THE TOMATO CROP

A scientific basis for improvement

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London New York
CHAPMAN AND HALL
1986

Grierson, D. and A.A. Kader. 1986. Fruit ripening and quality, pp. 241-280. In: J.G. Atherton and J. Rudich (eds.), The Tomato Crop, Chapman and Hal, London.

CHAPTER 6

Fruit ripening and quality

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6.1 INTRODUCTION

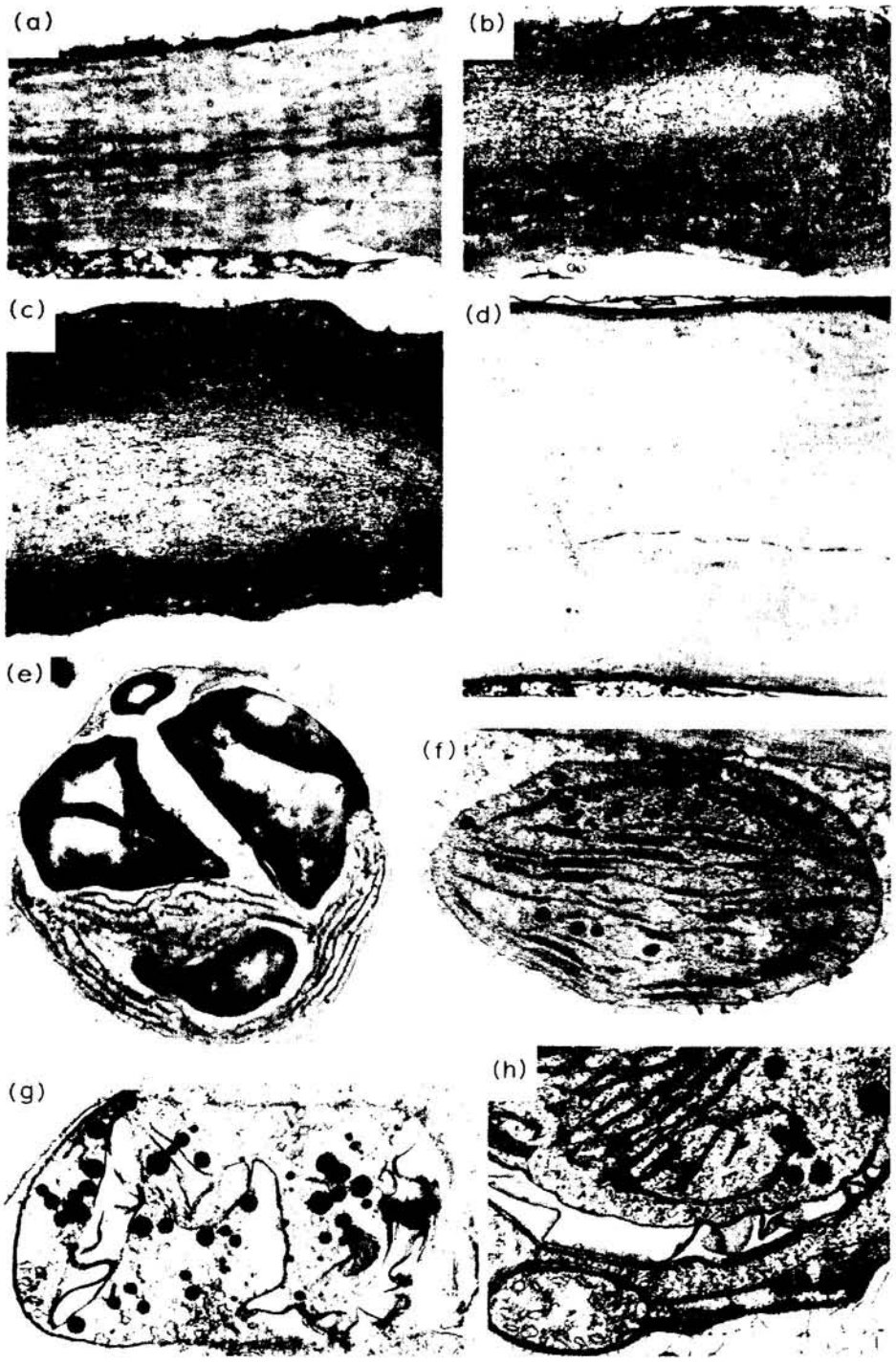
The conversion of a tomato fruit from the mature green to fully ripe state involves dramatic changes in colour, composition, aroma, flavour and texture. Ripening used to be thought of simply as the result of a series of degradative processes, probably because some of the more obvious changes require the action of hydrolytic enzymes. However, it is now clear that ripening is dependent on a wide range of separate synthetic as well as degradative reactions. These include alterations in metabolism and gene expression which have a dramatic effect on fruit quality. The changes are highly coordinated; they occur in the majority of the cells of the fruit and involve every subcellular compartment. The various facets of ripening appear to be coordinated and regulated by plant hormones but may be modified by genetic and environmental factors. In this chapter the general features of tomato ripening are outlined first, together with a discussion of the cellular mechanisms regulating the process. This is followed by a consideration of the ways in which genotype, growing conditions, disease, and post-harvest history can influence specific quality attributes.

6.2 PHYSIOLOGY AND BIOCHEMISTRY OF RIPENING

6.2.1 Cell ultrastructure and function

(a) Mature green fruit

Massive cell expansion occurs during the growth of tomato fruits. Typical pericarp parenchyma cells at the mature-green stage measure 300–500 μm or more across, have relatively thick cell walls and a thin layer of cytoplasm surrounding a central vacuole. The cytoplasm of each cell is bounded on the inside by the tonoplast membrane and on the outside by the plasmalemma. Occasionally the cytoplasm of adjacent cells can be seen to be in communication by strands of protoplasm, called plasmodesmata, which pass through the cell walls. Each cell contains a number of organelles including a nucleus, many mitochondria and chloroplasts, ribosomes, and endoplasmic



reticulum membranes. The cell walls consist of a middle-lamella region separating the primary and secondary walls of adjacent cells. The cell wall complex generally appears densely packed and consists of cellulose fibrils embedded in a matrix of pectic substances, hemicellulose and proteins (Fig. 6.1).

The chloroplasts are bounded by two membranes. Inside these are separate thylakoid membranes, containing chlorophyll and proteins in the photosynthetic light reaction centres. The thylakoids are frequently stacked together to form grana, but these are not as extensive as found in a typical leaf chloroplast of a C3 plant. Prominent starch grains can be seen in the chloroplasts of immature fruit but these become reduced at the mature green stage (Fig. 6.1). Some chloroplast proteins are synthesized inside the chloroplast envelope using the unique DNA, RNA polymerase, ribosomes and other protein-synthesizing machinery present within these organelles. Other essential proteins are formed outside the chloroplasts and transported across the envelope. One important function of the chloroplasts is to fix carbon dioxide present in the fruit. However, they also store starch and synthesize a range of important compounds, including some of the plant hormones.

The plastids also contain the major pigments of the tomato fruit, the chlorophylls and the carotenoids. The predominant pigments in green fruits comprise a mixture of chlorophylls *a* and *b* (Edwards and Reuter, 1967). The total chlorophyll in green fruit is about 13 μg g fr.wt.$^{-1}$ and the chlorophyll *a*/*b* ratio is greater than unity (Watada *et al.*, 1976b). Carotenoids are only present in small amounts. More than half of them are xanthophylls at the immature-green stage but this proportion decreases towards maturity (Rabinowitch, Budowski and Kedar, 1975). Alpha- and beta-carotene are also present in small quantities (Meredith and Purcell, 1966).

The nucleus, which is relatively small compared to the size of the cell, is surrounded by a distinct membrane which is perforated at intervals by nuclear pores. It contains a prominent nucleolus, where ribosomes destined for the

Figure 6.1 Ultrastructural changes during tomato ripening. (a)–(d) show changes in the cell wall. In (a) the middle-lamella region can be seen as a slightly darker-staining region. Solubilization of the pectin begins here about 2–3 days after the start of ethylene synthesis (b) and becomes more extensive during the next few days (c). Eventually (after 8–12 days, d) the wall is extensively solubilized and swollen. (e)–(h) show changes in the plastids during development and ripening. Large starch grains and some thylakoid membranes can be seen in the chloroplast of immature green fruit (e). At the mature-green stage (f) prominent thylakoids and lipid droplets are visible and starch is reduced or absent. During chromoplast formation the thylakoids disappear and membranes enclosing lycopene crystals appear (g). A portion of a chromoplast is also shown in (h), together with a mitochondrion in the lower left of the picture. Magnificiation × 22 000 (a), × 24 000 (b), × 23 000 (c), × 15 000 (d), × 12 000 (e), × 33 000 (f), × 39 000 (g), × 52 000 (h). Photographs courtesy of Philip Crookes and Mary Purton.

cytoplasm are assembled, and the chromosomes which, in fruit cells, are not highly condensed. During fruit growth and maturation the RNA polymerase enzymes in the nucleus transcribe many different genes to produce messenger RNA molecules, transfer RNAs and ribosomal RNAs. These molecules are transported through the nuclear pores to the cytoplasm where they form polyribosomes, synthesizing a range of enzymes and structural proteins required in the cytoplasm, membranes, cell organelles and cell walls.

(b) Changes during ripening

Several important changes in ultrastructure occur during ripening. Cell wall changes begin in the region of the middle lamella (Fig. 6.1), which appears to become less electron-dense. This is equated with the initiation of cell-wall solubilization by the softening enzyme polygalacturonase (Crookes and Grierson, 1983) and the accumulation of water-soluble pectin. Hydrolysis of the pectin is probably facilitated by the action of pectin esterase, which renders it more susceptible to degradation by polygalacturonase. Hydrolysis of cellulose by cellulase may also be involved in wall degradation, but the most important softening enzyme in tomato is polygalacturonase. As ripening continues, solubilization of the wall becomes more extensive (Fig. 6.1) and in very ripe fruit the cell walls are fragile. This produces a soft, juicy texture but renders the fruit tissue susceptible to mechanical damage and penetration by pathogens. There is also a striking reduction in size of the starch grains present in the chloroplasts during ripening and they eventually disappear altogether (Fig. 6.1). At about the same time the thylakoid membranes begin to show fewer grana, and lipid droplets, which may be derived from the thylakoids, begin to appear. These changes herald the beginning of the transformation of the chloroplasts into chromoplasts (Harris and Spurr, 1969). They lose their chlorophyll and eventually the familiar features of the thylakoids disappear and are replaced by an internal membrane system with a characteristic 'wavy' appearance (Simpson *et al.*, 1976; Crookes and Grierson, 1983; Fig. 6.1). Sometimes these membranes appear needle-shaped but may also be arranged concentrically. The red pigment, lycopene, accumulates in association with this internal membrane system as the chromoplast develops, and β-carotene, which has a different solubility, accumulates in lipid globules.

Ripening used to be thought of essentially as a degenerative process, involving senescence and the general breakdown of the cell, leading ultimately to cell death. Apparent evidence of degeneration was sometimes observed with the electron microscope and cited as evidence in support of this view. Although it is certainly true that the cells of ripening fruit will eventually die if left for long enough, the process takes several weeks. During all this time the nucleus, mitochondria, chromoplasts, plasmalemma and polyribosomes all remain intact and active. In some cases they even increase their metabolic activity. A more correct interpretation of the ultrastructural evidence is that various parts of the cell are simply transformed in appearance as the composition of the cells

becomes modified. The cells may become more fragile as a result of cell-wall degradation, and possibly other changes, so that they are more easily damaged during preparation for electron microscopy. However, the use of gentle techniques for sample preparation has shown that there is very little breakdown of cell organization during the early stages of ripening (Simpson *et al.*, 1976; Crookes and Grierson, 1983).

