# 2.16

# Biotechnological Approaches to Improve Nutritional Quality and Shelf Life of Fruits and Vegetables

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## 16.1 INTRODUCTION

Fruits and vegetables, which can be consumed as fresh and as processed products, are important ingredients of a healthy diet. They are valuable sources of vitamins, minerals, antioxidants, and fiber. The important quality factors of fruits and vegetables are their color, flavor, texture, and nutritive value. Consumers always prefer to buy fruits and vegetables of high quality. As used by the industry, quality is a concept involving measurable attributes: degree of purity, firmness, flavor, color, size, maturity, condition, or any other distinctive attributes or characteristics of the product (1). The qualities of the produce bought by the consumer are influenced by many factors, such as the cultivar, the environmental conditions affecting growth, cultural practices, exposure to pests and diseases, time of harvesting, and postharvest and storage conditions used. Today, with the advancement of technology in several areas, the only factor on which the grower has no control of is the environment of the field conditions. Heredity (the identity of the cultivar) plays a major role in determining the quality of fruits and vegetables, as evidenced by the various varietal differences in quality. Even though traditional crop breeding is still being used as one means of crop improvement, continuing advances in knowledge and technology have dramatically expanded the biotechnological tools available for genetic improvement and production of vegetables and fruits. The term biotechnology is broad, encompassing a wide range of disciplines in science. In this chapter, the focus will be on plant transformation, where genes are modified or transferred by molecular means, and the resulting improvements in fruit and vegetable quality. Because this chapter is focused on genetic engineering, we will be discussing only the parameters and mechanisms that affect nutritional quality and shelf life (other than pests and diseases), and which can be improved or modified by genetic engineering. The discussion will also be based on two important crops; potatoes and tomato.

## 16.2 POTATOES

Potatoes are ranked fourth in production of all agricultural commodities in the world and yield more dry matter and protein per hectare than the major cereal crops (2). They are consumed as fresh and processed products, and used as raw material for many industrial purposes such as starch extraction. Potato chips and french fries are two of the most popular processed potato products. The consumer preference for these products is influenced by the color and crispness of these products. The primary problem associated with potato processing is the nonenzymatic browning of the product that occurs under the high temperature conditions used during frying, when reducing sugar levels are high in the tissue, a phenomenon known as Maillard reaction (3). The reducing sugars, glucose and fructose, combine with the $\alpha$-amino groups of amino acids at the high temperatures used in frying operations, resulting in darker and more bitter flavored french fries and chips that are unacceptable to

the consumer. The ideal content of reducing sugars is 0.1% of the tuber fresh weight; 0.33% is the upper acceptable limit (4). A four year study was conducted to determine the compositional differences during low temperature storage between low sugar accumulating and high sugar accumulating cultivars in relation to potato chip processing quality (5). Pearson analysis of the above data showed that chip color was most closely correlated with reducing sugar concentration. Multiple regression analysis revealed that the relative contribution of each of the parameters studied, such as sucrose, reducing sugars, nitrogen, protein, ascorbic acid and dry matter content, varied greatly among cultivars and selections evaluated and from season to season (5).

### 16.2.1 Factors Affecting Accumulation of Reducing Sugars in Potatoes

The factors that affect the sweetening or breakdown of starch into sucrose and its component reducing sugars glucose and fructose are drought, excess nitrogen during growth, high temperature at harvest, handling, aging, identity of the cultivar, anaerobic conditions, and low temperature during post harvest storage (6).

### 16.2.2 Low Temperature Sweetening in Potatoes

Low temperature sweetening (LTS) in potato tubers is a phenomenon that occurs when tubers are stored at temperatures below 10°C in order to minimize respiration and sprouting. LTS results in the accumulation of starch breakdown products, primarily sucrose and the reducing sugars glucose and fructose (7), which cause Maillard browning during potato chip frying operations (3,8). Fry color of Russet Burbank and Shepody potatoes has been shown to be more closely associated with glucose concentration than with fructose, total reducing sugars, sucrose, or total sugars (9). In order to avoid Maillard browning, processing potatoes are generally stored at temperatures around 10°C; but at this storage temperature potato tubers will sprout. To prevent sprouting during storage, the processing tubers are treated with chemical sprout inhibitors. However, due to health and environmental concerns, there is increasing pressure to reduce the use of chemical sprout inhibitors. The only solution to avoid this problem is using cultivars that are resistant to LTS.

Low temperature storage of potato tubers has many advantages, such as control of sprout growth and senescent sweetening, reduction of physiological weight loss due to decreased respiration and losses associated with bacterial and fungal pathogens, and extended marketability. Low temperature storage has several advantages, but the main drawback associated with it is the accumulation of reducing sugars and the resulting browning of processed products such as chips and fries.

The mechanism responsible for the initiation and subsequent regulation of LTS in potato tubers has not been fully elucidated. Many theories have been proposed to explain LTS based on starch metabolism, sucrose metabolism, glycolysis and the oxidative pentose phosphate pathway (PPP), and mitochondrial respiration (10,11), as well as membrane instability, lipid peroxidation, and electrolyte leakage (12–15). It has been suggested that in mature, cold stored potato tubers, the glycolytic or respiratory capacity plays a key role in the ability of potatoes to regulate their sugar concentration (16). Although LTS has not been elucidated at the molecular level, many factors may play a role in it. For example, chilling may influence compartmentation and membrane permeability by altering the phase transition of lipids in the bilayer, resulting in the leakage of key ions such as inorganic phosphates. This can alter the activity and synthesis of key enzymes involved in the metabolic pathways, ultimately resulting in LTS (17).

Many theories have been postulated and documented to explain LTS at the level of carbohydrate metabolism in stored potato tubers (7,11,12,17,18). The mechanism is complex and may involve the interaction of several pathways of carbohydrate metabolism and the

genes that regulate these pathways. This discussion focuses on a theoretical model of the mechanisms involved in LTS based on information available about the roles of the major tuber carbohydrate metabolic pathways as well as changes in membrane stability (Figure 16.1).

### 16.2.3 Metabolism of Starch in Tuber

Starch is the major component in the main crop plants of the world, as well as an important raw material for many industrial processes. Potato tubers contain 60–80% starch, of which sugars represent only a small fraction (up to 3% on a dry weight basis) (19). There is evidence that the principal event in LTS is the cold induced synthesis of sucrose (7,12). The carbon needed for the synthesis of sucrose and reducing sugars for LTS is generally, but not always, provided by a net breakdown of starch. An increase in potato tuber sugar content occurs early during cold storage; over two to three months at storage temperatures of 1–3°C, tubers can convert as much as 30% of their starch content (20). In mature King Edward tubers stored at 2°C, the sugar content increased from 0.3 to 2.5% in three months, with the initial appearance of sucrose followed by reducing sugars (19). Coffin et al. (21) found that sucrose content increased within two days of 5°C storage for both mature and immature tubers of four cultivars, while fructose and glucose content increased more slowly. Pollock and ap Rees (22) reported an increase in both sucrose and reducing sugar content within 5 days in tubers stored at 2°C, and after 20 days storage, the sugar content was approximately

