be considered to have a non-climacteric type of respiratory pattern. The division of fruit into two classes on the basis of their respiratory pattern is an arbitrary classification but has served to stimulate considerable research to discover the biochemical control of the respiratory climacteric.

TABLE 3.2 CLASSIFICATION OF SOME FRUIT ACCORDING TO THEIR RESPIRATORY BEHAVIOUR DURING RIPENING

CLIMACTERIC FRUIT	NON-CLIMACTERIC FRUIT
Apple (<i>Malus domestica</i>)	Cherry: sweet (<i>Prunus avium</i>)
Apricot (<i>Prunus armeniaca</i>)	sour (<i>Prunus cerasus</i>)
Avocado (<i>Persea americana</i>)	Cucumber (<i>Cucumis sativus</i>)
Banana (<i>Musa sp.</i>)	Grape (<i>Vitis vinifera</i>)
Blueberry (<i>Vaccinium corymbosum</i>)	Lemon (<i>Citrus limon</i>)
Cherimoya (<i>Annona cherimola</i>)	Pineapple (<i>Ananas comosus</i>)
Feijoa (<i>Feijoa sellowiana</i>)	Satsuma mandarin (<i>Citrus unshiu</i>)
Fig (<i>Ficus carica</i>)	Strawberry (<i>Fragaria sp.</i>)
Kiwi fruit (<i>Actinidia deliciosa</i>)	Sweet orange (<i>Citrus sinensis</i>)
Mango (<i>Mangifera indica</i>)	Tamarillo (tree tomato)
Muskmelon (<i>Cucumis melo</i>)	(<i>Cyphomandra betacea</i>)
Papaya (<i>Carica papaya</i>)	
Passionfruit (<i>Passiflora edulis</i>)	
Peach (<i>Prunus persica</i>)	
Pear (<i>Pyrus communis</i>)	
Persimmon (<i>Diospyros kaki</i>)	
Plum (<i>Prunus sp.</i>)	
Tomato (<i>Lycopersicon esculentum</i>)	
Watermelon (<i>Citrullus lanatus</i>)	

Effect of ethylene

Climacteric and non-climacteric fruits may be further differentiated by their response to applied ethylene and by their pattern of ethylene production during ripening. It has been clearly established that all fruit produces minute quantities of ethylene during development. However, climacteric fruits produce much larger amounts of ethylene during ripening than non-climacteric fruits. This difference between the two classes of fruit is further exemplified by the internal ethylene concentration found at several stages of development and ripening (Table 3.3). The internal ethylene concentration of climacteric fruits varies widely, but that of non-climacteric fruits changes little during development and ripening. Ethylene, applied at a concentration as low as 0.1–1.0 µL/L for 1 day, is normally sufficient to hasten full ripening of climacteric fruit (Figure 3.5), but the magnitude of the climacteric is relatively independent of the concentration of applied ethylene. In contrast, applied ethylene merely causes a transient increase in the

TABLE 3.3 INTERNAL ETHYLENE CONCENTRATIONS MEASURED IN SEVERAL CLIMACTERIC AND NON-CLIMACTERIC FRUITS

FRUIT	ETHYLENE (µL/L)
<i>Climacteric</i>	
Apple	25–2500
Pear	80
Peach	0.9–20.7
Nectarine	3.6–602
Avocado	28.9–74.2
Banana	0.05–2.1
Mango	0.04–3.0
Passionfruit	466–530
Plum	0.14–0.23
Tomato	3.6–29.8
<i>Non-climacteric</i>	
Lemon	0.11–0.17
Lime	0.30–1.96
Orange	0.13–0.32
Pineapple	0.16–0.40

SOURCE From S.P. Burg and E.A. Burg (1962) The role of ethylene in fruit ripening, *Plant Physiol.* 37: 179–89. (With permission.)

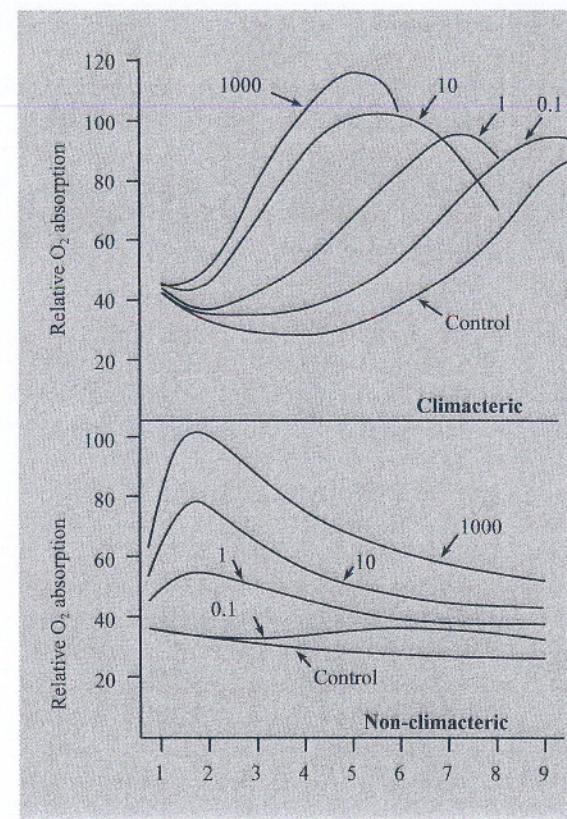


Figure 3.5
Effects of applied ethylene on respiration of climacteric and non-climacteric fruits.
SOURCE J.B. Biale (1964) Growth, maturation, and senescence in fruits, *Science* 146: 880–88. (With permission.)

respiration of non-climacteric fruits, the magnitude of the increase depending on the concentration of ethylene (Figure 3.5). Moreover, the rise in respiration in response to ethylene may occur more than once in non-climacteric fruits in contrast to the single respiration increase in climacteric fruits.