6.2.2 Chemical composition

Some of the important changes in composition that occur during tomato ripening are listed in Table 6.1. Many of these can be shown to take place when mature-green fruit are detached from the plant and allowed to ripen. It therefore follows that at least some aspects of ripening depend on the metabolism of components already existing in the fruit and are not dependent on the import of materials from the parent plant. However, this is not to say that the quality of fruit that ripen after being picked is identical in every respect with that of fruit allowed to come to full ripeness on the plant (see Section 6.4).

In general the dry matter content of ripe fruit is within the range 5–7.5% (Davies and Hobson, 1981) and 100 g of raw tomato contains about 20 calories. The major constituents are listed in Table 6.2. Note the high proportion of sugars and organic acids, which make a major contribution to the taste of the fruit (see Section 6.3.3). Although the vitamins only account for a small proportion of the total dry matter they are highly significant from the nutritional point of view (see Section 6.3.4) and are listed separately in Table 6.3. In addition there are more than 200 individual volatile constituents, but the significance and relative importance of these in flavour and aroma is far from clear (see Section 6.3.3).

The production of the normal red colour of ripe fruit is due to the destruction of chlorophyll and the extensive accumulation of the carotenoids β-carotene and lycopene as the chloroplasts are transformed to chromoplasts. Although the plastids of green fruit do synthesize small quantities of carotenoids, their accumulation in fruit involves the operation of a separate set of enzymes that is switched on at the onset of ripening (Fig. 6.2). Several of these enzymes are

TABLE 6.1 *Changes in composition during ripening*

Degradation of starch and production of glucose and fructose
Loss of chlorophyll
Synthesis of pigments such as β-carotene and lycopene
Increase in soluble pectins resulting from wall softening and degradation
Production of flavour and aroma compounds
Increase in ratio of citric acid to malic acid
Increase in glutamic acid
Breakdown of the toxic alkaloid α-tomatine

TABLE 6.2 *Composition of ripe tomato fruit (% of dry matter). (From Davies and Hobson, 1981.)*

Sugars	
Glucose	22
Fructose	25
Sucrose	1
Alcohol insoluble solids	
Protein	8
Pectic substances	7
Hemicellulose	4
Cellulose	6
Organic acids	
Citric acid	9
Malic acid	4
Minerals	
(mainly K^+, Ca^{2+}, Mg^{2+}, P)	8
Others	
Lipids	2
Dicarboxylic amino acids	2
Pigments	0.4
Ascorbic acid	0.5
Volatiles	0.1
Other amino acids, vitamins and polyphenols	1.0

TABLE 6.3 *Vitamin content of ripe tomato fruit (range of values per 100 g fruit). (From Davies and Hobson, 1981.)*

Vitamin A (β-carotene)	900–1271 i.u.*
Vitamin B_1 (thiamine)	50–60 μg
Vitamin B_2 (riboflavin)	20–50 μg
Vitamin B_3 (pantothenic acid)	50–750 μg
Vitamin B_6 complex	80–110 μg
Nicotinic acid (niacin)	500–700 μg
Folic acid	6.4–20 μg
Biotin	1.2–4.0 μg
Vitamin C	15 000–23 000 μg
Vitamin E (α-tocopherol)	40–1200 μg

* 1 i.u. (international unit) = 0.6 μg β-carotene.

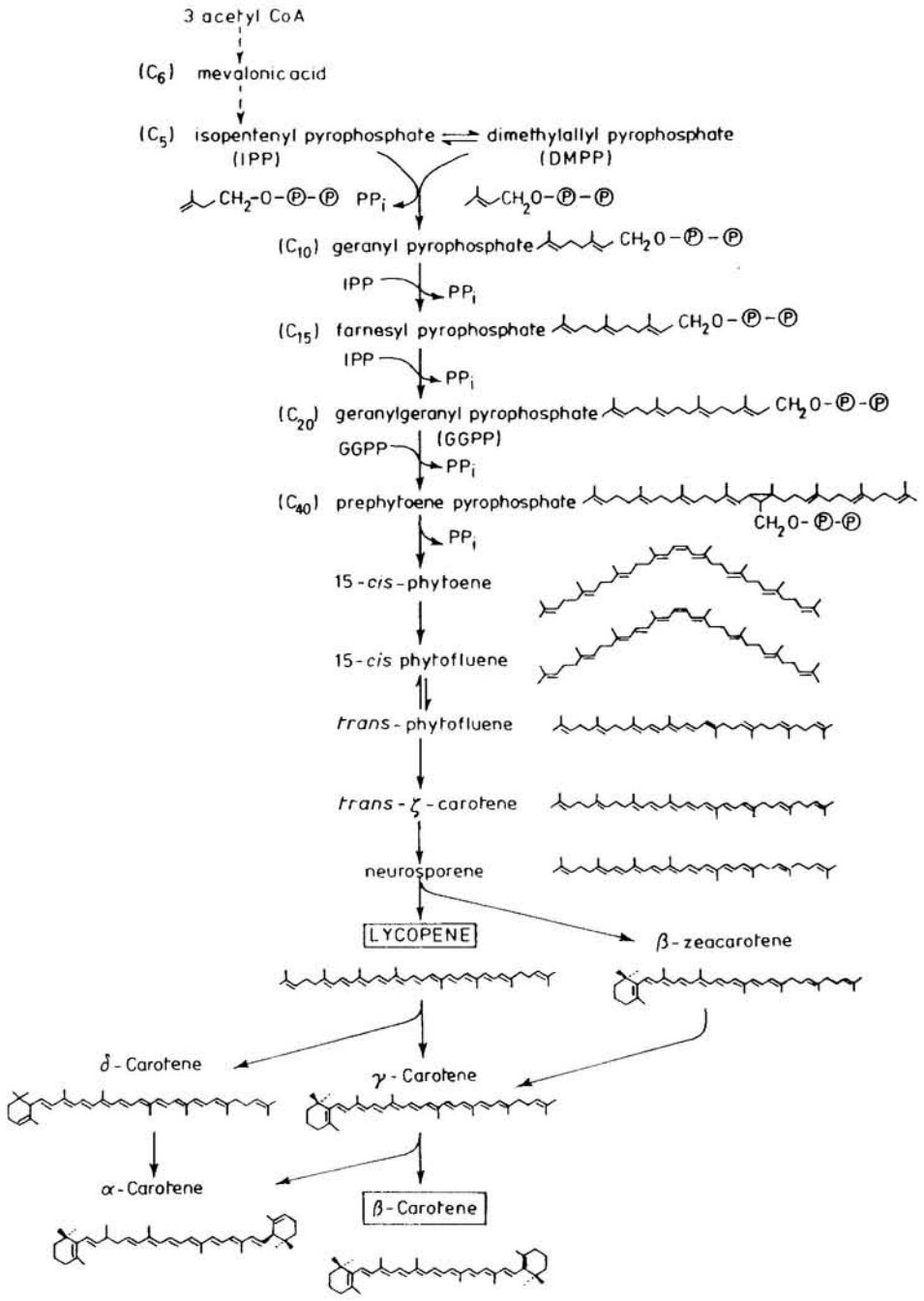


Figure 6.2 Synthesis of carotenoids in tomato fruit.

encoded by nuclear genes and their mRNAs are translated in the cytoplasm and the polypeptides are transported into the plastids. The precursors for carotenoid biosynthesis are derived from acetyl CoA which is converted into a series of reactions to mevalonic acid, which in turn is converted into isopentenyl pyrophosphate (a C_5 compound) in the plastids. Isomerization of isopentenyl pyrophosphate produces dimethylallyl pyrophosphate and these two molecules are condensed, with the elimination of pyrophosphate, to form geranyl pyrophosphate (a C_{10} compound). Further additions of isopentenyl pyrophosphate produce farnesyl pyrophosphate (C_{15}) and geranylgeranyl pyrophosphate (C_{20}). Two molecules of geranylgeranyl pyrophosphate are then combined to form prephytoene pyrophosphate (C_{40}). The above reactions are carried out by enzymes that are either soluble or peripherally associated with the inner plastid membranes. The prephytoene pyrophosphate is converted into 15-*cis*-phytoene which undergoes dehydrogenation to produce 15-*cis*-phytofluene. This is followed by a series of dehydrogenation steps, with removal of two hydrogens at a time from alternative sides of the molecule, to generate trans-ζ-carotene, neurosporene and lycopene (Fig. 6.2.). This series of dehydrogenation reactions is probably carried out by a multifunctional dehydrogenase enzyme associated with the inner chromoplast envelope membrane. Lycopene is not the end product of the biosynthetic pathway and undergoes cyclization to produce either δ-carotene or γ-carotene; a second ring closure generates β-carotene and α-carotene respectively (Fig. 6.2). An alternative route to γ- and β-carotene *via* β-zeacarotene probably also operates in tomatoes.

During fruit ripening, α- and β-carotene reach peak concentrations at the breaker and light-red stages (Meredith and Purcell, 1966). The orange colour in half-ripe fruit is attributable to the increase in β-carotene content, whereas the red colour of ripe fruit is due to the subsequent rapid accumulation of lycopene, which reaches 40–180 $\mu g\ g^{-1}$ fresh weight in different varieties and accounts for 50–76% of the total pigments (Davies and Hobson, 1981). Other pigments such as ζ- and γ-carotene also accumulate in ripening fruit, as do the colourless precursors phytoene and phytofluene (Goodwin, 1980). Numerous other minor pigments also occur, but do not contribute significantly to the final colour. The accumulation of lycopene during ripening is puzzling, since it is an *intermediate* in the biosynthesis of β-carotene. This apparent anomaly could be explained if there were separate enzyme systems for the production of lycopene and β-carotene. The finding that the synthesis of lycopene, but *not* β-carotene, is inhibited by temperatures within the range 30–35 °C supports the idea of two enzyme systems (Goodwin and Jamikorn, 1952). The accumulation of lycopene could also be explained by inhibition of the cyclization step leading to the production of the carotenes (Fig. 6.2). In this connection it is interesting to note that the herbicide CPTA (2-*p*-chlorophenylthiotriethylammonium chloride) causes the accumulation of lycopene, even in fruits that do not normally turn red, by inhibiting the cyclization reaction (Goodwin, 1980).

There is considerable variation in colour and uniformity in different varieties and this is under genetic control (Table 6.4, Goodwin, 1980; see also Chapter 2). In the *greenflesh* mutant, for example, the degradation of chlorophyll is reduced, resulting in a red–brown fruit (Table 6.4). In *tangerine* tomatoes, the poly*cis* isomers *cis*-ζ-carotene, proneurosporene and prolycopene accumulate

TABLE 6.4 *Tomato ripening-mutants*

Name	Chromosome	Phenotype of fruit homozygous for the mutation
Ripening inhibitor (*rin*)	5	Fruit do not ripen fully: they turn yellow and only soften very slowly. There is little or no synthesis of polygalacturonase and no rise in respiration or ethylene synthesis. Fruit lack the normal tomato flavour and store for a very long time
Non-ripening (*nor*)	10	Similar to *rin* but the final fruit colour is pale orange
Never-ripe (*Nr*)	9	'Ripe' fruit are orange and soften slowly: synthesis of lycopene and polygalacturonase is much reduced and fruit store for a long time
Greenflesh (*gf*)	8	Ripe fruit appear red–brown in colour because chlorophyll loss is incomplete but are otherwise normal
Yellow flesh (*r*)	3	Ripe fruit are yellow because lycopene is not synthesized but are otherwise normal
Alcobaca (*alc*)	10	Fruit ripened attached to the vine are pale red. The flavour is almost normal but storability is increased due to a slow softening rate, which is probably related to reduced polygalacturonase. Fruit picked mature green show reduced ethylene production and respiration and only ripen to a yellow colour
Longkeeper	10	Fruit ripen to a golden-orange–red colour. Polygalacturonase activity, softening and carotenoid synthesis is much reduced and, as the name implies, fruit store for a long time
Tangerine (*t*)	10	Fruit flesh is a rich tangerine colour owing to the replacement of lycopene by prolycopene. The fruit are otherwise normal
Uniform ripening (*u*)	10	This gene eliminates the dark-green shoulder from immature fruit, thus preventing 'green-back'

For further information see Chapter 2 and Rick (1980).