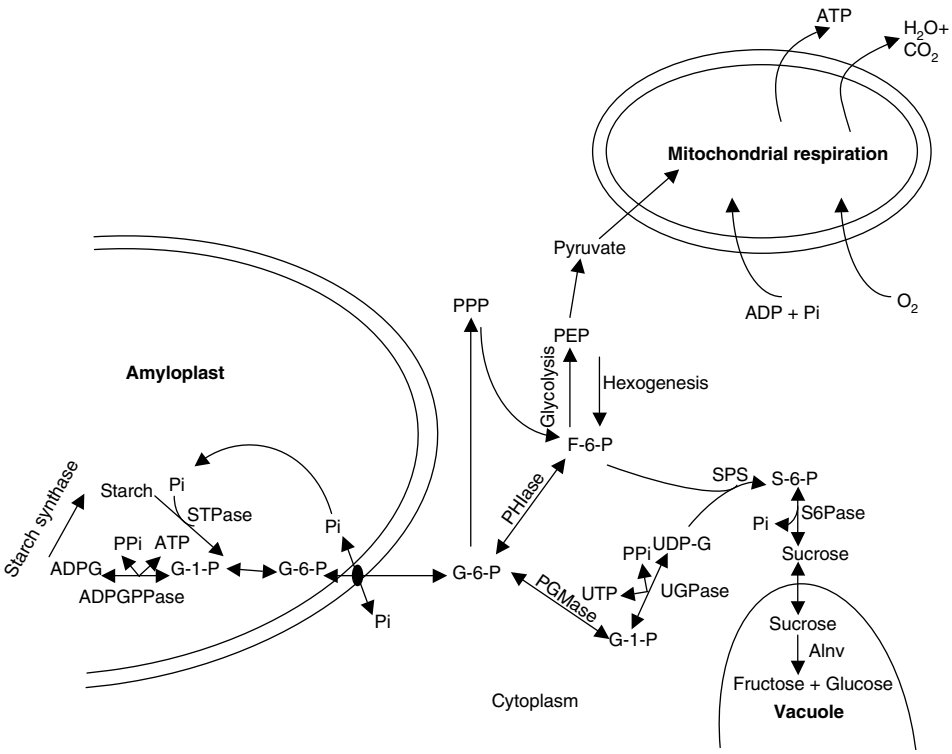


**Figure 16.1** Starch-sugar interconversion in potato tubers. ADPG, ADP-glucose; ADPGPPase, ADP-glucose pyrophosphorylase; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; STPase, starch phosphorylase; F-6-P, fructose 6-phosphatase, SPS, sucrose-6-phosphate synthase; S-6-P, sucrose-6-phosphate; S6Pase, sucrose-6-P phosphatase; Ainv, acid invertase; PPP, pentose phosphate pathway; PEP, phosphoenolpyruvate (adapted from Sowokinos, 2001)

six times greater than at day 0. The sweetening response of tubers to low temperatures is fairly consistent, but is influenced by cultivar, locality, and conditions prior to cold storage. Isherwood (19) related energy requirements to possible biosynthetic pathways and concluded that sucrose was formed from starch when potato tubers were moved from 10° to 2°C and that starch was reformed when tubers were moved back from 2° to 10°C, although different metabolic pathways were involved. Reconditioning of tubers is sometimes used to improve chipping quality by decreasing the level of reducing sugars (20). After cold storage, potatoes are reconditioned at 18°C where sugar content decreases and starch content increases as sugars are resynthesized to starch. However, the response to reconditioning is neither consistent nor completely restorative, and tends to be cultivar dependent.

Preconditioning treatment has been used to lessen chilling injury in chilling sensitive plants. Storage at 10°C prior to cold storage can acclimatize tubers and lessen the LTS effect (8). Katahdin tubers preconditioned at 15.5°C for one to four weeks before 0°C storage did not show a change in sugar accumulation patterns or respiration rates (23). The use of intermittent warming (15.5°C for one week following 0°C for three weeks) decreased sugar levels and respiration rates to levels lower than those of tubers stored continuously at 0, 1 and 4.5°C, although sugar levels were not low enough for desirable chipping potatoes.

#### *16.2.3.1 Starch Synthesis*

Starch is synthesized in plastids (amyloplasts) upon tuber initiation, and both the number of starch grains and the grain size increase during tuber growth (Figure 16.1, Figure 16.2). Starch consists of two types of glucose polymers, the highly branched amylopectin, and relatively unbranched amylose. Potato starch is comprised of 21–25% amylose and 75–79% amylopectin (24). Starch is synthesized from ADPglucose by the concerted action of ADPglucose pyrophosphorylase (ADPGPase), starch synthase, and the starch branching enzymes (25). Following the conversion of sucrose into hexose phosphates in the cytosol, glucose-6-phosphate is transported into the amyloplast where it is converted into glucose-1-phosphate. A study involving antisense inhibition of plastidial phosphoglucomutase supported the theory that carbon from the cytosol was imported into potato tuber amyloplasts in the form of glucose-6-phosphate (26). Glucose-1-phosphate is subsequently converted to ADPglucose by ADPGPase. The starch synthases catalyze the polymerization of the glucose monomers into α-1,4-glucans using ADPglucose as a substrate, while the starch branching enzyme catalyzes the formation of the α-1,6-linkages of amylopectin (25).

ADPGPase is often referred to as a rate-limiting step in starch synthesis (25). It is subjected to allosteric activation by 3-P-glycerate and inhibition by inorganic phosphate (27). Strategies to alter the starch metabolism in tubers by genetic manipulation of ADPGPase may be helpful in reducing the accumulation of reducing sugars during LTS. It has been reported that transgenic tubers with an 80–90% reduction in ADPGPase activity have reduced starch content relative to wild type tubers (28,29). The reduction in ADPGPase activity resulted in a major reduction of carbon flux, with increased flux to sucrose and decreased flux to starch. Stark et al. (30) have reported that hexose accumulation was greatly reduced in cold stored tubers with overexpression of the mutated ADPGPase gene, glgC16, from *E. coli*. The glgC16 gene produces a mutant form of ADPGPase that is less responsive to allosteric effectors. It has been suggested that the observed decrease in hexose concentration could be due to the higher starch biosynthetic capacity of the transgenic tubers. Lorberth et al. (31) developed transgenic potatoes with decreased levels of R1 protein, a starch granule bound protein capable of introducing phosphate into starch-like glucans. By reducing the activity of the R1 protein using antisense technology, the phosphate content of starch was reduced, resulting in a starch that