The significance of ethylene for fruit ripening was established during the early part of the 20th century, when heaters burning kerosene were used to degreen — or colour yellow — California lemons. Denny, in 1924, found that while warmth was needed, the real cause of degreening was ethylene, and many workers soon demonstrated that ethylene could hasten the ripening of many fruits. Ethylene was regarded as an external agent that could promote the ripening of fruits, but in 1934 Gane found that fruit and other plant tissues produced extremely small quantities of ethylene. This finding was achieved despite the use of insensitive and time-consuming methods of analysis, which

generally relied on trapping the ethylene with mercuric perchlorate, releasing the trapped ethylene and measuring it by manometry.

Research into the involvement of ethylene in fruit ripening was greatly stimulated by the development of sensitive gas chromatographic techniques for the measurement of low levels of ethylene, so that it is now possible to measure quantitatively as little as 0.001 µL/L in a 1 mL gas sample (see Appendix IV). This obviates the need for collecting or absorbing ethylene over a lengthy period of time.

Ethylene biosynthesis

Ethylene has been shown to be produced from methionine via a pathway that includes the intermediates S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC). The conversion of SAM to ACC by the enzyme ACC synthase is thought to be the rate limiting step in the biosynthesis of ethylene. However, in higher plants, ACC can be removed by conjugation to form malonyl ACC or glutamyl ACC. The addition of ACC to preclimacteric (unripe) fruit generally results in only a small increase in ethylene evolution, showing that another enzyme, the ethylene-forming enzyme (EFE or ACC oxidase), is required to convert ACC to ethylene. ACC oxidase is a labile enzyme that is sensitive to oxygen. Factors that affect the activity of ACC synthase include fruit ripening, senescence, auxin, physical injuries and chilling injury. This enzyme is believed to be a pyridoxal enzyme because it requires pyridoxal phosphate for maximal activity and is strongly inhibited by aminoxyacetic acid (AOA), rhizobitoxine and its analogue and L-2-amino-4-(2-aminoethoxy)-trans-3-butenoic acid (AVG), which are known inhibitors of pyridoxal phosphate-dependent enzymes. ACC oxidase is inhibited by anaerobiosis, temperatures above 35°C and cobalt ions. Small amounts of ethylene can also be formed in plant tissues from the oxidation of lipids involving a free-radical mechanism.

Mode of action

Ethylene is a plant hormone that acts in concert with other plant hormones (auxins, gibberellins, kinins and abscisic acid) to exercise control over the fruit ripening process. Most is known about the relation of ethylene to fruit ripening because the availability of the sensitive gas chromatographic method for measurement of ethylene has enabled detailed studies of this relationship. The relationship of the other plant hormones to ripening is as yet not clearly defined.

It has been proposed that two systems exist for the regulation of ethylene biosynthesis. System 1 is initiated or perhaps controlled by an

unknown factor that is probably involved in the regulation of senescence. System 1 then triggers system 2, which is responsible, during ripening of climacteric fruits, for the production of the large amounts of ethylene that are necessary for the full integration of ripening. System 2 is an autocatalytic process, with the production of ethylene triggering further production. Non-climacteric fruits do not have an active system 2, and treatment of climacteric fruits with ethylene circumvents system 1.

As in the case of other plant hormones, ethylene is believed to bind to specific receptor(s) to form a complex that then triggers ripening. Ethylene's action can be affected by altering the amount of receptor(s) or by interfering with the binding of ethylene to its receptor. Detailed studies of the structural requirements for biological activity of ethylene receptors led to the proposal that binding takes place reversibly at a site containing a metal, possibly copper. From kinetic studies on the responses of plant tissue to added ethylene it has been proposed that the affinity of the receptor for ethylene is increased by the presence of oxygen and decreased by carbon dioxide. The occurrence of a metal-containing receptor has not been confirmed but the proposition is supported by studies with silver ion. Treatment of fruit, flowers and other tissues with silver ion has been shown to inhibit the action of ethylene. The need for specific structural requirements for ethylene action has been demonstrated by treating tissues with analogues and antagonists of ethylene. The gaseous cyclic olefines, 2,5-norbornadiene and 1-methylcyclopropene (1-MCP), have been shown to be highly effective inhibitors of ethylene action. 1-MCP has been shown to bind irreversibly to the ethylene receptors in sensitive plant tissues and a single treatment with low concentrations for a few hours at ambient temperatures confers protection against ethylene for several days.

The pattern of changes in ethylene production rates and the internal concentrations of ethylene in relation to the onset of ripening have been observed in several climacteric fruits. In one type of fruit (e.g. banana, tomato and honey dew melon), the ethylene concentration rises before the onset of ripening, which was defined as the initial respiratory increase. In the second type (e.g. apple, avocado and mango), ethylene does not rise before the increase in respiration. In honey dew melon, the internal ethylene concentration rises from the preclimacteric level of 0.04 µL/L to 3.0 µL/L, at which concentration the fruit commences to ripen. The low concentrations of ethylene present in unripe fruit and the evident involvement of system 2 ethylene in ripening indicate that treatments that prevent ethylene from reaching a triggering concentration should delay ripening. Ripening has been delayed in green

banana fruit for up to 180 days at 20°C when the fruit were ventilated continuously with an atmosphere of 5 per cent carbon dioxide, 3 per cent oxygen, and 92 per cent nitrogen.