(Goodwin, 1980) and in the *delta* mutant there are reduced concentrations of lycopene and high levels of δ-carotene. Other mutants exist that are deficient in various aspects of carotenoid biosynthesis and appear white, yellow or orange when ripe (Chapter 2, and Goodwin, 1980).

Citric acid concentration increases to a maximum at the mature-green stage and remains at this level during ripening, whereas malic acid concentration decreases. As a result, the ratio of malic acid to citric acid falls from 1.3 to 0.6 in the whole fruit during ripening and citric acid accounts for well over half the total acidity in a ripe fruit (Davies and Hobson, 1981) (see Section 6.4.1). There are cultivar differences in the ratio of these two acids (Davies, 1965; see Section 6.3.3).

A number of factors, such as genotype, irradiance and potassium nutrition during fruit growth, and temperature during ripening, affect colour, acidity and sugar content (Section 6.4).

6.2.3 The respiratory climacteric and ethylene production

Fruits can be classified as 'climacteric' or 'non-climacteric' on the basis of their respiratory behaviour during ripening (Biale and Young, 1981). Non-climacteric fruit, such as citrus, pineapple and strawberry, show no marked change in respiration while others, including apple, pear, banana and tomato, show a characteristic increase. Climacteric fruit show a drop in respiration to a pre-climacteric minimum, prior to ripening. At the onset of ripening, respiration increases, rises to a maximum, called the climacteric peak, and subsequently declines slowly (Fig. 6.3). In some climacteric fruits this increase in respiration is truly massive but CO_2 production in tomatoes only increases by a factor of two during ripening, to about 20 μl CO_2 g^{-1} fruit h^{-1}.

The significance of the respiratory climacteric and the biochemical control mechanisms responsible for increased respiration are not very clear. Experiments with isolated fruit mitochondria indicate that they remain fully functional during ripening and may even show an increased capacity for the phosphorylation of ADP to ATP (Hulme, Jones and Wooltorton, 1963; Lance *et al.*, 1965). Furthermore, a second 'alternate pathway' for electron transport, which is resistant to inhibition by cyanide, operates during ripening (Lance, 1981). It has been suggested that mitochondrial activity may increase due to alterations in the availability of substrates and cofactors (Romani, 1975) and changes in respiratory enzymes have been demonstrated (Chalmers and Rowan, 1971; Rhodes, 1980, 1983). The carbon sources for respiration include carbohydrates and also organic acids. For example, the metabolism of malic acid increases during tomato fruit ripening (Davies and Maw, 1972; Jeffery *et al.*, 1984).

In addition to a stimulation of respiration, climacteric fruit also exhibit an increase in ethylene synthesis during ripening. Ethylene production is generally high at the time of anthesis and for a short time after this. It then

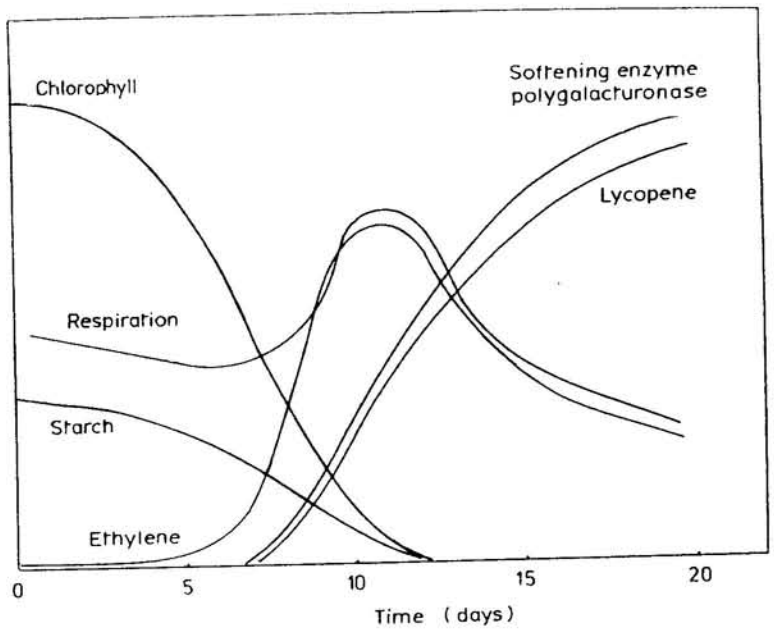


Figure 6.3 Changes in metabolism and composition during ripening. (Redrawn from Grierson and Covey, 1984.)

declines to a low level (less than 0.05 nl g^{-1} fruit h^{-1}) during later tomato growth and maturation. Wounding or damage by pathogens stimulates ethylene synthesis but in intact, healthy fruit an increase in ethylene production (to 2–10 nl g^{-1} h^{-1}) only occurs at the onset of the respiratory climacteric (Fig. 6.3). Indeed, an increase in the synthesis of CO_2 and ethylene are normally the first indications of the beginning of ripening, before any other events are manifested. Changes in concentration of these gases can be detected by analysing samples taken from the internal atmosphere of an individual fruit with a syringe. In addition, since 97% of the gas exchange from a detached tomato occurs through the calyx scar (Cameron and Yang, 1982) an increase in production of CO_2 and ethylene can be monitored by analysing samples of gas diffusing from fruit after they are picked.

There are conflicting reports about whether the increase in respiration in different climacteric fruits precedes the rise in ethylene synthesis or *vice versa* (Burg, 1962; Biale and Young, 1981). The discrepancies may be due to species variation or differences in the methods and sensitivity of the analytical equipment used in these measurements. Recent studies on the internal gas atmosphere of tomatoes, however, show that a rise in ethylene production undoubtedly precedes the respiration increase (Sawamura, Knegt and Bruinsma, 1978).

Many characteristic ripening changes begin to occur at about the same time as the increases in respiration and ethylene production (see Fig. 6.3). There are several reasons (discussed in Section 6.2.6) for believing that ethylene accelerates these other changes in ripening fruit. When applied to green bananas ethylene reduces the preclimacteric period and hastens the onset of the respiratory rise and ripening. Application of ethylene also stimulates ripening of green tomatoes, but they are much more resistant to the induction of ripening by ethylene than many other fruit, especially at the immature-green stage (McGlasson, Wade and Adato, 1978).

6.2.4 Pathway and regulation of ethylene synthesis

It was recognized many years ago that ethylene was probably synthesized from the sulphur-containing amino acid methionine, but the intermediate steps in the pathway were only worked out relatively recently from studies of apple tissue by Adams and Yang (1979). They showed that under anaerobic conditions, when ethylene synthesis is inhibited, ^{14}C from methionine accumulated in a compound which turned out to be a key intermediate. This compound, identified as ACC (1-amino-cyclopropane-1-carboxylic acid), was shown to be converted into ethylene by plant tissues in the presence of oxygen (Yang, 1981). The biochemical pathway from methionine to ethylene involves the synthesis and breakdown of two intermediates, SAM (*S*-adenosylmethionine) and ACC. This is achieved in three separate reactions (Fig. 6.4). The main features of the process are that carbon from methionine is released as ethylene and the sulphur is recycled, via methyl thioribose, to regenerate methionine. Nearly all plant cells contain methionine and SAM, which are essential for a wide range of biochemical reactions, and only the last two steps in Fig. 6.4 (the synthesis and breakdown of ACC) are specific for ethylene production. The formation of ACC from SAM is catalysed by ACC synthase. This is a soluble enzyme with a molecular weight of about 55 000 (Yang, 1981; Acaster and Kende, 1983) which requires pyridoxal phosphate for activity. The ACC produced by this enzyme is broken down by an 'ethylene forming enzyme' which requires oxygen and utilizes water molecules and hydroxyl ions. Although the latter enzyme has not been fully characterized there is some evidence that it may be associated with the cell membrane (Yang, 1981).

Most plant cells, including those of unripe tomatoes, synthesize small amounts of ethylene all the time. The endogenous levels of ACC and ACC synthase are very low but, when the cells are supplied with ACC, they are capable of an increase in ethylene production (Yang, 1981). These observations suggest that in unripe fruit the biochemical pathway shown in Fig. 6.4 operates at a low level and is mainly limited by the activity of ACC synthase. The finding that there is a large increase in both ACC synthase activity and ACC concentration during tomato ripening, and that this is correlated with

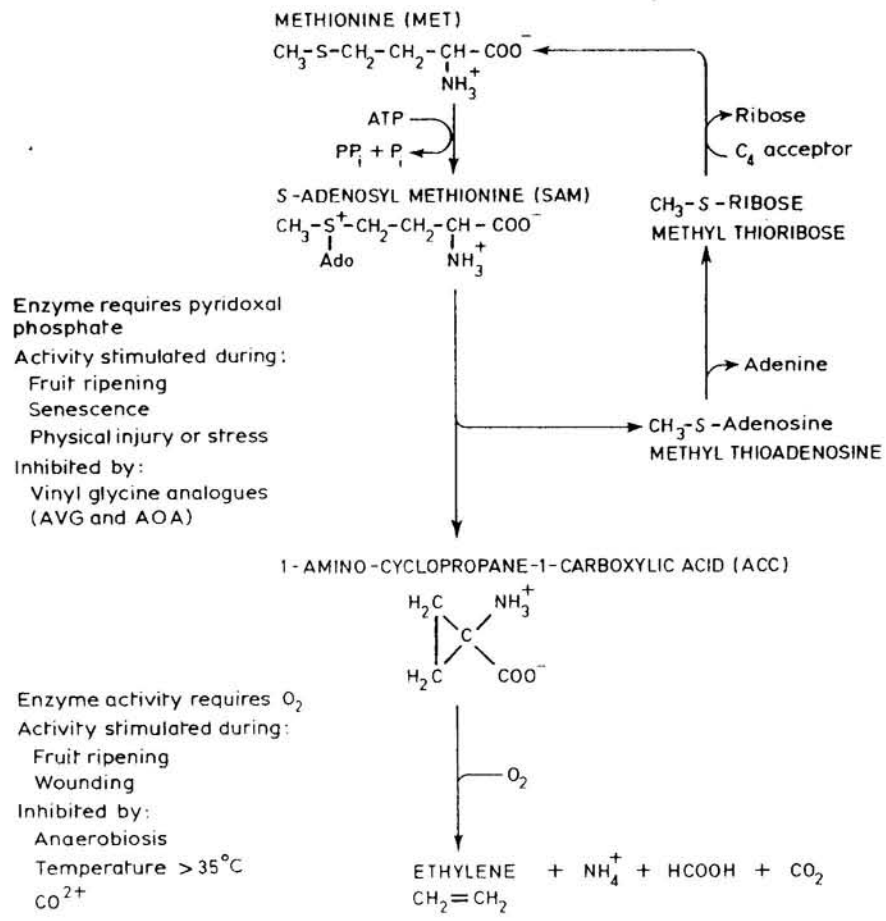


Figure 6.4 Ethylene biosynthesis.

increased ethylene synthesis (Hoffman and Yang, 1980; Su *et al.*, 1984) supports this interpretation. However, an increase in the activity of the 'ethylene forming enzyme' may also contribute to enhanced ethylene production during ripening (Yang, 1981, Yang and Hoffman, 1984).