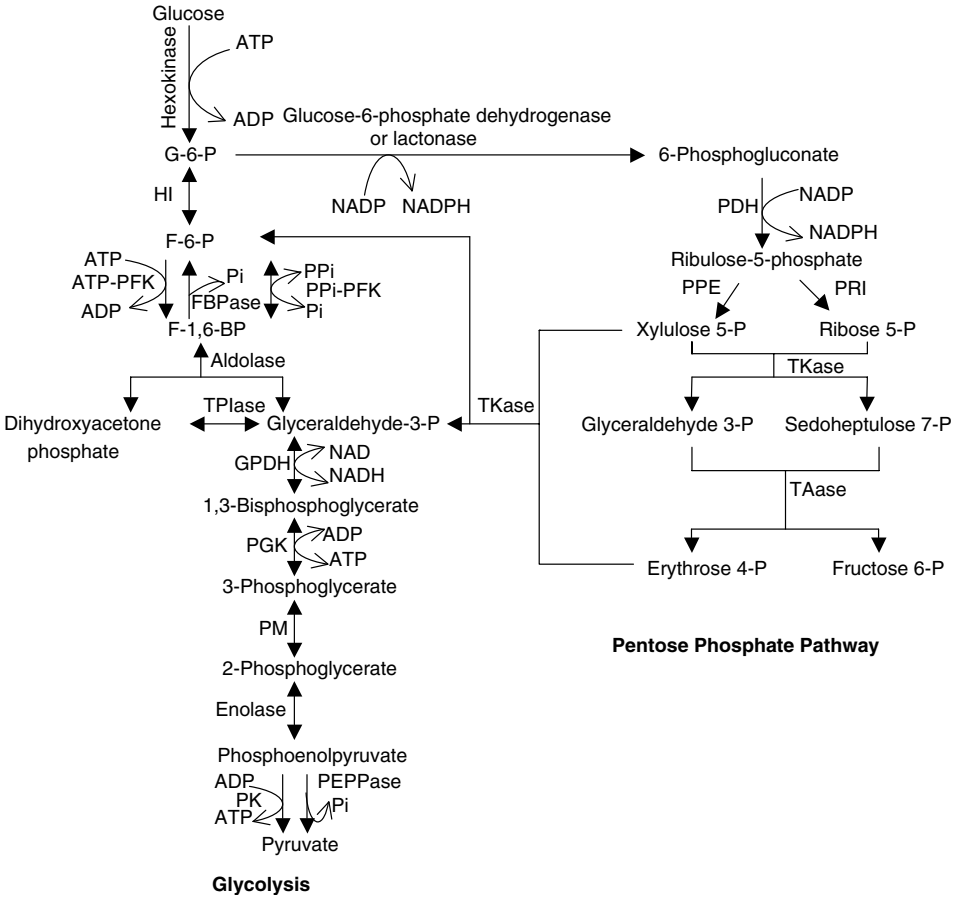


**Figure 16.2** Interactions between glycolysis and pentose phosphate pathway. G-6-P, glucsoe 6-phosphate, HI, hexose phosphate isomerase, F-6-P, fructose 6-phosphate, ATP-PFK, ATP-dependent phosphofructokinase, FBPase, fructose 1,6-bisphosphatase, PPi-PFK, PPi-dependent phosphofructose kinase, F 1,6-BP, fructose 1,6 bisphosphate, TPlase, triose phosphate isomerase; GPDH, glyceraldehyde 3--phoshate dehydrogenase; PGK, phosphoglycerate kinase; PM, phosphoglycero mutase; PK, pyruvate kinase; PDH, 6-phosphogluconate dehydrogenase; PPE, phosphopentose epimerase; PRi, phoshoribo-isomerase; Tkase, transketolase; Taase, transaldolase.

was less susceptible to degradation at low temperatures relative to the starch of wild type tubers. It has been observed that after two months of storage at 4°C, the transgenic tubers contained up to ninefold lower concentrations of reducing sugars compared to the wild type. However, the commercial value of the modification of starch could not be assessed because the authors did not analyze the processing quality of transgenic tubers.

### *16.2.3.2 Starch Degradation*

The differential response of potato cultivars to LTS may be the result of starch properties that affect the ability of enzymes to degrade it. The various starch properties ascribed are:

1. Chemical modifications of glucose units by attachment of covalently bound phosphate. Phosphate esters are attached to C3 or C6 glucosyl residues of amylopectin

in the larger interbranch chains and are absent around the branching points. This affects the cleavage sites and degradation product patterns (32).

2. Surface property alteration caused by the negative surface charge from surface phosphate, lipid, or protein can affect the properties of enzymes and other soluble compounds (33).
3. Association with starch-metabolizing enzymes such as endoamylase activity in cotton leaves (34) and starch synthase in potato tubers (35).
4. Physical characteristics of starch. Isolated starch grains from two cultivars differing in their sensitivity to LTS showed an increase in starch grain size over time with the disappearance of smaller starch grains while ND860-2, the resistant cultivar had a consistently smaller mean starch grain size (36,37). Higher levels of amylose in ND 860-2 were believed to be responsible for a more ordered crystallinity within the starch granule, decreased thermomechanical analysis swelling, increased resistance to gelatinization and decreased susceptibility to α-amylase hydrolysis.

In addition to the various properties of starch, which affect its degradation, other factors such as enzymes responsible for starch degradation during LTS have been studied. The pathway of starch breakdown during LTS is not well established. The degradation of starch is believed to occur in the amyloplast (38). The widespread distribution of α-glucan phosphorylase, α-amylase, *β*-amylase, and maltase (39) suggests that starch breakdown could be phosphoryltic, hydrolytic, or both. However it is assumed that starch breakdown in cold stored potato tubers is mainly conducted by starch phosphorylase, because sucrose is the first sugar to accumulate upon transfer of tubers to chilling temperatures (19). Amylase activities are too low at such cold temperature to catalyze the required rate of starch degradation (40), and no increase in either maltose or polymers of glucose larger than maltose, the common products of amylolytic starch degradation (41), have been observed during LTS (42).

Two types of potato phosphorylases are recognized based on glucan specificity, monomer size, and intracellular location. They are noninterconvertible proteins with different primary structures and different immunological properties (38). Type 1 isozyme, also known as type H, is localized in the cytoplasm, has a low affinity for maltodextrins, has a high affinity for branched polyglucans, and cross reacts with type H phosphorylase from potato leaves. Type 2 isozyme, also known as type L, is located in the amyloplast, has a high affinity for maltodextrins, has a low affinity for branched polyglucans, and cross reacts with type L enzyme from the leaf (38,43). Type L and type H isozymes do not cross react immunologically. The function of these isozymes in starch degradation and LTS is unknown. There are reports which suggest that starch breakdown during LTS is phosphorylitic. Kumar et al. (44) have demonstrated that although the activities of cytosolic and plastidic isozymes of starch phosphorylase were reduced by up to 70% in transgenic potatoes expressing antisense cDNA constructs of starch phosphorylase, this did not affect the accumulation of reducing sugars during 4°C storage.

Other studies suggest that starch breakdown in potato tubers during cold storage is not solely due to the activity of starch phosphorylase. Cochrane and coworkers, (45) using a modified amylase assay, found that α- and *β*-amylases and α-glucosidase activities were much higher in tubers stored at the colder temperature (4°C) than those stored at 10°C, and in cultivars known to be more susceptible to LTS. It was considered inappropriate to correlate reducing sugar content and amylase activity, because the formation of reducing sugars is influenced by many other cold labile processes in the tuber. Reducing sugar content and the

activities of α- and β-amylases and debranching enzymes were measured by Cottrell et al. (46) over 139 days in five cultivars of potato tuber stored at 4 and 10°C. The activities of these enzymes were always greater at 4 than at 10°C, but cultivars that accumulated high levels of reducing sugars did not always display the greatest level of hydrolytic enzyme activity (46). It has been reported that the onset of sugar accumulation in tubers during low temperature storage coincided with an increase in the activity of one specific isoform of amylase, the β-amylase in the cultivar Desirée (47–49). β-Amylase activity was present at low levels in tubers stored at 20°C, and increased from four- to fivefold within 10 days of storage at 3°C. However, no specific role has been established for this cold induced β-amylase in LTS.