It is well known that as many fruits develop and mature, they become more sensitive to ethylene. For some time after anthesis (flowering), young fruit can have high rates of ethylene production. Early in the life of fruit the concentration of applied ethylene required to initiate ripening is high, and the length of time to ripen is prolonged but decreases as the fruit matures (Table 3.4). The tomato is an extreme example of tolerance to ethylene. Banana and melons, in contrast, can be readily ripened with ethylene even when immature. Little is known about the factor(s) that control the sensitivity of the tissue to ethylene.

The earlier concept that an initial triggering of ripening by a single dose of ethylene is sufficient to ensure ripening is not now the case since application of silver ions, which block the ethylene receptor, will not only block the initiation of ripening by exogenous ethylene but also will arrest the ripening process during its progress. For example, colour development and enzyme synthesis will cease. Furthermore, one of the effects of storage under modified atmospheres (see Chapter 6) is for levels of ethylene produced by the fruit to diminish along with changes in colour and texture changes, while changes in sugars and acids

TABLE 3.4 EFFECT OF MATURITY ON THE TIME TO RIPEN FOR TOMATOES

MATURITY AT HARVEST (DAYS AFTER ANTHESIS)	DAYS TO RIPEN	
	TREATED WITH ETHYLENE	CONTROL
17	11	*
25	6	
31	5	15
35	4	9
42	1	3

* Had failed to ripen when experiment was terminated.

Note: Time to ripen was determined between anthesis and the first detectable red colour (first colour stage). Fruit were treated continuously with 1000 µL/L ethylene.

SOURCE J.M. Lyons and H.K. Pratt (1964) Effect of stage of maturity and ethylene treatment on respiration and ripening of tomato fruits, *Proc. Am. Soc. Hort. Sci.*, 84: 491–500. (With permission.)

responsible for some of the flavour proceed normally. It is apparent, therefore, that ethylene is only one of the regulatory components involved in ripening.

Ripening has long been considered to be a process of senescence and to be due to a breaking down of the cellular integrity of the tissue. Some ultrastructural and biochemical evidence supports this view. It is widely accepted that ripening is a programmed phase in the development of plant tissue, with altered nucleic acid and protein synthesis occurring at the commencement of the respiratory climacteric resulting in new or enhanced biochemical reactions operating in a coordinated manner. Both views fit with the known degradative and synthetic capacities of fruit during ripening. In view of the ample evidence of the ability of ethylene to initiate biochemical and physiological events, it is evident that ethylene action is regulated at the level of gene expression.

Genetic control of ripening

It will be no surprise that ripening is under genetic control in the cells of the fruit. In recent years, research has begun to unravel the complex process of the genetic control of ripening. There is good evidence that there is a marked increase in protein synthesis and nucleic acid synthesis (especially messenger RNA synthesis) preceding and during the early stages of the respiratory climacteric for climacteric fruits such as avocado, banana and tomato. Gel electrophoresis has been used to compare the presence of certain proteins during fruit growth and ripening, and has demonstrated that the levels of some proteins increase during ripening and that new types of proteins are synthesised during the ripening process. Similarly, mRNAs increase during ripening, and *in vitro* translation of these mRNAs can be shown to produce the new proteins. All this evidence supports the view that ripening is under genetic control.

Additional evidence comes from a series of natural mutants of tomato and variants produced by genetic transformation that exhibit abnormal ripening behaviour. A number of non-ripening or slow ripening mutants have been identified that appear to affect the processes of ethylene synthesis, ethylene perception and/or signal transduction leading to abnormal colouring (often yellow or pale red), lack of softening or low ethylene production (see Table 3.5). These mutants have been extensively used to determine some of the fundamental processes involved in ripening, particularly softening. A number of different hydrolase enzymes break down the carbohydrate polymers (e.g. pectins, celluloses, hemicelluloses) responsible for the structural integrity of cell walls. Among these is the enzyme polygalacturonase (PG), which

hydrolyses the $\alpha(1\rightarrow 4)$ linkage between galacturonic acid residues in pectins. Early research with the tomato mutants suggested this could be the primary enzyme responsible for softening. However, as with many systems, further work has shown that softening is more complex, probably also involving a number of other mechanisms including the hydrolase enzyme, pectinesterase, which demethylates galacturonic acid residues in pectins, the release of Ca^{2+} , which is important in cross-linking the polymer chains in the cell wall, and the consequent swelling in the middle lamella of the adjacent cell walls, allowing the cells to move apart. This gives the softer mouthfeel characteristic of a ripe fruit.

TABLE 3.5 MUTANTS OF TOMATO WITH ABNORMAL RIPENING BEHAVIOUR

MUTANT	LOCATION ON CHROMOSOME	FRUIT PHENOTYPE COMPARED WITH WILD TYPE
Ripening inhibitor (<i>rin</i>)	5	normal growth, slowly turns pale yellow; very low ethylene production; little softening, very low PG activity; does not ripen after exposure to ethylene; high oxygen causes light pink colour
Never ripe (<i>Nr</i>)	9	normal growth, slowly turns orange-red; limited softening, ethylene production, PG and lycopene synthesis
Non ripening (<i>nor</i>)	10	more extreme than <i>rin</i> ; final colour is deep yellow; very low ethylene production; contains <1% of wild type PG; high NaCl causes faster ripening, deep orange colour and some softening

SOURCE Adapted from G. Hobson and D. Grierson, G.B. Seymour, J.E. Taylor and G.A. Tucker (eds) (1993) *Biochemistry of fruit ripening*, Chapman & Hall, London. pp. 405–42.