Increased ethylene synthesis by tomatoes begins in cells in the vicinity of the seeds or in the columella when the fruit starts to ripen. The gas diffuses through the intercellular spaces and induces adjacent cells to begin ethylene production. This 'autocatalytic' action of ethylene in inducing its own synthesis appears to play a part in coordinating ripening behaviour of different cells within the fruit. The large increase in ethylene synthesis is important because it stimulates the cells to undergo other physiological and biochemical changes required for ripening. In this sense, control of ethylene synthesis is the key to

the control of ripening (see Section 6.2.6). Evidence that the natural mechanism involves the regulation of mRNA and enzyme synthesis is discussed below.

6.2.5 Changes in gene expression during ripening

(a) Ripening mutants

Many tomato mutations that affect a variety of aspects of ripening have been studied by plant breeders (Rick, 1980). Some of these are listed in Table 6.4. The occurrence of mutations that alter ripening behaviour shows that specific genes exist which determine separate facets of the process. Some of these mutations, such as *gf* and *r*, appear to affect single aspects of ripening, whereas others, including *rin*, *nor*, *Nr*, appear to regulate a number of processes simultaneously. The *rin* and *nor* mutations are particularly interesting: plants homozygous for either of these mutant alleles give rise to yellow or pale orange fruit respectively which produce very little of the red pigment lycopene. They soften only very slowly because they do not produce the major softening enzyme polygalacturonase (Tigchelaar, McGlasson and Buescher, 1978; Grierson, Tucker and Robertson, 1981; Tucker and Grierson, 1982). In addition, they have no respiratory climacteric and do not show increased ethylene synthesis during ripening. Such mutants have a twofold value. First, they may help to further our understanding of how normal ripening is controlled. Secondly, since the fruit remain perfectly sound for many months, they are valuable in breeding programmes designed to increase fruit shelf life (see Section 6.4.1).

(b) The involvement of RNA and protein synthesis

Numerous enzymes have been shown to change in activity during fruit ripening (Sacher, 1973) and some of these have clear physiological relevance. For example, an increase in ACC synthase is implicated in ethylene synthesis (see Section 6.2.4) and the appearance of polygalacturonase is correlated with cell-wall degradation. It is assumed that changes in other enzymes are involved in processes such as chlorophyll degradation, metabolism of starch, and pigment synthesis, but this has not been directly demonstrated. There are a number of possible ways in which these enzyme changes might be controlled, including regulation of transcriptional, translational, or post-translational events. Thus, alterations in the activity of existing inactive enzymes could be caused by changes in membrane permeability, availability of substrates and cofactors or altered location of the enzymes. Alternatively, they could be brought about by an increase in the number of enzyme molecules, involving changes in the rates of protein synthesis and degradation. This might involve the utilization of stored mRNAs or the appearance of new ones. There is no compelling reason to assume that any one type of control mechanism predominates: ripening is a

complex process and several different mechanisms are probably involved in its control (Rhodes, 1983). Nevertheless, there is now good evidence for the appearance of novel mRNAs at the onset of ripening which direct the synthesis of enzymes required to catalyse various ripening-related reactions.

Studies with several different climacteric fruits have provided evidence that an increase in protein synthesis occurs at the onset of ripening (Rhodes, 1980, 1983; Grierson, Tucker and Robertson, 1981; Grierson, 1983). In tomatoes there is a general stimulation of incorporation of amino acids into protein during ripening (De Swardt, Swanepoel and Duvenage, 1973). It is quite difficult to establish unequivocally which enzymes are being synthesized as a result of this metabolic activity because experimental verification, required for each enzyme, is difficult and time consuming. However, clear evidence has been presented for *de novo* synthesis of invertase and polygalacturonase (Iki *et al.*, 1978; Tucker and Grierson, 1982) and it seems probable that several other enzymes, including ACC synthase, will eventually be shown to belong to this group.

The synthesis of polygalacturonase is particularly important since it is responsible for almost all of the fruit softening in tomatoes. There are three isoenzyme forms which are structually related (Tucker, Robertson and Grierson, 1980, 1981; Ali and Brady, 1982; Crookes and Grierson, 1983). Polygalacturonase is absent from green fruit and accumulates in large quantities during ripening. The appearance of the enzymes is correlated with the onset of cell wall degradation (Fig. 6.1) and mutants deficient in polygalacturonase synthesis show little softening (Table 6.4). The purified tomato enzymes have been shown to degrade isolated cell walls *in vitro* (Themmen, Tucker and Grierson, 1982) and the walls of green-fruit cells *in situ* (Crookes and Grierson, 1983). Natural ethylene synthesis begins before polygalacturonase appears and exogenous ethylene causes the accumulation of the enzyme in mature fruit (Fig. 6.5). Studies involving enzyme purification and utilization of specific antibodies raised against polygalacturonase show that the enzyme activity increases in parallel with the number of enzyme molecules, thus confirming that it is synthesized *de novo* and is not simply activated during ripening (Tucker and Grierson, 1982).

The synthesis of at least some of the ripening enzymes involves changes in RNA metabolism. De Swardt, Swanepoel and Duvenage (1973) showed that there is a stimulation of incorporation of radioactive precursors into RNA during tomato ripening and Rattanapanone, Grierson and Stein (1977) demonstrated that this was associated with the production of high-molecular-weight ribosomal RNA, poly(A)-containing mRNA and soluble RNA. Analysis of the products of *in vitro* translation provided the first evidence for changes in mRNA during ripening (Rattanapanone, Speirs and Grierson, 1978). Further investigation showed that several abundant mRNAs in green fruit disappear and several new mRNAs appear as ripening begins (Speirs *et al.*, 1984; Grierson *et al.*, 1985b). These new mRNAs are associated with

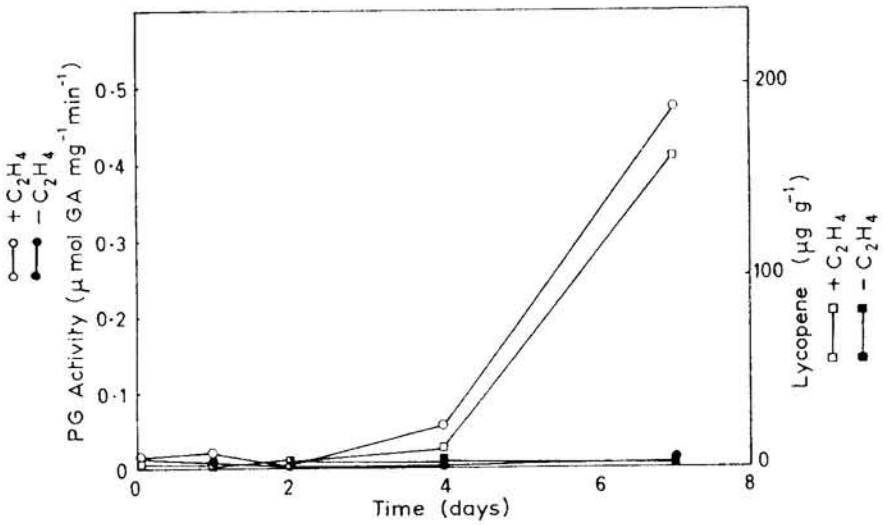


Figure 6.5 Effect of ethylene on synthesis of lycopene and polygalacturonase. (Redrawn from Grierson and Tucker, 1983.)

polyribosomes, which increase in relative abundance during ripening. Recently it has been shown that the mRNA coding for polygalacturonase is one of those that appears during ripening (Grierson *et al.*, 1985b and 1986) but the function of the others is not known. It is probable that they encode other enzymes important for the ripening process.

6.2.6 Coordination and regulation of ripening reactions

(a) Ripening involves a series of separate reactions

Normal ripening involves a series of apparently unrelated biochemical reactions that are switched on and coordinated during the climacteric period. The separate identity of these changes is manifested in a variety of ways. First, they occur in separate cell compartments such as the nucleus, cytoplasm, chloroplast and cell wall (Fig. 6.1). Secondly, there are specific mutations that affect one or a few ripening changes without influencing others (Table 6.4). Thirdly, controlled atmosphere storage (see Section 6.5.1) allows changes like starch degradation to continue while other aspects of ripening are prevented (Jeffery *et al.*, 1984). Fourthly, treatment with gibberellic acid delays chlorophyll loss but has no effect on either respiration or ethylene production (Dostal and Leopold, 1967). All these observations tend to support the view that ripening is made up of a variety of distinct processes and yet our experience is that they all tend to occur at the same time. It therefore seems reasonable to conclude that some factor in the fruit is responsible for the initiation and coordination of these changes.

(b) The significance of the respiratory climacteric

Since one of the first signs of ripening is the beginning of the respiratory climacteric, it would be reasonable to consider that this might promote ripening by providing abundant energy to drive the many biosynthetic reactions involved. However, an increase in respiration is clearly not essential in order for ripening to take place since non-climacteric fruit ripen perfectly well without one (Biale and Young, 1981). Furthermore, the application of the protein synthesis inhibitor cycloheximide to pears and bananas has no effect on the respiration rise but does inhibit some other aspects of ripening (Frenkel, Klein and Dilley, 1968; McGlasson *et al.*, 1971). This suggests that other factors, apart from increased respiration, play an important part in ripening.

In a number of climacteric fruits, including tomatoes, the increase in respiration has been shown to be preceded by a rise in ethylene production (see Section 6.2.3). Furthermore, exposure of many plant tissues to ethylene, including leaves, storage roots, flowers and non-climacteric fruit, has been shown to induce a rapid respiration rise which persists for the period of exposure to ethylene (Solomos, 1983). This suggests that the rise in endogenous ethylene production in climacteric fruit may actually cause the respiration increase. This is significant, in view of the observations that ethylene also accelerates the accumulation of ripening-related mRNAs (Grierson *et al.*, 1985a and 1986) and the synthesis of polygalacturonase (Grierson and Tucker, 1983).

(c) Ethylene and other factors

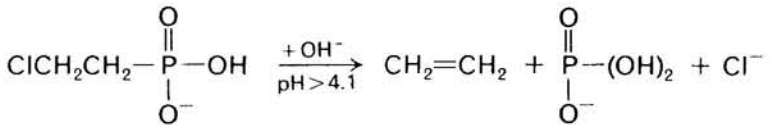
The mechanism whereby ethylene stimulates respiration, mRNA accumulation and enzyme synthesis is unknown. It is possible that it dissolves in one or more of the membranes of the plant cell and affects permeability or transport of small molecules which in turn stimulate important regulatory processes. However, there is also evidence for specific ethylene binding sites or receptors, which, in the presence of ethylene are thought to stimulate a coupling reaction that alters the activity of biochemical pathways required for ripening. This may also stimulate mRNA and protein synthesis. Ethylene binding sites have been isolated and partially characterized from several plant tissues, including tomato fruit. Some of these receptors may have an affinity for silver as well as ethylene. Significantly, low concentrations of silver inhibit ripening, presumably by competing with the binding of ethylene by these receptors.

There are several objections to the proposal that ethylene is the sole initiator of ripening, however. First, there is evidence that certain compounds in the fruit, which perhaps come from the parent plant, delay ripening by decreasing sensitivity to ethylene. Thus, the threshold level of ethylene required to induce ripening of mature-green tomatoes is lower in detached fruit compared to those remaining in the plant (Sawamura, Knegt and Bruinsma, 1978). Secondly, immature tomatoes do not ripen readily in response to added ethylene.