**16.2.3.2.1 Effect of Inorganic Phosphates** The intracellular compartmentalization of Pi has been suggested to influence carbon partitioning in nonphotosynthetic potato tubers in a manner similar to its role in photosynthetic tissues. It has been observed that increased inorganic phosphate in the amyloplast shifted metabolic activities toward starch degradation rather than accumulation (Figure 16.1) (17). Increased Pi concentration inhibits ADPGPase and enhances starch breakdown by α-glucan phosphorylase. A high concentration of Pi was found in tuber amyloplasts (50), and Pi in cold stored tubers was later found to be cleaved off from starch (51). Higher levels of Pi were found in Russet Burbank potatoes stored at 5.5 than at 15.5°C (52). A highly significant correlation was found between the Pi content and the accumulation of reducing sugars. Amyloplasts were found to have high concentrations of Pi, citrate, $Cl^-$, and $K^+$. It was suggested that Pi leaks from the amyloplast to the cytoplasm during cold storage and induces higher sugar concentration levels in tubers during LTS (53).

Another source of Pi in plant cells is the vacuole (54). The major portion of Pi is stored in potato tuber vacuoles where it is compartmentalized away from the cytoplasm. Loughman (55) examined the respiratory changes of potato tuber slices and found that the larger part of Pi in the cell was localized in the vacuole, and did not take part in the steady state metabolism of the cell. However, the Pi in the vacuole may become available for metabolism in the cytoplasm during cold storage when ionic pumps that utilize ATP in the tonoplast become unable to maintain ionic gradients (56). This scenario could happen by passive leakage of Pi into the cytoplasm or when the membrane becomes leaky due to changes in the properties of the lipid bilayer. Increased Pi in the cytoplasm could affect the metabolism by mobilizing carbon from the amyloplast into the cytoplasm in exchange for Pi transported into the amyloplast by the hexose phosphate translocator (Figure 16.1) (57). Inside the amyloplast stroma, Pi can serve as a substrate for the continued phosphorlysis of starch, via α-glucan phosphorylase, forming additional molecules of G-1-P.Elevated cytoplasmic levels of Pi initiated by leaky membranes, coupled with reduced levels of fructose 2,6,bisphosphate during cold stress, would direct carbons away from glycolysis and favor the buildup of hexose phosphates for gluconogenic reactions (17).

### 16.2.4 Starch–Sugar Balance

The close relationship between starch and sugar levels when potatoes are cooled from 10 to 2°C and then rewarmed from 2 to 10°C after an interval, has given rise to a misleading concept of a starch to sugar balance in which the overall change between the two compounds is seen as being reversible (19). All the available evidence suggests that sucrose is formed from starch by an irreversible pathway, and that starch is formed from sucrose by separate, but likewise irreversible, routes. The very close relationship between starch and sucrose in stored potatoes may be due to the fact that starch is the only possible source of carbon for sugar synthesis in the cold (7,11,12,17). There is a strong evidence to indicate that G-6-P is transported into the amyloplast of potato tubers to support starch synthesis (26). Thus the pathways of starch and sugar biosynthesis compete for the same pool of precursors.

A net flux of carbon from starch synthesis into sucrose occurs in cold stored tubers as evidenced from the use of radiolabels in experiments (4,58). The coexistence of the pathways of sucrose synthesis and starch breakdown in stored tubers may be regulated by fine control mechanisms. In potato discs incubated with $^{14}C$ glucose at 3°C and 15°C, a large proportion of label is recovered in starch. At low temperature, in a cold sensitive, high sugar-accumulating cultivar, the ratio of $^{14}C$ recovered in sucrose to that recovered in starch increased (4,58), but was unaffected in a cold tolerant, low sugar-accumulating cultivar (4,58). This suggests that genotypic variation in the capacity to maintain an active starch synthesizing system may help in alleviating the rate of sucrose accumulation.

### 16.2.5 Sucrose Metabolism

Sucrose is the first sugar to accumulate during LTS. Its accumulation in potato tubers has been recorded within hours of their placement at LTS inducing temperatures, with the accumulation of reducing sugars occurring a few days later (19). Sucrose synthesis occurs in the cytoplasm of the tuber either by sucrose 6-P synthase (SPS), or by sucrose synthase (SS) and the hexose phosphates required for this are transported from the amyloplast via a phosphate translocator (Figure 16.1) (26,59).

Pressey (60) reported that SS activities decreased after harvest, and continued to do so under low temperature storage conditions. SPS activity also decreased if tubers were held at warm temperatures but rapidly increased when tubers were held at low temperatures. This observation indicates that SPS is the enzyme responsible for sucrose synthesis at low temperatures. Pollock and ap Rees (22) reported that sucrose synthesis during LTS is catalyzed by SPS and not by SS. This was also confirmed by $^{13}C$ NMR studies (61).

The increase in sucrose synthesis upon transferring the tubers to low temperature has been associated with the increased expression of an isoform of SPS (SPS-1b, 127 kDa) (62). The cold induced increase in the SPS-1b isoform was found to correlate well with the change in the kinetic properties of the enzyme. The major isoform found in tubers stored at room temperature is a 125-kDa protein (SPS-1a). Reconditioning of the tubers at 20°C resulted in the disappearance of the cold induced SPS isoform after 2–4 days (49). An increase in the total amount of SPS transcript was observed at low temperature in each of these studies. SPS from potato tubers has been shown to be subject to fine regulation by allosteric effectors and protein phosphorylation (63). Potato tuber SPS is allosterically activated by G-6-P and inhibited by protein phosphorylation.

Antisense technology has been used as an effective tool to investigate the roles of enzymes that lead to the production of sucrose, as well as reducing hexoses such as glucose and fructose in LTS. Many researchers investigating LTS mechanisms have used this technology to substantiate the role of enzymes in the carbohydrate metabolic pathway. For example, in experiments involving transgenic tubers where the SPS activity was reduced by 70–80% either by antisense or cosuppression, cold sweetening was reduced by inhibiting the increase of the cold induced isoform of SPS (64). The authors also observed that the $V_{max}$ of SPS was 50 times higher than the net rate of sugar accumulation in wild type tubers, and found that SPS is strongly substrate limited, particularly for UDP-G (Figure 16.1). These results indicate that the rate of cold sweetening in wild type tubers is not strongly controlled by the overall SPS activity or the overall amount of SPS protein. Alterations in the kinetic properties of SPS during cold temperature storage were more effective in stimulating sucrose synthesis than changes in SPS expression. The observation that changes in the kinetic properties of potato tuber SPS coincide with the onset of sugar accumulation points to the fact that the fine regulation of SPS may be more important than coarse regulation in controlling the ability of a cultivar to sweeten during cold storage.

However, it should be noted that SPS may not be the only candidate that regulates sugar accumulation during LTS, because other factors that affect the availability of hexose phosphates, such as glycolysis and the pentose phosphate pathway, may have key roles to play (Figure 16.1, Figure 16.2).