BIOCHEMISTRY OF RESPIRATION

All living organisms require a continuous supply of energy. This energy enables the organism to carry out the necessary metabolic reactions to maintain cellular organisation, to transport metabolites around the tissue and to maintain membrane permeability. In addition, a continuous supply of the organic molecules needed for synthetic reactions in the cells is required.

Aerobic metabolism

Most of the energy required by horticultural produce is supplied by aerobic respiration, which involves the oxidative breakdown of certain organic substances stored in the tissues. A common substrate for respiration is glucose and, if it is completely oxidised, the overall reaction is:



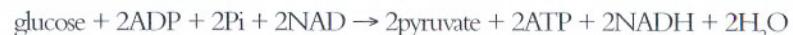
Respiration is essentially the reverse of photosynthesis, by which energy derived from the sun is stored as chemical energy, mainly in carbohydrates containing glucose. Full utilisation of glucose involves two main reaction sequences in respiration:

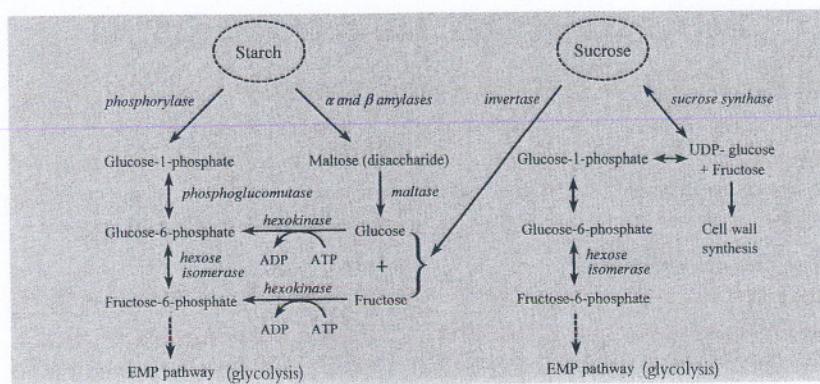
1. glucose \rightarrow pyruvate by the enzymes of the Embden-Meyerhof-Parnas (EMP) pathway, or glycolysis, which are located in the cytoplasm;
2. pyruvate \rightarrow carbon dioxide by the tricarboxylic acid (TCA) cycle, the enzymes of which are located in the mitochondria.

Free glucose is conventionally the compound involved in the initial oxidative step, but it is not the storage form of carbohydrate in the plant. Starch, a polymer of glucose, is often the main storage carbohydrate, and it must be degraded first to glucose by enzymes such as the amylases and maltase or to glucose-1-phosphate by the enzyme phosphorylase. Some commodities have a high sucrose content, which can be hydrolysed to glucose and fructose by the enzyme invertase or via sucrose synthase to UDP-glucose and so to glucose-1-phosphate. Interconversion of sucrose and starch is also possible in many plant tissues. Figure 3.6 provides a generalised scheme for the initial conversions of the storage carbohydrates. Glucose and fructose released from starch and sucrose are oxidised to other respiratory substrates (Figure 3.7).

Glycolysis (EMP sequence)

A simplified view of glycolysis is shown in Figure 3.7. The overall reaction system can be balanced as:

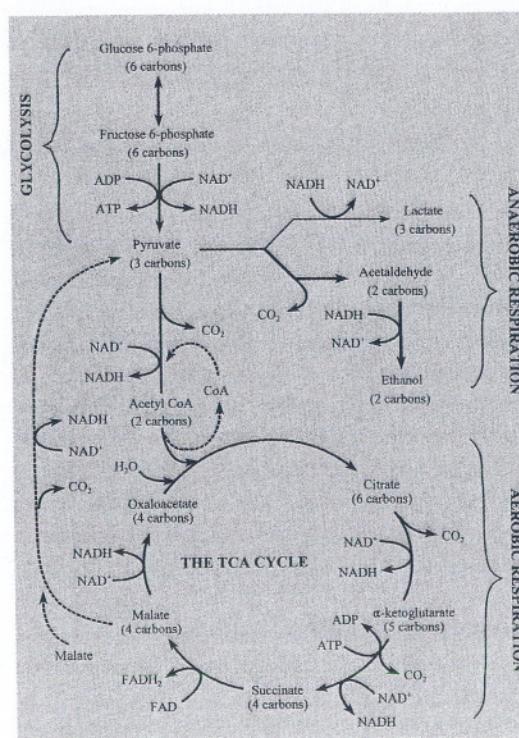


**Figure 3.6**

Starch and sucrose degradation to form the 6-carbon sugars, glucose and fructose, for oxidation via glycolysis. Some of the enzymes involved are indicated, as are the phosphorylation reactions of glucose and fructose. Sucrose may also be degraded to UDP-glucose (uridine diphosphoglucose), a key nucleotide sugar involved in cell wall synthesis, starch/sucrose and other important carbohydrate interconversions.