Thirdly, if ethylene is the initiator of ripening, and it promotes its own synthesis, it is difficult to see how ripening would ever begin, unless one postulates the involvement of at least one additional factor. Furthermore, the *rin* tomato, which ripens incompletely, does not synthesize ethylene and has no respiratory climacteric (Table 6.4). When these fruit are placed in ethylene a respiratory climacteric is induced but the fruit still do not ripen normally. This supports the suggestion that ethylene may cause the climacteric but also indicates that some other factors in addition to ethylene are necessary in order for ripening to proceed.

One or more of the other naturally occurring groups of plant growth regulators (i.e. auxins, gibberellins, cytokinins, or inhibitors such as abscisic acid) could influence ripening, either by preventing ethylene synthesis, by antagonizing the stimulatory effect of ethylene, or by controlling other important reactions. There is evidence that applications of gibberellins and cytokinins can sometimes delay aspects of ripening whereas abscisic acid can accelerate it (McGlasson, Wade and Adato, 1978). However, although there are characteristic changes in growth regulators during ripening (McGlasson, Wade and Adato, 1978; McGlasson and Franklin, 1979) none of these has clearly been shown to be involved in the natural control of the process.

The manipulation of ripening is of obvious commercial interest. However, it is easier to stimulate ripening than to retard it. The best method for promoting ripening is to treat harvested mature-green fruit with ethylene gas or to spray plants before harvest with the synthetic ethylene-generating compound ethephon (2-chloroethanesulphonic acid, also called ethrel). This chemical is taken up by the plant and at pH 4.1 and above (a condition that is met in the cytoplasm) it breaks down to produce ethylene



This stimulates the ripening of mature fruit. Generally ethephon is applied several days before mechanical harvest of field-grown tomatoes or to end-of-season glasshouse crops. However, as noted above, immature fruit do not ripen readily in response to ethylene. The application of ACC (Fig. 6.4) also stimulates ethylene production but it is not an economic alternative to ethephon.

Treatments that reduce the rate of metabolism or interfere with ethylene synthesis or action can retard or inhibit ripening. Thus, reducing the temperature slows the rate of ripening. However, this may prevent the full development of quality attributes such as colour and flavour, and below 12.5 °C chilling injury may result (see Section 6.5.2). Reduced temperature in conjunction with controlled atmosphere (e.g. 90% N_2, 5% CO_2, 5% O_2) has also been used to retard ripening of mature-green tomato fruit but the

final quality may not be high (see Section 6.5.1 and 6.5.2). Under these conditions the synthesis of ethylene is very significantly reduced. It is noteworthy in this respect that the final step in the ethylene biosynthetic pathway requires O_2 (Fig. 6.4). Vinyl glycine analogues such as AVG and AOA (inhibitors of ethylene biosynthesis, Fig. 6.4) have been used to retard ripening experimentally and silver ions infiltrated into mature-green fruit also inhibit ripening (Hobson *et al.*, 1984), presumably by blocking the ethylene receptors (see above). However, silver and vinyl glycine analogues are toxic and are of no commercial value. Thus, unfortunately, there is no totally satisfactory method for prolonged storage of fresh tomatoes that maintains high fruit quality. The best approach to this problem is probably by conventional breeding or genetic engineering. So far, the use of non-ripening mutants has not produced dramatic results (see Section 6.4.1).

The picture that emerges from the foregoing observations is that fruit have an underlying programme of development that is under genetic control. The respiratory climacteric may fuel ripening but does not initiate it. Ethylene, which is produced naturally, stimulates ripening but other substances in the fruit may also be involved in controlling it. Eventually the cells of mature-green fruit initiate a complex series of macromolecular changes which culminate in the catalysis of ripening events. Coordination of ripening behaviour involves communication both between cell compartments and from cell to cell. We do not understand in detail how ethylene works but it appears to act as an accelerator and coordinator of ripening. Among other things, it stimulates mRNA and enzyme production and promotes its own synthesis.

Thus, ripening involves an intricate and delicately balanced series of biosynthetic as well as degradative reactions, including the expression of ripening genes, which continue right up until the moment of consumption. This view contrasts sharply with the earlier idea that ripening was caused by a breakdown of tissue organization leading to cell degeneration. The quality of the final product can vary substantially, according to variety, disease and environmental conditions, and production, harvesting and handling procedures. Ways in which these factors influence quality are considered in the following sections.

6.3 QUALITY COMPONENTS AND EVALUATION PROCEDURES

Quality is established by all of the characteristics and attributes that are involved in satisfying the demands, needs and expectations of the person making the judgment. Thus producers are concerned that their tomatoes have good appearance and few visual defects. For them, a useful tomato cultivar must also score highly on yield, disease resistance, ease of harvest, and shipping quality. Quality of appearance, firmness, ripening behaviour, and shelf-life are the factors most important to receivers and market distributors. Consumers on

the other hand, consider good quality tomatoes as those that look good, are firm, and offer good flavour and nutritive value. Although consumers buy tomatoes on the basis of appearance and firmness, their satisfaction and repeat purchases, however, are dependent upon good eating quality.

Various components of quality considered in this section are used in tomato fruit evaluation in relation to specifications for grades and standards, selections in breeding programmes, and evaluation of fruit responses to various environmental factors and post-harvest treatments. The relative importance of each of these quality attributes depends on intended use (fresh or processed) and consumer preferences and all are affected by genotype, environment and post-harvest handling (see Sections 6.4.1, 6.5.1).

6.3.1 Appearance

Factors affecting appearance are colour, size, shape, defects and decay. The external colour of tomatoes is the result of both flesh and skin pigmentation. A pink tomato has a colourless skin and red flesh whereas a red tomato has a yellow skin and red flesh. There are tomato genotypes which have pink–purple, orange, dark yellow, light yellow, yellow with pink end, and other colours. However, most consumers prefer the deep, uniform red-coloured tomatoes. Since colour is an indicator of tomato ripeness, several subjective rating scales and colour charts have been developed for classifying ripeness stages of tomatoes. For example, the six classes of tomato ripeness included in the US Standards (USDA, 1976) are shown in Table 6.5. Objective methods of tomato colour evaluation include light reflectance measurement (Kader and Morris, 1978) and light transmittance techniques (Worthington, 1974; Chen and Studer, 1977). Determination of pigment content (e.g. chlorophyll, lycopene, β-carotene) can also be used to indicate tomato colour changes. Such determinations can be done non-destructively by light absorbance techniques (Watada *et al.*, 1976b).

TABLE 6.5 *Ripeness classes of tomatoes*

Score	Class	Description*
1	Green	Entirely light- to dark-green, but mature
2	Breaker	First appearance of external pink, red or tannish-yellow colour; not more than 10%
3	Turning	Over 10% but not more than 30% red, pink or tannish-yellow
4	Pink	Over 30% but not more than 60% pinkish or red
5	Light-red	Over 60% but not more than 90% red
6	Red	Over 90% red; desirable table ripeness

* All percentages refer to both colour distribution and intensity.

Preference for a given size of tomatoes varies among consumers and depends, to some extent, on the intended use of the fruits. The range of fruit sizes varies among cultivars. Within each cultivar when tomatoes are picked green, the smaller fruits are likely to be more immature. Thus, ripening and ethylene production rates are highly correlated with fruit size. However, if fruits are harvested at the breaker stage or more advanced stages of ripeness, no effect of size is noticeable on the ripening rate or composition and flavour at the table-ripe stage.

Tomato cultivars differ greatly in fruit shape and may be spherical, oblate, elongated or pear-like. In addition to these descriptive terms for shapes, the ratio of polar diameter to equatorial diameter and the ratio of maximum and minimum equatorial diameters can be used as indexes of shape. Whereas fruit shape has no direct effect on flavour or textural quality of tomatoes, it may have an indirect effect because of the internal fruit structure (pericarp/locular material ratio) associated with a given shape (see Section 6.3.3). An angular shape is undesirable because it reflects immaturity or puffiness. Shape defects are usually related to poor pollination and irregular development of some locules. Such misshapen or rough, ridged fruits are considered defective and are usually discarded.

The appearance of tomatoes is greatly influenced by the presence and magnitude of defects. Minor blemishes that would not detract from eating quality are acceptable, but more serious defects can influence appearance, firmness, shrivelling, and susceptibility to decay. Defects originating before harvest

TABLE 6.6 *Rating scale for internal tissue damage due to bruising*

Score	Degree of severity
1	*None*, no visible internal damage
2	*Slight*, one or more locules showing disorganized gel
3	*Moderate*, <5% of exposed tissue appears water soaked; <30% of locular gel is disorganized, shrunken or stringy
4	*Severe*, 5 to 20% of exposed tissue appears water soaked; 30 to 60% of locular gel is disorganized, shrunken or stringy
5	*Extreme*, >20% of exposed tissue appears soaked; >60% of locular gel is disorganized, shrunken or stringy

TABLE 6.7 *Rating scale for overall visual quality*

9	*Excellent*, essentially no symptoms of deterioration
7	*Good*, minor symptoms of deterioration, not objectionable
5	*Fair*, deterioration evident, but not serious
3	*Poor*, serious deterioration, limit of saleability
1	*Extremely poor*, not usable

TABLE 6.8 *Rating scale for physical damage*

Score	Degree of severity
1	*None*, no symptoms of any physical injuries
2	*Slight*, minor symptoms of physical injury which would not affect retail price
3	*Moderate*, symptoms of physical injury are evident; retail price may be affected
4	*Severe*, serious physical injuries; not marketable without substantial price reduction
5	*Extreme*, unusable; no market value

include puffiness or boxiness, blossom-end rot or catfacing and other scars, radial and concentric growth cracks, insect and bird damage, sunscald, excessive softening, and irregular ripening. Since no objective methods are available for evaluating defects, subjective rating scales are used. For general visual quality, other than colour, a hedonic scale of 1 to 9 (Table 6.7) is a convenient and useful tool for grading. Scores are usually given to individual fruits so as to have a measure of variability within the sample.

Physical damage can occur during harvest and post-harvest handling steps. It is not only unsightly, but also increases moisture loss and decay, and can result in less desirable flavour (MacLeod, Kader and Morris, 1976a, b). Subjective scoring systems can be used to describe the severity of physical damage (Tables 6.6, 6.7 and 6.8) and internal tissue damage due to bruising.

The presence of decay is a very serious defect which renders tomatoes unmarketable. This factor is considered in more detail in Section 6.6.

6.3.2 Firmness

After visual appearance, the most important factor in tomato quality is firmness which is closely associated with ripeness stage. Most consumers prefer firm fruits which do not lose too much juice when sliced, and which do not have tough skin. Firmness affects susceptibility of tomatoes to physical damage and consequently their shipping ability. The textural quality of tomatoes is influenced by skin toughness, flesh firmness, and internal fruit structure (pericarp/locular material ratio) which vary greatly among cultivars. The production of the cell wall solubilizing enzyme polygalacturanase during ripening plays a significant role in texture changes (see Section 6.2.1).

Sensory evaluation of textural quality involving both finger feel, mouth feel and slicing characteristics are all related to firmness. Objective evaluation methods for tomato firmness can be destructive or non-destructive. Destructive methods measure tissue resistance to force of penetration (fruit firmness testers, penetrometers), shearing (shear press), cutting, compression, or their combinations. The Instron Universal Testing Machine can be used to

measure any of these parameters. Instruments for non-destructive determination of fruit firmness measure resistance to compression (deformation) force applied at a single or multipoints on the fruit (Stenvers, Rudolphij and Bruinsma, 1973; Kader, Morris and Chen, 1978b).