UDP-glucose pyrophosphorylase (UGPase) is a cytosolic enzyme that catalyzes the formation of UDP-G, one of the substrates required for the synthesis of sucrose (Figure 16.1). Depending on the physiological state of the tubers (i.e, growth or post harvest storage), the UGPase reaction may be directed toward the synthesis or degradation of starch (10). During the process of cold sweetening, it has been suggested that UDP-G and PPi have regulatory roles in directing carbon flux into glycolysis, starch synthesis, hexose formation, or a combination of the three (17,65). The activity of UGPase has been correlated with the amount of glucose that tubers of different cultivars accumulate in cold storage (12), leading to the assumption that this enzyme might be a control point for low temperature sweetening, as it regulates the rate of SPS and sucroneogenesis by controlling the levels of UDP-G (17,66).

Genetic manipulation to down regulate the expression of UGPase in potato tubers has resulted in contrasting results based on the physiological stage of the tubers. In two separate experiments in which the UGPase activity was reduced by 30–50% compared to their wild types, the transgenic tubers accumulated lower levels of sucrose during storage relative to wild type tubers at 4°C and 12°C (67) and at 6°C and 10°C (68). It has been suggested that by limiting the rate of UDP-G synthesis, UGPase may exert control over the flux of carbon toward sucrose during the cold storage of tubers. These observations are supported by the results of Hill et al. (47) who observed that following the initiation of cold sweetening, the concentration of UDP-G changed in parallel with the concentration of sucrose.

In contrast to the above results, Zrenner et al. (69) observed that carbohydrate metabolism of growing tubers was not affected when the transgenic plants had a 96% reduction in UGPase activity as compared with the wild type plants. No significant changes were observed in the levels of fresh mass, dry mass, starch, hexose phosphates, or UDP-G at harvest relative to the wild type tubers. It was reported that 4–5% of UGPase activity was still in considerable excess compared to the activity of other glycolytic enzymes in the tuber, and the antisense construct may have to reduce UGPase to negligible levels in transgenic potatoes before any phenotypic differences are noticeable (70).

It should be noted that the flow of carbon is different based on the physiological state of the tuber. In the growing tuber, most of the incoming sucrose is used for the synthesis of starch, while in the stored tuber the hexose–phosphate produced from starch degradation is converted into sucrose. This explains the different responses obtained by Zrenner et al. (69), Spychalla et al. (67), and Borovkov et al. (68). In cold stored tubers, when the rate of starch breakdown exceeds the rates of glycolysis and respiration, the conversion of G-1-P to UDP-G is the only means of controlling the level of hexose phosphates. Hence it is possible that a significant effect of reduced UGPase activity may be observed only in tubers acting in the direction of sucrose synthesis, such as during post harvest storage.

Two UGPase alleles have been identified in potato tubers: UgpA and UgpB (67). In a survey conducted on a number of American and European cultivars and selections stored at 4°C, it was observed that a relationship existed between the allelic polymorphism of UGPase and the degree of sweetening. The genotypes that resist sweetening during cold storage have demonstrated a predominance of the allele UgpA; the genotypes susceptible to sweetening have a predominance of the allele UgpB (68).

In order to assess the role of UGPase in LTS, Sowokinos (13) cloned UGPase from 16 American potato cultivars and selections that have varying degrees of cold sweetening ability during storage at 3°C. It was observed that cultivars that were resistant to LTS possessed

a UgpA: UgpB allelic ratio of 4:0 or 3:1. The cultivars demonstrating LTS revealed a ratio of 1:3 or 0:4 in favor of the UgpB allele. Sowokinos (13) also observed that the cold sensitive potato cultivars expressed up to three acidic isozymes of UGPase (UGP1, UGP2, UGP3) with UGP3 being the most abundant. In addition to the three isozymes present in the sensitive cultivars, the cold resistant cultivars possessed another two isozymes, UGP4 and UGP5 that were more basic in nature. Sowokinos (18) studied the physicochemical and catalytic properties of the purified UGP4 and UGP5 isozymes, and suggested that the differences in sugar accumulation between the cultivars and selections that are either sensitive or resistant to LTS may be partially due to the unique nature of expression and catalytic properties of the isoforms in resistant lines, including pH optimum, substrate affinities for G-1-P and UTP, $V_{max}$, and the magnitude of product inhibition with UDP-G. The overall effect of these differences in isozyme expression is that it may decrease the rate of UDP-G formation, resulting in a lower accumulation of reducing sugars in the cold resistant clones.

#### *16.2.5.1 Sucrose Degradation*

Sucrose plays a pivotal role in plant growth and development because of its function in translocation and storage, and the increasing evidence that sucrose (or some metabolite derived from it) may play a nonnutritive role as a regulator of cellular metabolism, possibly by acting at the level of gene expression (71). As mentioned earlier, sucrose is the first sugar to form during LTS, and the source of glucose and fructose accumulation appears to be the degradation of sucrose (72). Sucrose is broken down by two types of enzymes in plants. By invertase action, it is hydrolyzed into glucose and fructose; whereas by the action of SS, it is converted into UDP-G and fructose in the presence of UDP (73).

Potato tubers are known to possess both alkaline and acid invertases. Acid invertase is localized in vacuoles, whereas alkaline (neutral) invertase is localized in the cytoplasm (72,74). Acid invertase isoforms that are ionically bound to the cell wall have also been identified (74). Alkaline invertases are sucrose specific, while acid invertases cleave sucrose at the fructose residue but can also hydrolyze other $\beta$-fructose containing oligosaccharides such as raffinose and stachyose (74).

Based on several observations of sucrose synthase and acid invertase activities in developing, mature, and cold stored tubers, and given the fact that sucrose is stored mainly in the vacuole, it is believed that sucrose synthase is responsible for sucrose degradation in developing tubers, whereas acid invertase is the principal enzyme responsible for the breakdown of sucrose into hexoses during LTS (60,70,73,75–77). Based on the widely established inverse correlation between sucrose content and vacuolar acid invertase activity, it is strongly believed that sucrose is broken down by acid invertase in the vacuole and the resulting glucose and fructose are transported into the cytosol for the formation of hexose phosphate by hexoskinase (72,78–80). It has been reported that glucose concentrations are frequently higher than fructose concentrations in stored potato tubers (81). Zrenner et al. (82) evaluated the glucose to fructose ratio of 24 different cultivars and found that the ratios were between 1.1 and 1.6, which is a strong indicator that invertase is the key enzyme responsible for the conversion of sucrose to hexose.

Zrenner et al. (82) studied the effect of soluble acid invertase activity in relation to the hexose to sucrose ratio in 24 different potato cultivars and observed a strong correlation between the hexose to sucrose ratio and the extractable soluble invertase activity. They also isolated a cold inducible acid invertase cDNA from potatoes and developed transgenic potatoes expressing the invertase cDNA in an antisense orientation. The subsequent 12–58% reduction of acid invertase activity compared to the wild type tubers resulted in an accumulation of sucrose and a decrease in the concentration of hexoses. The hexose to

sucrose ratio was found to decrease with decreasing invertase activities; however, the total amount of soluble sugars did not significantly change. Based on these observations, it was concluded that invertases do not control the total combined amount of glucose, fructose, and sucrose in cold stored potato tubers, but are involved in the regulation of the ratio of hexoses to sucrose (82). Greiner et al. (83) strongly inhibited the activity of cold induced vacuolar invertase in potato plants by repressing the activity, or by the expression of a putative vacuolar invertase inhibitor from tobacco (Nt-inh), in potato plants under the control of the CaMV 35S promoter. It was possible to decrease the cold induced hexose accumulation up to 75% without affecting tuber yield. Although the concentration of sugar produced during cold induced sweetening was decreased, the level of hexose accumulated was still in excess of what is commercially acceptable for the production of potato chips and fries. The observation that antisense expression of acid invertase did not control the total amount of soluble sugars in cold stored potato tubers (82) indicates that other factors in the carbohydrate metabolism may influence the regulation of the total amount of sugars accumulated, and that acid invertase could be only one of the enzymes involved in starch–sugar conversion.