Figure 3.7

A simplified scheme for aerobic respiration of carbohydrate reserves in plants via glycolysis and the TCA cycle. Malate from the vacuole or mitochondria can be directly metabolised to pyruvate by NAD⁻ or NADP-specific malic enzyme (ME). Pyruvate produced by ME or from fructose 6-phosphate can be oxidised via the TCA cycle or by anaerobic respiration. The conversion of intermediates in these pathways to CO₂ and the reduction of NAD and FAD to NADH and FADH₂, respectively, are also indicated. NADH and FADH₂ are oxidised via the electron transport chain (see Figure 3.8).



The energy liberated from the reaction system is trapped and stored in adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH), with oxidation of each molecule of NADH giving 3ATP (Figure 3.8). The total energy liberated by the conversion of glucose to pyruvate is, therefore, equivalent to 8ATP. The energy is subsequently made available to the plant by breaking a phosphate bond in the reverse reaction:



This energy can then be used in a wide range of synthetic reactions and metabolic interconversions in the plant.

TCA cycle (tricarboxylic acid cycle)

A simplified view of the full cycle is shown in Figure 3.7. The overall reaction system is:

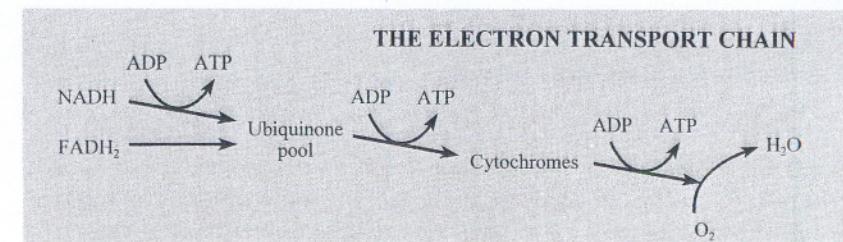


The energy of the original glucose molecule (giving 2x pyruvate) liberated from the TCA cycle is 30ATP (compared with 8ATP from the EMP sequence). The carbon dioxide produced in respiration is derived from the TCA cycle under aerobic conditions and involves a consumption of oxygen. The rate of respiration can therefore be measured by the amount of carbon dioxide produced or oxygen consumed.

The total chemical energy liberated during the oxidation of 1 mole of glucose is approximately 1.6 MJ. About 90 per cent of this energy is preserved within the plant system, and the remainder is lost as heat. Respiration is therefore an efficient converter of energy compared with man-made devices for energy conversion. For example, in the petrol engine more than 50 per cent of the liberated energy is lost as heat.

Figure 3.8

NADH and FADH₂ are oxidised via the electron transport chain to produce water and ATP from ADP. Three ATP molecules are produced from NADH and two from FADH₂.



Other respiratory pathways

The oxidative pentose phosphate pathway (OPPP) converts glucose-6-phosphate to fructose-6-phosphate and glyceraldehyde-3-phosphate and CO_2 through a complex cyclic reaction pathway involving 4-, 5-, 6- and 7-carbon sugar phosphates (Figure 3.9). Although it is not considered to be a major respiratory pathway in fruits, the OPPP does provide ribose-5-phosphate for nucleotide and nucleic acid synthesis, erythrose-4-phosphate for shikimic acid and aromatic amino acid biosynthesis and NADPH to drive a variety of synthetic reactions. The OPPP is of greater importance in leafy vegetables and ornamentals in which it can account for a significant proportion of tissue respiration, perhaps in the range of 10–20 per cent.

The vacuoles of many fruit and vegetables contain high concentrations of organic acids, particularly malic and citric acids, which can be used as respiratory substrates. These acids can be utilised directly by the TCA cycle in the mitochondria. Malic acid can also undergo reductive decarboxylation with the evolution of carbon dioxide and production of NADPH or NADH and pyruvate by malic enzyme present in the cytosol or in the mitochondria (Figure 3.7).

Electron transport system and oxidative phosphorylation

The NADH and FADH_2 produced as a result of certain reactions in glycolysis and the TCA cycle are oxidised through the electron transport system, which is a complex set of dehydrogenase enzymes, electron carrier compounds such as ubiquinones and cytochromes, and cytochrome oxidase bound in the mitochondrial membranes (Figure 3.8). This process utilises oxygen in the final step catalysed by cytochrome oxidase, and is responsible for the physiologically measurable uptake of oxygen. The system is tightly linked to the production of ATP (from ADP and Pi), and hence is termed oxidative phosphorylation.

A number of other oxidase enzymes are present in plants and their fruit tissues. These include polyphenol oxidases, ascorbate oxidases and others, but their role in respiration is unclear, though they do contribute to the oxygen uptake of plant tissues to varying degrees.

Respiratory quotient (RQ)

The complete oxidation of malate



generates more carbon dioxide than the amount of oxygen consumed, whereas oxidation of glucose generates an equal amount of carbon dioxide for the oxygen consumed. This relationship becomes important

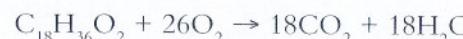
when measuring respiration by gas exchange, in which the carbon dioxide evolved and/or oxygen consumed is measured (i.e. it is possible to record different values for respiration depending on which gas is monitored). Ideally both gases should be measured simultaneously.

The concept of respiratory quotient (RQ) has been developed to quantify this variation, where:

$$\text{RQ} = \text{CO}_2 \text{ produced (mL)} / \text{O}_2 \text{ consumed (mL)}$$

For the complete oxidation of glucose, $\text{RQ} = 1.0$, whereas for malate $\text{RQ} = 1.3$.