6.3.3 Flavour

Tomato flavour involves the perception of the taster as influenced by the aromas of many chemical constituents. Sugars, acids and their interactions are important to sweetness, sourness, and overall flavour intensity in tomatoes (DeBruyn, Garretsen and Kooistra, 1971; Stevens *et al.*, 1977b). Fructose and citric acid are more important to sweetness and sourness than glucose and malic acid, respectively. High sugars and relatively high acids are required for best flavour. High acids and low sugars will produce a tart tomato while high sugars and low acids will result in a bland taste. When both sugars and acids are low, the result is a tastless, insipid tomato.

The pericarp portion of tomato fruit contains more reducing sugars and less organic acids than the locular portion. Thus, cultivars with a large locular portion and with high concentrations of acids and sugars have better flavour than those with a small locular portion (Stevens, Kader and Albright-Holton, 1977) (Fig. 6.6).

Differences in amino acid concentrations associated with fruit ripeness when picked do not appear to be directly related to flavour differences (Kader *et al.*, 1978c). The possible contribution of other constituents, such as minerals, ascorbic acid and alkaloids, has not been investigated.

Volatile compounds are important to not only the aroma but also the overall flavour of tomatoes. No single volatile compound or a small number of volatiles appear to be responsible for the characteristic aroma of fresh tomatoes (Buttery, *et al.*, 1971). Dirinck, *et al.*, (1976) found panel evaluations of aroma quality to be related to the concentrations of n-hexanal, trans-2-hexenal, cis-3-hexen-1-ol, 2-isobutylthiazole, and some unidentified C_{12}–C_{16} volatile compounds. Of more than 200 peaks on the gas chromatograph (GC), Hayase, Chung and Kato (1984) identified 130 compounds. Hexanal, trans-2-hexenal, 2-isobutylthiazole, 2-methyl-2-hepten-6-one, geranylacetone, and farnesylacetone, were identified by the GC-sniff method as those volatiles that are important contributors to fresh tomato aroma. Concentration of these compounds increased with ripening.

The 'off-flavour' character found in tomatoes of certain cultivars when picked green and ripened off the plant appears to be related to higher concentration of some volatiles, especially 2-methyl-1-butanal which has a flavour threshold concentration of 0.1 to 1 ppm.

There is a relationship between tomato fruit colour and its volatile composition, especially those volatile compounds which are formed by

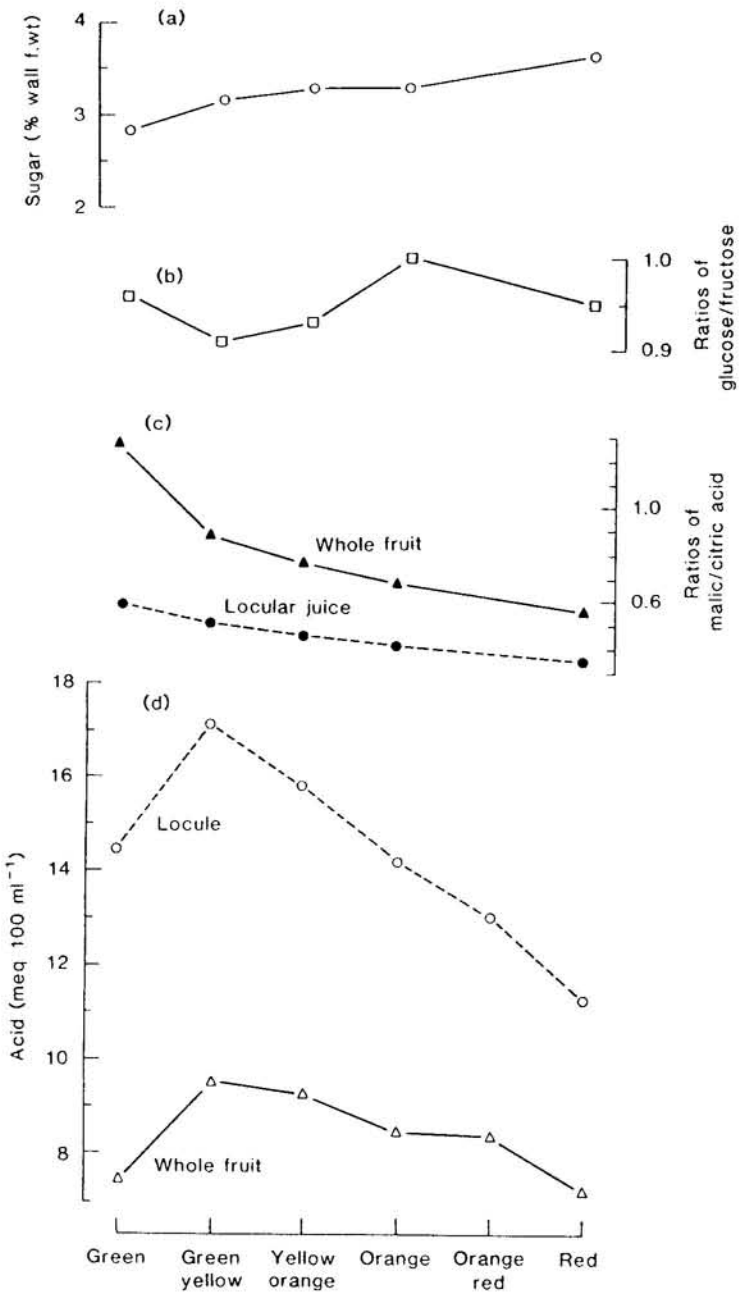


Figure 6.6 Changes in (a) sugar content, (b) ratio of glucose to fructose, (c) ratio of malic acid to citric acid and (d) acidity in fruit tissue during tomato fruit ripening. (After Davies and Hobson, 1981.)

oxidation of carotenoids. High β-carotene cultivars (e.g. 'Caro Red') and high δ-carotene cultivars (e.g. 'Gold Jubilee') have a distinctly different volatile composition and flavour than red cultivars.

6.3.4 Nutritional value

Tomatoes are important sources of vitamins A and C, more because of the large amount consumed than their average content of these two vitamins (Table 6.3). A 100 g tomato can supply about 20% and 40% of the US recommended daily allowances of vitamins A and C, respectively, for adults. It is possible to select tomato genotypes that are rich in vitamins A and C. Cultivars with very high vitamin A content have been developed, but their orange colour limits consumer acceptance.

6.3.5 Safety

Safety factors include naturally occurring toxicants and contamination with chemical residues, heavy metals, and micro-organisms of public health significance. Tomatine (Table 6.1) is a steroidal glycoalkaloid which is found in all tomato genotypes (Davies and Hobson, 1981). Young developing tomato fruits accumulate tomatine, but as ripening begins, alkaloid degradation occurs and tomatine concentration declines to less than 0.04%, on a fresh weight basis. The LD_{50} value for tomatine is estimated to be 0.5 g per kg body weight. Thus, in ripe tomatoes tomatine does not pose a safety hazard.

6.4 PREHARVEST FACTORS INFLUENCING QUALITY

Many preharvest, harvest, and post-harvest factors influence the composition and quality of tomatoes. These include inherent (genetic) and environmental factors such as climatic conditions (temperature, light, pollutants) and cultural practices (soil type, nutrient and water supply, use of agricultural chemicals, harvesting method). Maturity stage at harvest (Table 6.5) and post-harvest handling procedures also affect tomato quality and its maintenance.

6.4.1 Genotype and environmental factors

Stevens, Kader and Albright (1979) found that differences among genotypes in sugars and acids are responsible for most of the differences in sweetness, sourness, and overall flavour intensity. They concluded that improved tomato flavour can be achieved via increased sugar and acid content. As mentioned earlier (Section 6.3.3), these are influenced by the ratio of pericarp to locule tissue and cultivars with large locules may have a better flavour. Light intensity and potassium content during the growing period also have a profound effect on sugars and acids. Although ripening itself can occur in the dark and is not

much influenced by light, sugar content is closely correlated with solar radiation during fruit growth and high irradiance leads to high concentrations of sugar in the fruit (Fig. 6.7). Furthermore, the acid concentration is related to potassium content, which can be modified by the application of nutrients (Fig. 6.8). Reduced soil moisture and salt stress increase sugar content in tomatoes while high nitrogen decreases it.

Fruit colour and firmness are also affected by environmental conditions (Davies and Hobson, 1981). Low temperatures tend to reduce lycopene synthesis (Koskitalo and Ormrod, 1972) and temperatures above about 30 °C can inhibit lycopene production altogether (Goodwin and Jamikorn, 1952; Tomes, 1963). This is not uncommon in field tomatoes and in poorly ventilated glasshouses. It is also important during fruit storage. Exposure of mature-green tomatoes to temperatures above 30 °C can result in irregular ripening (yellow

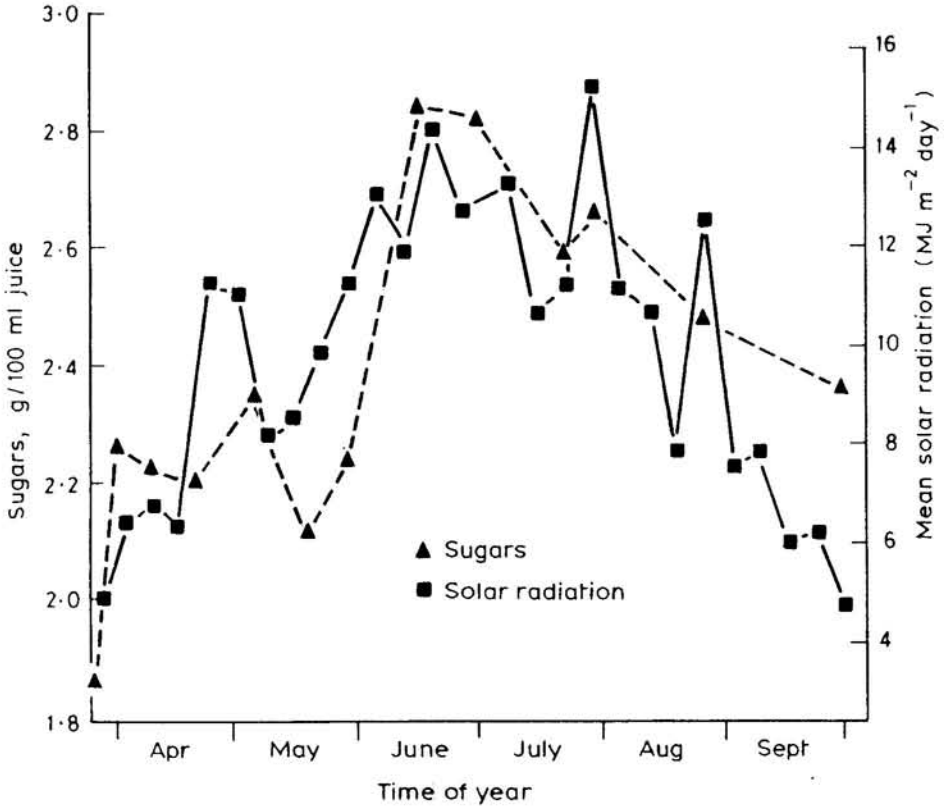


Figure 6.7 Seasonal trends in the sugar content of the fruit juices of tomato (cv. 'Grenadier') together with integrated data for solar radiation. (After Winsor and Adams, 1976.)