Metabolism is more rigorously regulated by intracellular compartmentalization in plants than in animals (84), and compartmentalization of the pathways of carbohydrate catabolism is realized to be a distinct feature of plant respiration (70). It is now believed that a "futile" cycling (simultaneous synthesis and degradation) of sucrose functions continuously to allow plants to respond rapidly to demand for carbon (85). This metabolic cycle may also be involved in LTS. For instance, it has been observed that in the first two weeks of 4°C storage, the initial rates of sucrose accumulation corresponded closely with the estimated rates of sucrose synthesis (47). The rate of total soluble sugar accumulation decreased with increasing duration of cold storage. It is suggested that sugar accumulation decreased because the rate of recycling equalled the rate of synthesis.

### 16.2.6 Glycolysis

The effects of cold exposure on the metabolism of potato tubers indicate that cold induced sweetening may at least in part be due to differential sensitivity to low temperature of the enzymes in the glycolytic pathway (86). The available data suggest that phophofructokinase (PFK) and pyruvate kinase are more sensitive to cold than are the other enzymes involved in the metabolism of hexose-6-phosphates (Figure 16.2), and by lowering the temperature, divert the latter to sucrose (Figure 16.1) (22,86,87). Another glycolytic enzyme that has been studied in relation to LTS is fructose-1,6-bisphosphatase (FBPase). Plants possess ATP dependent (ATP-PFK) and PPi dependent (PPi-PFK) phosphofructokinases (88).

PPi-PFK is a cytosolic enzyme and experiments to examine the role of PPi-PFK during the aging of tissue slices from potato tubers (starch-storing tissue) and carrot roots (sucrose-storing tissue) showed that both vegetables showed the same pattern of changes of phosphorylated metabolites and fructose 2,6-bisphosphate. But, the consumption of PPi by tubers and the production of PPi in carrots indicated PPi-PFK control of the glycolytic flux in tubers and catalysis of the opposite reaction in carrot roots (89). PPi-PFK is activated by fructose-2,6-bisphosphate which does not affect ATP-PFK (90). The activity of PPi-PFK is often equal to or exceeds that of ATP-PFK (72,90). It has been reported that the activity of PPi-PFK was ten times that of ATP-PFK in developing potato tubers, and hence it is suggested that glycolysis may proceed regardless of the activity of ATP-PFK (91). The maximum activity of PPi-PFK has also been shown to be greatly reduced in tubers stored at a low temperature of 5°C due to a decrease in PPi-PFK affinity for fructose 2,6-bisphosphate, an increase in sensitivity to fructose 2,6-bisphosphate as an activator,

and a decrease in fructose 2,6-bisphosphate concentration at decreasing temperature (92). By contrast, in another study, no evidence was found for a cold induced inhibition of PPi-PFK in tubers stored at 2°C and 8°C (93). Hence it was postulated that PPi-PFK contributes to LTS by regulating the PPi concentration below inhibitory levels, facilitating the formation of UDP-G and subsequent synthesis of sucrose (Figure 16.1 and Figure 16.2).

ATP-PFK has been implicated in the regulation of LTS (94,95). It is responsible for the irreversible ATP dependent conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. Potato tubers have been reported to possess four isozymes of ATP-PFK (96). It has been reported that the temperature coefficient ($Q_{10}$) of three of the four isoforms were higher at 2–6°C than at 12–16°C, indicating the cold lability of these isoforms and their roles in the accumulation of hexose phosphate and sucrose synthesis in LTS (95). This result supports the suggestion of Bryce and Hill (97) that ATP-PFK dominates the control of glycolysis, and thereby respiration, in plants. However, the observation that respiration of potato tubers increases concomitantly with the initial increase in sugar concentration (19,98), and the fact that the conversion of fructose-6-phosphate is also catalyzed by PPi-PFK (ure 16.2) indicate multiple regulatory controls in the biosynthesis of sugar phosphates.

Genetic manipulation of the two PFKs was carried out further to explore their roles in LTS. About 88–99% inhibition of PPi-PFK expression was obtained in stored tubers by antisense expression of PPi-PFK cDNA (99). Even though the transformation resulted in higher levels of hexose phosphates in transgenic tubers compared to their wild type tubers, no difference was observed between these tubers in the rates of sucrose and hexose accumulation, and the total amounts of sugars accumulated at 4°C. Besides, no change was observed in the maximum catalytic activities of ATP-PFK or other enzymes of glycolysis (pyruvate kinase) or sucrose breakdown (invertase and sucrose synthase) in the antisense tubers. This observation suggests that compensation occurs at the level of fine metabolic regulation rather than gene expression. The above results indicate that PPi-PFK may not control the rate of glycolysis at low temperatures, and that tubers possess excessive capacity to phosphorylate fructose-6-phosphate. The results are also not in agreement with the theory proposed by Claassen et al. (93), that PPi-PFK is involved in regulating the PPi concentration, as no evidence was observed to substantiate that the antisense and wild type tubers contained different PPi concentrations (99).

Expression of the *E. coli* pfkA gene in potato tubers resulted in a 14- to 21-fold increase in the maximum catalytic activity of ATP-PFK, without affecting the activities of other glycolytic enzymes (100). It was also found that no corresponding decrease in the concentration of hexose phosphate was observed, while the pool sizes of other glycolytic intermediates increased three- to eightfold. In another study, it was reported that a substantial increase in ATP-PFK activity did not affect the flux through glycolysis or a flux between glycolysis and the PPP (101). The above results suggest that ATP-PFK may not limit the rate of respiration of potato tubers. ATP-PFK is potently inhibited by phosphoenolpyruvate, and hence ATP-PFK activity may be dependent upon the activity of enzymes that metabolize phosphoenolpyruvate (PEP) such as pyruvate kinase and phosphoenolpyruvate phosphatase. The above contention is in agreement with the findings of Thomas et al. (102). Using metabolic control analysis (MCA) on tuber glycolysis, Thomas et al. (102) observed that ATP-PFK exerts little control over glycolytic flux, while far more control of flux resides in the dephosphorylation of PEP.

Fructose 1,6-bisphosphatase is localized in the plastids and in the cytosol. Cytosolic FBPase is involved in hexogenesis, converting fructose-1,6-bisphosphate to fructose-6-phosphate, which is used by SPS as one of the substrates for the production of sucrose-6-phosphate (Figure 16.1 and Figure 16.2). FBPase is potently inhibited by fructose-2,6-bisphosphate (90,103), a metabolite which is also a potent activator of PPI-PFK (104). In a study to

investigate the role of FBPase in LTS, it was observed that there was a rapid increase in the levels of sucrose and reducing sugars in tubers stored at 2°C, but no change in FBPase activity, relative to 8°C storage.