An alternative substrate could be long-chain fatty acids, for example, stearic acid:

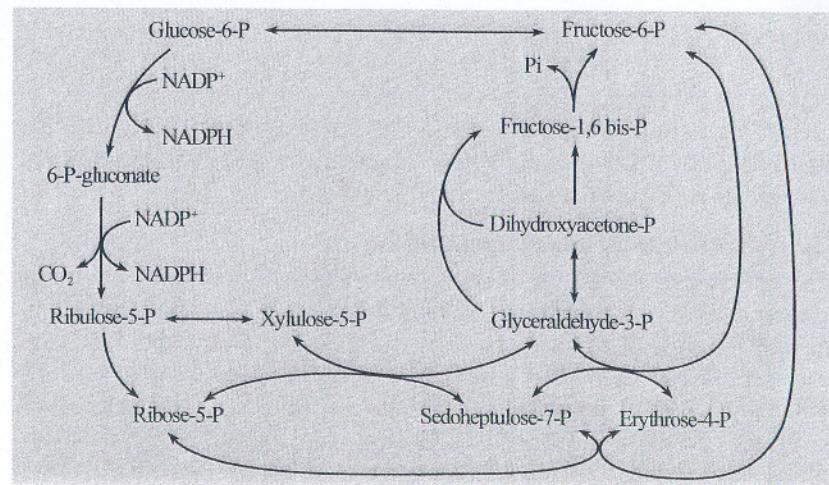


These fatty acids have much less oxygen per carbon atom than sugars and, therefore, require a greater oxygen consumption for the production of carbon dioxide. The RQ equals 0.7 for the above reaction.

Measurement of RQ itself can give some guide to the type of substrate that is being respired: a low RQ suggests some fat metabolism and a high RQ suggests organic acids. Changes in RQ during growth and storage can also indicate a change in the type of substrate that is being metabolised.

Figure 3.9

Oxidative Pentose Phosphate Pathway. This diagram illustrates one of several variants of this pathway, located in the cytosol.



Anaerobic metabolism (fermentation)

The aerobic respiratory pathways utilise oxygen and are the preferred pathways. The normal atmosphere is rich in oxygen, so the amount of oxygen available in the tissue is unlimited. Under various storage conditions the amount of oxygen in the atmosphere may be limited and insufficient to maintain full aerobic metabolism. Under these conditions the tissue can initiate anaerobic respiration, by which glucose is converted to pyruvate by the EMP pathway. But pyruvate is then metabolised into either lactic acid or acetaldehyde and ethanol in a process termed fermentation (Figure 3.7). The oxygen concentration at which anaerobic respiration commences varies between tissues and is known as the extinction or anaerobic compensation point. The oxygen concentration at this point depends on several factors, such as species, cultivar, maturity and temperature. Anaerobic respiration produces much less energy per mole of glucose than aerobic pathways, but it does allow some energy to be made available to the tissue under adverse conditions. An increasing RQ is generally indicative of a switch to fermentation reactions. Anaerobic fermentation will usually lead to production of off-flavours and odours in fruit and vegetables.

Metabolites for synthetic reactions

The respiratory pathways are not only used for the production of energy for the tissue. Carbon skeletons are required for many synthetic reactions in the cell, and these skeletons can be removed at several points. For example, α -ketoglutarate may be converted to the amino acid glutamate, from which several other amino acids may be produced for protein synthesis; succinate may be diverted into the synthesis of various heme pigments including chlorophyll. The loss of α -ketoglutarate and succinate from the TCA cycle for synthetic reactions would eventually lead to the stopping of the cycle. Therefore, C4 acids are fed into the cycle. These are produced principally by the fixation of carbon dioxide into phosphoenol-pyruvate to give oxaloacetate. Alternatively, vacuolar reserves of malate, for example, may be utilised.

Genetic control of plant metabolism

The environmental control of plant metabolism has been used for a long time in a more or less empirical fashion by the use of low temperature. For example, to store fruits and vegetables and to bring about rapid cooling of leafy vegetables, in particular, after harvest from the field. Cooling markedly reduces the rate of respiration of the tissues, generally prolonging their useful life (Chapter 4). Controlled atmosphere (CA) and modified atmosphere (MA) storage of fruits and vegetables (Chapter 6), using elevated

concentrations of carbon dioxide and lowered concentrations of oxygen compared to air, also are used to slow respiratory processes in produce.

More subtle means of manipulating the postharvest behaviour of fruits and vegetables are now becoming available by using mutant varieties or genetic manipulation to develop transgenic plants in which particular enzymes (gene products) can be either virtually eliminated or enhanced. A notable example is the reduction in the activity of the enzyme polygalacturonase, which is involved in the breakdown of the cell wall during ripening of the tomato. This research has resulted in the commercial production and marketing of the FLAVR SAVR™ tomato, which remains firmer longer during ripening so that it can be kept on the plant longer before picking. The object of this is to enhance the flavour compared with tomatoes picked at an early stage of colouring to enable the fruit to withstand the mechanical handling during transport and marketing. Transgenic fruits containing ACC deaminase and antisense ACC synthase, ACC oxidase and polyphenoloxidase have also been produced. The first three transformations reduce ethylene production and slow ripening, while lowered polyphenoloxidase activity reduces browning of damaged tissue.