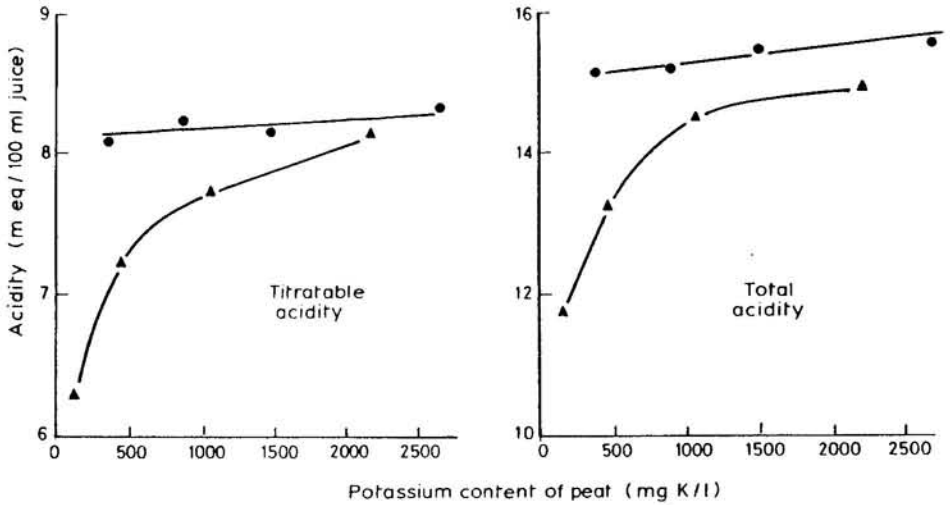


Figure 6.8 Relationship between the potassium content of the peat compost used to grow tomato plants and the titratable and total acidities of the fruit juices. Liquid feeds of 100 ppm K (▲), 300 ppm K (●). After Adams, Davies and Winsor, 1978.)

and greenish-yellow areas on red ripe fruits). This results from inhibition of lycopene synthesis at temperatures above 30 °C and inhibition of carotenoid synthesis at 40 °C or higher. Ethylene production and synthesis of the softening enzyme polygalacturonase are also inhibited at such temperatures. Tolerance to high temperature exposure varies among cultivars and with temperature and duration of exposure. For tolerant cultivars, high temperature injury can still be reversible after up to 2 days at 40 °C, 4 days at 35 °C, or 6 days at 30 °C if the tomatoes are transferred to optimum ripening temperatures (20 to 25 °C).

Genotypic variation in fruit firmness at harvest and softening pattern is an important factor in determining shipping ability and post-harvest-life of tomatoes. Cultivars that maintain good firmness beyond the table-ripe stage will permit fruit picking at more advanced ripeness stages which have better flavour.

While hybrid tomatoes heterozygous for the *rin* and *nor* non-ripening genes (Table 6.4) produce fruits with greatly extended shelf-life, they may have limitations in terms of flavour quality. In a comparison of *rin* and *nor* hybrids with their parents and standard cultivars, Strand, Morris and Heintz (1983) found the hybrids can have acceptable quality. Taste-life is extended by the *nor* gene, but not nearly as much as shelf-life, while the two *rin* hybrids tested did not have an extended taste life. The effects of both *rin* and *nor* on firmness, flavour, shelf-life, and taste-life are dependent however on the genetic background into which they are incorporated.

6.4.2 Physiological disorders

A brief description follows of the symptoms, causes, and possible control procedures for each of the important disorders which detract from tomato quality. For more details see McColloch, Cook and Wright (1968) and Hobson, Davies and Winsor (1977).

(a) Blossom-end rot

Symptoms begin as a small, water-soaked spot at or near the blossom scar of green tomatoes. As the spot enlarges the affected tissues dry out and become light brown to dark brown. Then, the lesion develops into a well-defined sunken spot with the affected tissues collapsed and leathery. Incidence and severity are influenced by calcium deficiency resulting from inadequate calcium supply in the soil and/or from growing conditions that reduce calcium translocation into the fruit. The incidence of blossom-end rot increases markedly when the concentration of calcium in the fruit falls below 0.08% (on a dry weight basis) while above 0.12% the disorder seldom occurs.

(b) Blotchy ripening

Blotchy or irregular ripening is characterized by green, greenish-yellow areas on apparently normal red fruit. It is usually confined to the outer walls, but in extreme cases radial walls can also be affected. Blotchy areas of fruit walls contain less organic acids, dry matter, total solids, starch, sugars, and nitrogenous compounds. However, the exact cause of blotchy ripening is still not known (Hobson, Davies and Winsor, 1977), although there is a relationship between the concentration of potassium and inorganic nitrogen in the soil and the even ripening of fruit (Fig. 6.9).

(c) Greenback

This is a separate disorder from blotchy ripening. The shoulders of the fruit near the calyx remain green for longer and ripen more slowly than the rest of the fruit. This is generally thought undesirable but in some countries it is actually preferred by consumers. Greenback can be abolished by incorporating the 'uniform ripening' gene (Table 6.4) into susceptible varieties.

(d) Solar injury

When tomatoes are exposed to direct sun radiation, fruit temperatures may increase by 10 °C or more above ambient air temperatures. If the fruit temperature exceeds 30 °C for long periods the affected part of the fruit becomes yellowish and remains so during ripening (solar yellowing see Section 6.4.1). When the temperature of an exposed fruit portion exceeds 40 °C, it becomes white and sunken (sunscald, sunburn, or sunscorch). Green fruits are more sensitive to solar injury than ripe fruits. This disorder can be reduced by using cultivars that have adequate foliage cover to shade the fruits.

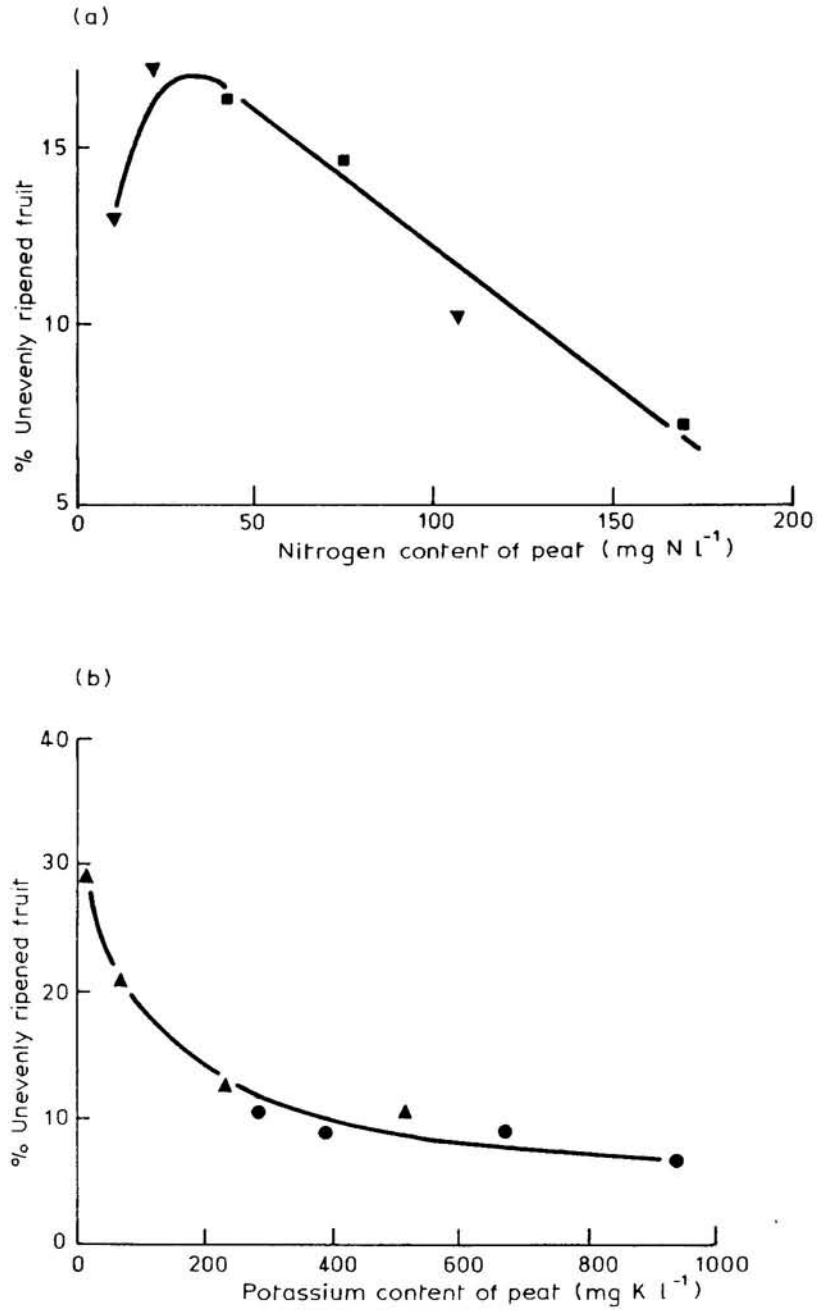


Figure 6.9 Relationship between nitrogen (a) and potassium (b) nutrition and uneven ripening. (After Adams, Davies and Winsor, 1978.)

(e) Cracking

Cracking and splitting tendency is genetically controlled and appears to be related to skin strength and stretching ability. Cracks may occur in circles around the stem scar (concentric cracking) or radiating from the stem scar (radial cracking). Cracking incidence is affected by soil moisture, rainfall, dew, and high temperatures. This disorder is not only unsightly but also increases susceptibility of affected fruits to pathogens and water loss. Use of cracking-resistant cultivars and avoidance of extreme fluctuations in water supply to the plants minimize losses due to this disorder.

(f) Puffiness

Puffiness (also known as hollowness or boxiness) refers to the existence of open cavities between the outer walls and the locular contents in one or more locules. Externally, puffy fruits appear slab-sided. Since puffy fruits are less dense than good fruits, separation by floatation in water is possible. The percentage of fruits affected is related to genotype and growing conditions which cause

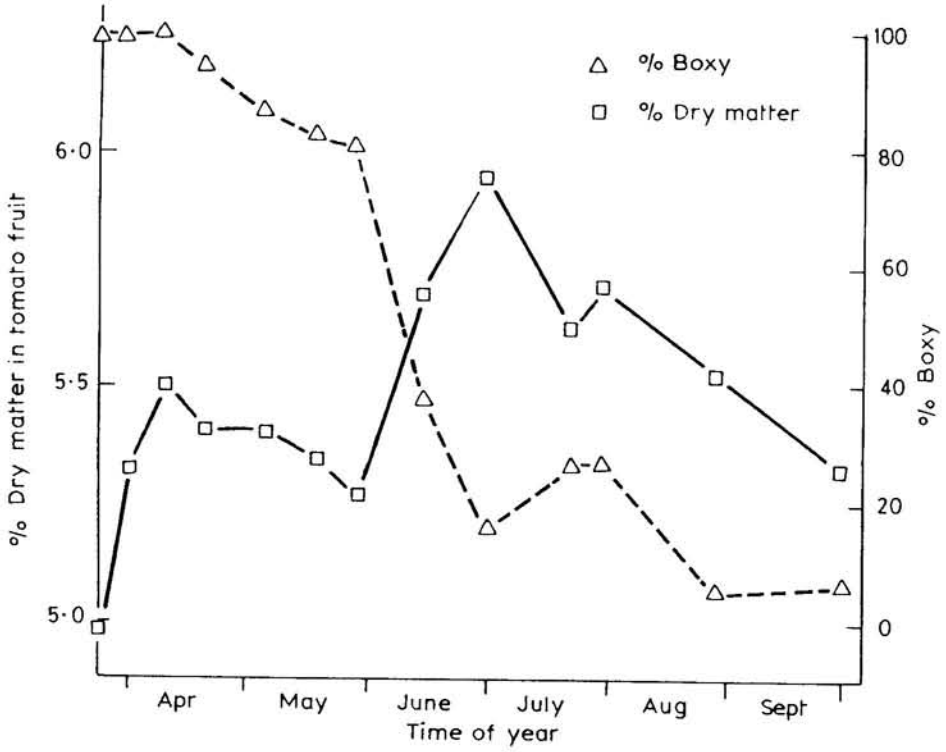


Figure 6.10 Seasonal trends in the proportion of boxy tomatoes and the dry matter content of fruit. (After Winsor and Adams, 1976.)

improper pollination, fertilization, or seed development. It is particularly important as a disorder in early-season glasshouse crops (Fig. 6.10).