In a study carried out to identify the regulatory steps in glycolysis, a decline in phosphoenolpyruvate and a rise in pyruvate were observed when potato tubers were stored under anoxic conditions (86). As this step is preceded by phosphofructokinase, pyruvate kinase cannot regulate glycolytic flux directly as it cannot control the entry of glucose-6-phosphate into glycolysis. However, pyruvate kinase could play a role in the regulation of the movement of carbon out of glycolysis and into the oxidative pentose phosphate pathway. It has been suggested that the cold lability of phosphofructokinase and pyruvate kinase could lead to a rapid reduction in hexose phosphate consumption, which could cause their diversion to sucrose (86).

The theory that the cold lability of enzymes in the glycolytic pathway diverts hexose 6-phosphate for sucrose production and thus to LTS cannot fully explain LTS, as it takes time for potatoes to sweeten fully (7). From the results of a study carried out by Marangoni et al. (16), by comparing LTS resistant (ND860-2) and LTS susceptible (Norchip) potato cultivars, it has been suggested that tubers with decreased invertase activity along with increased glycolytic or respiratory capacity, should be more tolerant to low temperature stress.

### 16.2.7 Oxidative Pentose Phosphate Pathway (PPP)

Although the PPP is usually depicted as being separate from glycolysis, the two pathways are intimately linked (Figure 16.2). They share the common intermediates glyceraldehyde 3-phosphate, fructose 6-phosphate and glucose 6-phosphate, and flow through either of the pathways will be determined by the metabolic needs of the cell. The main function of PPP is to generate NADPH for various biosynthetic reactions (105). It was proposed by Wagner et al. (106) that for low sugar accumulating cultivars, the PPP may provide a means of preventing the accumulation of high levels of sugars when tubers are stored below 10°C (by bypassing phosphofructokinase). However, no differences were observed in the specific activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase among LTS resistant and LTS susceptible potato cultivars stored at 4°C and 12°C, respectively (107). It was observed that the LTS resistant cultivars exhibited higher activities of G6PDH and 6PGDH, relative to the LTS susceptible cultivars.

### 16.2.8 Mitochondrial Respiration

During storage of potato tubers below 5°C, in addition to changes in sugar accumulation patterns, respiration changes have also been observed (108). It has been reported that the cold resistant potato clone ND860-2 has shown a higher respiration rate throughout storage compared to the cold susceptible Norchip (109). Respiration decreases as storage temperature decreases, but at storage below 5°C, respiration is stimulated. There is a brief respiratory burst attributed to the combined effect of cyanide resistant (alternative pathway) and cytochrome mediated pathways (108), followed by a subsequent decrease in respiration rate to a new steady state (19,99).

It has been suggested that during chilling stress, an alternative oxidase pathway may play a protective role in the mitochondrion by preventing both an over reduction of the respiratory chain and the consequent production of reactive oxygen species that cause cellular damage (110). It has been suggested that the alternative pathway operates only during periods of high cellular energy charge, or when there is an imbalance between the supply of carbohydrates and the requirement for carbohydrates for structural growth, energy production, storage, and osmoregulation (111,112). There is also evidence which suggests

that physical characteristics of the cellular membrane (i.e., mitochondrial membranes) may activate the alternative pathway (113). In a study carried out by Amir et al. (99) to study the relationship between respiration rate, sugar content, and ATP levels in cold stored tubers, an immediate decrease in respiration rate was observed upon storage at 4° C. The respiratory minimum was concomitant with an ATP maximum which is followed by a respiratory burst and a rapid decline in ATP content. This evidence suggests the presence of an active alternative pathway in cold stressed tubers. Expression of the alternative pathway is known to increase with decreasing temperatures (114). It has been suggested that sucrose formation could serve as an effective sink for excess ATP via the alternative pathway (113). In agreement with Solomos and Laties (113), it was observed that low $O_2$ levels, which inhibit the alternative pathway, were effective in suppressing sugar accumulation in tubers stored at 1°C (115) which suggests that LTS may be directly linked to the onset of cyanide resistant respiration.

### 16.2.9 Compartmentation and Stress Induced Membrane Changes

Membranes play an integral role in the response of plant tissues to chilling and freezing. It has been proposed that the thermotropic phase transition of membrane lipids might play an initiative role in the chilling sensitivity of plants (116–118). With further exposure to chilling, the phase separated biomembranes become incapable of maintaining ionic gradients and cellular metabolism becomes disrupted. The occurrence of phase separation as the initial event in chilling injury has been demonstrated in cyanobacterium *Anacystis nidulans* (119). It has been argued that such a phase separation would not occur in plant cells because they contain high levels of polyunsaturated fatty acids in their membranes. However, a positive correlation has been observed between chilling sensitivity of herbaceous plants and the level of saturated and transmonounsaturated molecular species of phosphatidylglycerol in thylakoid membranes (120,121).

It is likely that the regulation of starch breakdown and of sucrose synthesis is to some extent achieved by compartmentation. Therefore, it is possible that low temperature sweetening is, at least in part, due to effects of cooling on such compartmentation (7). In potatoes, studies have been performed on the effects of cold storage on lipid composition and membrane permeability (122,123), the associated biophysical changes of amyloplast membranes (124,125) and mitochondrial membranes (126), and lipid peroxidation (15,127–129). The results of these studies are described below.

#### *16.2.9.1 Lipid Composition*

Phospholipids and glycerolipids are the major potato lipids (130). It has been found that the combined proportion of polyunsaturated fatty acids (linoleic and linolenic) for all potato varieties examined consistently represents 70–76% of the total fatty acids, which help maintain membrane fluidity at lower temperatures (130).

In plants stressed by low temperature or other factors, the survival of the plant is based on the ability of the plant to maintain or reestablish membrane fluidity (131). Fatty acid desaturases play a central role in regulating the level of unsaturation of fatty acids in membrane lipids, which helps maintain membrane fluidity or refluidizes the membranes that have become rigid due to low temperature exposure (132). Bonnerot and Mazliak (133) reported cold induced oleyl-PC desaturase activity in microsomes from 16 h aged slices of potato tubers stored at 4°C for 3 months. Spychalla and Desborough (134) reported that the total amount of linoleic and linolenic acids remained constant, but the ratio of linolenic to linoleic increased over storage time for both tubers stored at 3°C and 9°C. Low temperature storage of potato tubers has been shown to increase the levels of