Apart from any concerns that consumers may have about eating genetically manipulated produce, these genetic techniques will need to be approached with some caution since plant metabolism is very adaptable due to the fact that there are usually alternate pathways by which a given metabolic product or intermediary metabolite may be produced. This plasticity of metabolism makes plants and their products able to modify their performance under a wide variety of conditions but will make the task of the genetic engineer more difficult. This is borne out by very recent studies in which particular enzyme levels have been modified in plants by genetic manipulation in order to study metabolic control. Formerly it was thought that a small number of key enzymes in each pathway regulated the flow of carbon through a particular pathway. This was thought to be achieved by low absolute concentrations of the regulatory enzyme, with its activity depending on one or more co-factors such as the ratio of oxidised to reduced nicotinamide nucleotides (NAD/NADH; NADP/NADPH) or phosphorylating co-factor (ADP/ATP). However, the virtual removal of a particular enzymic activity by genetic manipulation is found to have little effect in a number of cases, so a combination of metabolic plasticity (mentioned above) and much more dispersed regulatory control mechanisms must be invoked.

CHEMICAL CHANGES DURING MATURATION

At some stage during the growth and development of fruit and vegetables, the consumer recognises that the produce has attained optimum eating condition. This desirable quality is not associated with any universal change, but is attained in various ways in different tissues (see Chapter 10).

Fruit

Climacteric fruit generally reach the fully ripe stage after the respiratory climacteric. However, it is the other events initiated by ethylene that the consumer associates with ripening.

Colour

Colour is the most obvious change that occurs in many fruits and is often the major criterion used by consumers to determine whether the fruit is ripe or unripe. The most common change is the loss of green colour. With a few exceptions — such as the avocado, kiwifruit and Granny Smith apple — climacteric fruits show rapid loss of green colour on ripening. Many non-climacteric fruits also exhibit a marked loss of green colour when they reach optimum eating quality, such as citrus fruit in temperate climates (but not in tropical climates). The green colour is due to the presence of chlorophyll, which is a magnesium–organic complex. The loss of green colour is due to degradation of the chlorophyll structure. The principal agents responsible for this degradation are pH changes (mainly due to leakage of organic acids from the vacuole), oxidative systems and chlorophyllases (Figure 3.10). Loss of colour depends on one or all of these factors acting in sequence to destroy the chlorophyll structure.

The disappearance of chlorophyll is often associated with the synthesis and/or revelation of pigments ranging from yellow to red. Many of these pigments are carotenoids, which are unsaturated hydrocarbons with generally 40 carbon atoms and which may have one or more oxygen atoms in the molecule. Carotenoids are stable compounds and remain intact in the tissue even when extensive senescence has occurred. Carotenoids may be synthesised during the development stages on the plant but remain masked by the presence of chlorophyll. Following the degradation of chlorophyll, the carotenoid pigments become visible. With other tissues, carotenoid synthesis occurs concurrently with chlorophyll degradation. Banana peel is an example of the former system and tomato of the latter.

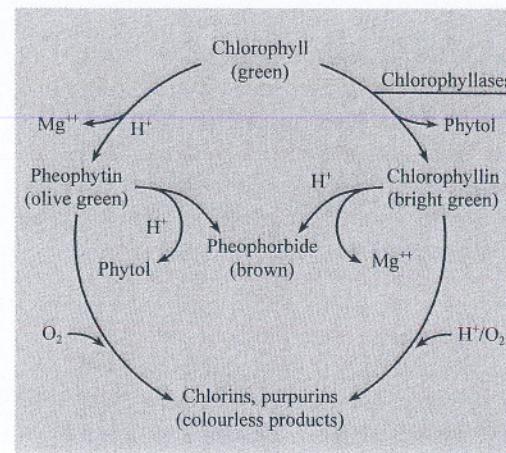


Figure 3.10
Some pathways for the degradation of chlorophyll.

Anthocyanins provide many of the red-purple colours of fruit, vegetables and flowers. Anthocyanins are water-soluble phenolic glucosides that can be found in the cell vacuoles of fruit and vegetables (e.g. beetroot), but are often in the epidermal layers (e.g. apples and grapes). They produce strong colours, which often mask carotenoids and chlorophyll.

Carbohydrates

The largest quantitative change associated with ripening is usually the breakdown of carbohydrate polymers, especially the near total conversion of starch to sugars. This alters both the taste and texture of the produce. The increase in sugar renders the fruit much sweeter and, therefore, more acceptable. Even with non-climacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar may be derived from sap imported into the fruit rather than from the breakdown of the fruit's starch reserves.

The breakdown of polymeric carbohydrates, especially pectic substances and hemicelluloses, weakens cell walls and the cohesive forces binding cells together. In the initial stages, the texture becomes more palatable, but eventually the plant structures disintegrate. Protopectin is the insoluble parent form of pectic substances. In addition to being a large polymer, it is cross-linked to other polymer chains with calcium bridges and is bound to other sugars and phosphate derivatives to form an extremely large polymer. During ripening and maturation, protopectin is gradually broken down to lower molecular weight fractions, which are more soluble in water. The rate of degradation of pectic substances is directly correlated with the rate of softening of fruit.

Organic acids

Usually organic acids decline during ripening as they are resired or converted to sugars. Acids can be considered as a reserve source of energy to the fruit, and would therefore be expected to decline during the greater metabolic activity that occurs with ripening. There are exceptions, such as bananas, where the highest level is attained when the banana is fully ripe, but the acidic level is not high at any stage of development compared with other produce.

Nitrogenous compounds

Proteins and free amino acids are minor constituents of fruit and, as far as is known, have no role in determining eating quality. Changes in nitrogenous constituents do, however, indicate variations in metabolic activity during different growth phases. During the climacteric phase of many fruits there is a decrease in free amino acids, which often reflects an increase in protein synthesis. During senescence, the level of free amino acids increases, reflecting a breakdown of enzymes and decreased metabolic activity.