(g) Gold fleck/pox syndrome

On immature and mature-green tomatoes, round, elongated or irregular dark green specks are noted over the entire fruit surface. These specks become golden yellow as the fruit ripens on or off the plant. In fruits ripened on the plant, the yellow specks may develop into necrotic tan coloured lesions (fruit pox). The first two stages of this disorder do not detract appreciably from the visual quality of tomatoes but fruit pox is unsightly and increases the chances for entry of pathogens into the fruit. Since susceptibility to this disorder appears to be genetically controlled, its importance can be reduced by avoiding susceptible cultivars (Ilker, Kader and Morris, 1977).

6.4.3 Harvesting factors

Harvesting method (hand vs mechanical) can influence the incidence and severity of physical injuries and the percentage of immature fruits. These factors, in turn, can adversely affect tomato quality.

Maturity at harvest is very important to composition and quality of tomatoes. This is especially a problem with tomatoes picked green since it is difficult to differentiate between mature- and immature-green fruits. Four maturity stages of green tomatoes are described in Table 6.9 on the basis of internal examination. Typical and advanced mature-green tomatoes will usually attain

TABLE 6.9 *Maturity classes of green tomatoes*

Maturity score	Class	Description based on internal examination	Average no. of days to reach the 'breaker' stage at 20 °C
1	Immature-green (IMG)	No jelly-like material in any of the locules; seeds are cut by a sharp knife upon slicing the fruit	>10
2	Partially mature-green (PMG)	Jelly-like material formed in at least one, but in less than all locules; seeds are well developed	>5 to 10
3	Typical mature-green (TMG)	Jelly-like matrix in all locules; seeds are not cut by a sharp knife upon slicing the fruit	>1 to 5
4	Advanced mature-green (AMG)	Typical mature-green with some internal red colouration	1

a much better flavour at the table-ripe stage than those picked at the immature- or partially-mature stages. The latter are also much more susceptible to physical injuries and water loss because of their thin cuticle.

Ripeness stage at harvest affects fruit composition and quality. Tomatoes accumulate acids, sugars, and ascorbic acid during ripening on the vine (Sakiyama and Stevens, 1976; Betancourt, Stevens and Kader, 1977). Tomatoes ripened on the plant have better flavour and overall quality than room-ripened tomatoes (Bisogni, Armbruster and Brecht, 1976). Tomatoes picked at less than table-ripe and ripened at 20 °C were evaluated by panelists as being less sweet, more sour, less 'tomato-like' and having more 'off-flavour' than those picked at the table-ripe stage. The magnitude of these differences varied greatly among cultivars (Kader *et al.*, 1977). Watada and Aulenbach (1979) found that the intensities of sensory attributes were similar in table-ripe tomatoes harvested at the mature-green and breaker stages. Intensities of sweetness, saltiness, and 'fruity-floral' flavour were higher in tomatoes harvested at the table-ripe stage than at earlier stages.

6.5 POST-HARVEST FACTORS INFLUENCING QUALITY

Post-harvest losses result from various physiological, physical, or pathological disorders. The magnitude of these losses varies greatly with production area, handling and distribution system, and duration between harvest and consumption. In a survey of tomato losses at the retail and consumer levels in the New York area, Ceponis and Butterfield (1979) found that losses ranged from 11.4 to 14.2%. The major causes of losses were diseases; principally *Alternaria*, *Rhizopus*, and grey mould rots and bacterial soft rot (see Section 6.6); followed by physical injuries and physiological disorders.

6.5.1 Fruit handling

Post-harvest losses in quality are related to immaturity at harvest, inadequate initial quality control, incidence and severity of physical damage, exposure to improper temperatures, and delays between harvest and consumption. Shortening the time from harvest to consumption can minimize loss of the characteristic aroma and development of off-flavours. Tomatoes subjected to bruising usually have more off-flavour and less 'tomato-like' flavour than those without physical damage (Kader *et al.*, 1978c).

Ripening rate can be reduced by cooling the fruit. The lowest temperature at which ripening, with good colour and flavour development, occurs is 12.8 °C. Above 30 °C, lycopene formation (red colour) is inhibited and the tomatoes turn yellow (Section 6.4.1). The optimum ripening temperature in terms of quality maintenance is 20 °C.

Ethylene treatment to accelerate ripening of green tomatoes at 20 °C results in higher ascorbic acid content at the table-ripe stage when compared with

fruits ripened without added ethylene (Watada, Aulenbach and Worthington, 1976; Kader *et al.*, 1978b).

Using a low-O_2 atmosphere to retard tomato ripening has less effect on flavour than ripeness stage at harvest. If O_2 concentration is reduced to 2% or lower increased off-flavours and uneven colour development will result. Controlled atmospheres reduce the loss of chlorophyll and the synthesis of lycopene, carotenoids and xanthophylls (Goodenough and Thomas, 1980).

Carbon monoxide at 5 to 10% in combination with 4% O_2 reduces post-harvest decay incidence and severity without influencing flavour of tomatoes (Kader *et al.*, 1978a). Mature-green tomatoes can be stored at 12.8 °C for up to 7 weeks under 4% O_2, 2% CO_2, and 5% CO and still retain an adequate marketing life at acceptable quality for 1 to 2 weeks at 20 °C. However, the flavour of these tomatoes is likely to be inferior to that of mature-green tomatoes ripened soon after harvest.

6.5.2 Physiological disorders

(a) Temperature injury

Exposure of tomatoes to temperatures below their freezing point (average = −1 °C) results in freezing injury. Symptoms include a water-soaked appearance, softening, and drying of the gelatinous locular materials. Temperatures above 30 °C are equally deleterious to ripening fruit (see Section 6.4.1).

Symptoms of chilling injury, which occur well above the freezing temperature, include failure of the fruit to ripen, irregular ripening, premature softening, surface pitting, browning of the seeds, and increased decay (especially *Alternaria* rot). Chilling injury occurs when tomaotes are exposed to temperatures above their freezing point and below 12.5 °C for a period of time depending on temperature; the lower the temperature the shorter the duration necessary to induce chilling injury. Symptoms become more noticeable after transfer to ripening temperatures. Ripe tomatoes are less susceptible to chilling injury than green tomatoes.

Exposure to chilling temperatures adversely affects tomato flavour (increased acidity, loss of characteristic aroma) before other symptoms of chilling become apparent. Temperature also influences softening and colour uniformity of tomatoes. Adequate air exchange in storage and ripening rooms is important in reducing off-flavours.

(b) Elevated CO_2 and reduced O_2 injury

Subjecting mature-green tomatoes to CO_2 levels above 3–5% for a duration dependent on cultivar can result in CO_2 injury. Symptoms include retarded and irregular ripening, premature softening, and appearance of brown spots at the blossom-end.

Irregularly shaped brown areas develop that may be superficial or slightly sunken when mature-green tomatoes are exposed to less than 2% O_2.

6.5.3 Physical injuries

Physical injuries can occur throughout the handling system between field and consumer. There are several types of physical damage that occur on tomatoes including cuts and punctures, scuffs and abrasions. These injuries are unsightly, and result in increased water loss and susceptibility to decay. The affected areas may fail to develop normal red colour. Physical stress also stimulates CO_2 and C_2H_4 production rates by mature-green tomatoes (MacLeod, Kader and Morris, 1976a).

Fruit tumours (waxy blisters) are blister-like growths on the surface of mature-green tomatoes, which occur singly or in groups. The 'tumours' collapse and turn light brown as fruits ripen. They are caused by rubbing of tomatoes against each other or against rough surfaces.

Bruising results from impacts against other surfaces or by vibration during transport. External symptoms include tissue softening, water soaking, or cracked locular walls. Often bruise damage is not detected until the fruit is cut and internal tissue examined. Internal damage symptoms include water-soaked placental and wall tissue, whitish to greenish locular gel, and shrunken and disorganized gel.

Deformation is a localized, permanently flattened area resulting from a pressure on the tomato during post-harvest handling. Ripe fruits are more subject to bruising and deformation than less ripe tomatoes.

6.5.4 Pathological disorders

Most pathological disorders found during post-harvest handling of tomatoes originate in the field before harvest. Incidence and severity of these disorders are increased by physical injuries and chilling injury which make the fruits much more susceptible to decay. Thus, post-harvest decay problems can be reduced by effective preharvest disease control procedures and by use of proper sanitation procedures, careful handling to minimize physical injuries, and avoiding exposure to chilling temperatures. The major pathological disorders are described briefly together with some specific control measures. Further consideration of tomato disease is given in Chapter 11.

Black mould, caused by *Alternaria alternata*, occurs only on ripe tomatoes that have been physically damaged, chilled, and/or stored for long periods. The spores can be on the fruit surface at harvest but will not grow until the fruit ripens. Another form of this fungus (*Alternaria alternata* f. *lycopersici*) may infect and cause lesions on green fruit of susceptible cultivars. Careful sorting in the packinghouse to eliminate fruits with growth cracks, mechanical injuries, sunscald, or blossom-end rot; and avoiding chilling injury can greatly reduce the incidence of this disease.

Buckeye rot, caused by *Phytophthora* spp., appears as slightly water-soaked areas with distinct brown zonations. Usually infection occurs on fruits in contact with soil especially following irrigation or rain. Use of plastic mulch reduces the incidence of phytophthora rot. No post-harvest fungicides are very effective against this fungus.

On green fruits grey mould, caused by *Botrytis cinerea*, appears as white circles surrounding a green center (known as ghost spots). The affected areas become water-soaked, then greyish-green to greyish-brown lesions develop. Infection with *Botrytis* occurs before harvest when tomato fruit comes in contact with infected plant parts and rot can occur in the field or after harvest. Thus, preharvest treatments with recommended fungicides for *Botrytis* control are essential to reducing post-harvest decay problems. Post-harvest application of 'Botran' can provide good chemical control of this fungus.

Rhizopus rot is caused by *Rhizopus stolonifer* which attacks only physically injured tomatoes. It appears as large lesions that are soft and may have grey, coarse mycelium which is usually dotted with black and white spore masses. This fungus can be effectively controlled with 'Botran' treatments.

Soil rot, caused by *Rhizoctonia solani*, appears first as a brownish red spot on the fruit surface in contact with soil. Later stages show sunken, dark-brown spots. Use of plastic mulches or staking the plants can eliminate decay problems due to this fungus.

The fungus *Geotrichum candida*, which causes sour rot, attacks only physically injured fruits. On green fruits, sour rot lesions have a dull, greasy, water soaked to bleached appearance. Post-harvest application of sodium orthophenylphenate can control this fungus.

Symptoms of bacterial soft rot, caused by *Erwinia carotovora* and other species of bacteria, begin with the appearance of a slightly depressed, water-soaked spot on the fruit. The spot enlarges rapidly to affect much of the tomato and affected tissues quickly become very soft and watery. Infection begins in the field or packinghouse. It spreads from decayed fruits to adjacent fruits during post-harvest handling, especially at higher than optimum temperatures.

Infection of tomato fruits with viruses, such as tobacco mosaic virus (TMV), results in irregular ripening (yellowish patches on the surface and brownish spots beneath the surface). The weight of fruit produced per plant may also be reduced. Several genes have been identified that are associated with TMV resistance and TMV-resistant varieties are available.

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