Aroma

Aroma plays an important part in the development of optimal eating quality in most fruit. Aroma is due to the synthesis of many volatile organic compounds (often known merely as volatiles) during the ripening phase. The total amount of carbon involved in the synthesis of volatiles is less than 1 per cent of that expelled as carbon dioxide. The major volatile formed is ethylene, which accounts for about 50–75 per cent of the total carbon in the volatiles; ethylene does not contribute to typical fruit aromas. The amount of aroma compounds is therefore extremely small. Chapter 2 discussed the nature of the compounds formed. Non-climacteric fruits also produce volatiles as they reach optimum eating quality. These fruits do not synthesise compounds that are as aromatic as those in climacteric fruit; nevertheless, the volatiles produced are still appreciated by consumers.

Vegetables

Vegetables generally show no sudden increase in metabolic activity that parallels the onset of the climacteric in fruit, unless sprouting or regrowth is initiated. The process of germination is sometimes deliberately applied to some seeds, such as mung bean, and the sprouted product is the marketed vegetable. Apart from obvious anatomical changes during sprouting, considerable compositional changes occur. The sugar level increases markedly as the result of the

rapid conversion of fats or starch. From a nutritional view, the increase in vitamin C in sprouted seeds can be valuable in diets with marginal vitamin C intakes.

Vegetables can be divided into three main groups: seeds and pods; flowers, buds, stems and leaves; and bulbs, roots and tubers. Some fruits are also consumed as vegetables; they may be either ripe (e.g. tomato, egg-plant) or immature (e.g. zucchini, cucumber, okra).

Seeds and pods, if harvested when fully mature, as is the practice with cereals, have low metabolic rates because of their low water content. In contrast, all seeds consumed as fresh vegetables, such as legumes and sweet corn, have high levels of metabolic activity because they are harvested at an immature stage, often with the inclusion of non-seed material, such as bean pod (pericarp). Eating quality is determined by flavour and texture and not by physiological age. Generally the seeds are sweeter and more tender at an immature stage. With advancing maturity, the sugars are converted to starch with the resultant loss of sweetness, the water content decreases and the amount of fibrous material increases. Seeds for consumption as fresh produce are harvested when the water content is about 70 per cent; in contrast, dormant seeds are harvested at less than 15 per cent water.

Edible flowers, buds, stems and leaves vary greatly in metabolic activity and hence in the rate of deterioration. Stems and leaves often senesce rapidly and therefore lose their attractiveness and nutritional value. Generally the most visible sign of senescence is degreening, resulting in yellowing due to underlying carotenoid pigments. Texture often becomes the dominant characteristic that determines both the harvest date and quality, with reduced turgor through water loss altering the texture. The natural flavour is often of less importance than texture, as many of these vegetables are cooked with salt or spices added. Growth processes such as cell division and expansion and protein and carbohydrate synthesis usually cease, and the metabolism goes into a catabolic or degradative mode.

Bulbs, roots and tubers are storage organs that contain food reserves required when growth of the plant is resumed (they are often held for the purpose of propagation). When harvested, their metabolic rate is low and, under appropriate storage conditions, their dormancy can be prolonged. The biochemistry of these storage organs is geared to a slow metabolic rate designed to provide the low levels of energy required to maintain life in the cells of these tissues during dormancy. Postharvest considerations are to maintain produce in the dormant state.

Ornamentals

Ornamentals can be grouped into cut flowers, cut foliage and pot plants. Others, of course, are planted in gardens and urban landscaping, but they are beyond the scope of this book.

Flowers usually have high rates of respiration through glycolysis and the TCA cycle based on sugar translocation from the leaves. For cut flowers, therefore, so-called preservative solutions, used by florists, contain sucrose as a carbohydrate source to help maintain the respiration rate and to extend storage life.

Pigments

The main pigments in ornamental foliage are similar to those in fruit and vegetables. Chlorophyll is the principle green foliage pigment and carotenoids constitute many of the yellow, orange and red pigments. However, the anthocyanins and related compounds are responsible for red, purple and blue colours in most flowers. The observed colours are largely related to the pH of the flower sap. Anthocyanins are red at a more acidic pH (below 7), whereas they tend to be blue above pH 7. This gives rise to the phenomenon in roses known as 'blueing', where a shift from red to blue colouration occurs with ageing. This is due to the depletion of sugars as a respiratory substrate and the switch to catabolism of proteins, with the release of free amino groups resulting in a shift to a more alkaline pH in the cell sap. The 'colour' white is due to a total reflectance of the visible spectrum and often results from the presence of highly aerated tissues, as in some flowers. Variegated leaves in some ornamentals, for example, are due to areas devoid of chloroplasts and may be white or orange/yellow, the latter colour due to the carotenoid pigments.

Ethylene

Flowers tend to have a short postharvest (vase) life. A major contributing factor is often attributed to their sensitivity to ethylene. Most ornamentals should be regarded as non-climacteric, although some produce a distinct ethylene peak and respiratory climacteric. There will therefore be differential responses to ethylene similar to fruit and vegetables. Thus some flowers, such as the non-climacteric delphinium, are highly sensitive to ethylene while the climacteric carnation is relatively tolerant. The major effect of ethylene is induced abscission. The short postharvest life of cut flowers is, however, confounded by limited carbohydrate reserves and an overall rapid rate of metabolism and development.

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