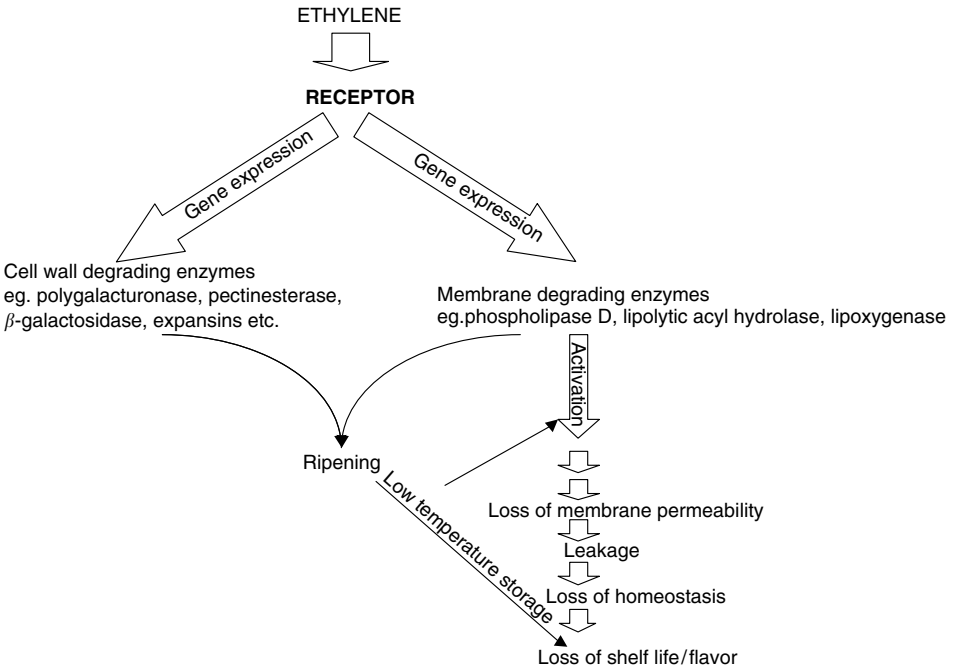


**Figure 16.3** Schematic diagram illustrating the early events in the membrane and cell wall degradation during fruit ripening.

monogalactosyl diacylglycerol and digalactosyl diacylglycerol (135–137). Hence, it is possible that one or more of the low temperature induced changes in the lipid composition of the membrane may contribute to LTS or confer resistance to LTS.

### *16.2.9.2 Membrane Permeability*

Several studies have reported that cold temperature damages membranes, resulting in the loss of compartmentation and homeostasis of the cell (138–140). As temperature decreases, membrane lipids undergo a phase transition from a liquid crystalline to a solid gel phase that results in cracks in the membrane, increases the membrane permeability and leakage of ions, and alters metabolism (141). The temperature range for the phase change in membranes is specific for each horticultural commodity and is a function of the heterogeneity of the lipid content, the ionic environment, and the presence of sterols and proteins (142).

From the earlier discussions, it is believed that LTS is caused by the effect of low temperature on many pathways of carbohydrate metabolism at the level of starch synthesis and breakdown, sucrose synthesis, hexogenesis, glycolysis, the PPP, and mitochondrial respiration. These pathways are also compartmentalized in the plant cell, and involve amyloplasts, the cytoplasm, vacuoles, and mitochondria. For the maintenance of homeostasis in normal cells, a tight control of the movement of the substrates or intermediates of these pathways is in place. However, when the plant, or a cell of the plant, experiences stress (such as low temperature stress as in the case of LTS), the normal metabolism of the cell will be lost, which might make the cell a candidate for readjustment at various levels. It is assumed that the changes caused by low temperature at the membrane level might be one of the factors contributing to LTS.

**16.2.9.2.1 Tonoplast Membrane Permeability** The "leaky membrane theory" of LTS suggests that the cause of LTS may be a leaky tonoplast membrane that allows Pi to be

leaked into the cytoplasm from the vacuole (17). A high concentration of Pi in cytoplasm is believed to mobilize carbon from the amyloplast to the cytoplasm, while cytoplasmic Pi participates with G-1-P in a reversible exchange across the amyloplast membrane. Increased Pi concentration in the amyloplast favors the α-glucan phosphorylase activated starch breakdown and inhibits ADPGPase mediated starch synthesis (17). In addition Pi affects fructose 2,6,-bisphosphate, phosphofructokinase, sucrose synthase, and UGPase (see section 16.2.3.2.1). The leaky membrane theory is been supported by subtle changes in fatty acid composition of potato membranes as well as by increases in electrical conductivity, which is an indicator of electrolyte leakage and membrane permeability. It has been reported that the relative change in electrical conductivity of four cultivars of potato paralleled the increase in sugar concentration when temperature was dropped from 20°C to 0°C (143). A difference in electrical conductivity was noticed among cultivars that had accumulated similar amounts of sugars. It was concluded that the increased electrical conductivity was not due to the increase in the accumulation of sugars, particularly because respiration rates were found to increase before the increase in sugar concentration and electrical conductivity.

Knowles and Knowles (122) studied the relationship between electrolyte leakage and degree of saturation of polar lipids in Russet Burbank seed tubers stored at 4°C, and observed an inverse linear relationship between the double bond index (DBI) and electrical conductivity ($r = -0.97$). The DBI revealed that the proportion of unsaturated fatty acids in membranes decreased over storage time with an accompanying increase in electrical conductivity. The authors concluded that the ability to increase membrane lipid unsaturation in storage could confer resistance to electrolyte leakage by maintaining the fluidity of the membranes. This result was supported by another study, in which it was observed that tubers stored at 3°C had greater increase in sugar content, total fatty acid saturation, and membrane permeability, as compared to tubers stored at 9°C (123). These studies suggest that high initial or high induced levels of lipid unsaturation could prevent increased membrane permeability during low temperature storage. It has been reported that cyanobacteria transformed with *des*A gene, which encodes a 12 acyl-lipid desaturase in *Synechocystis* PCC6803, did not show any significant changes in photosynthetic activity below 10°C, whereas in the wild type cells, the photosynthetic activity was decreased irreversibly (132).

**16.2.9.2.2 Amyloplast Membrane Permeability** It has been suggested that the low temperature induced defects in amyloplast membrane allow α-glucan phosphorylase from the cytoplasm to enter the amyloplast and degrade starch, resulting in the accumulation of sugars during LTS (144,145). Electron spin resonance study, used to examine the amyloplast membrane in potato tubers stored at 5.5 and 15.5°C, showed a strong relationship between membrane permeability and starch to sugar conversion (146). Studies using spin labeled probes revealed that at low temperatures, membranes exhibited decreased lipid fluidity. O'Donoghue et al. (125) observed that membrane lipid phase transitions were higher for Norchip (an LTS susceptible cultivar) than ND 860-2 (an LTS resistant cultivar) at both 4 and 12°C storage, and Norchip amyloplast membranes were more ordered at 4 than 12°C. The drop in double bond index (DBI) was 93% for Norchip while only 70% drop was observed for ND 860-2 due to loss of linoleic and linolenic acid. It was suggested that low temperature caused Norchip membranes to undergo deterioration to a greater extent than ND 860-2 membranes and this could have contributed to LTS. By contrast, it has been reported that the amyloplast membrane breaks down during senescence but remains relatively intact during LTS (147–149). Based on TEM examination of membranes from LTS resistant and LTS susceptible cultivars of potatoes stored at 5 and 10°C, Yada et al. (149) concluded that LTS is not the result of amyloplast membrane breakdown. However, it is likely that changes in membrane can take place at the molecular composition or organizational level, which can affect the permeability or transport properties, or both, and can contribute to LTS.

### 16.2.10 Free radicals and Antioxidant Enzymes

Evidence from several lines of research suggests that a variety of toxic oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals are produced in plants exposed to various environmental stresses such as high and low temperatures, drought, light, and exposure to pollutants causing oxidative damage at the cellular level (150). Lipid peroxidation is considered to be one of the reasons for membrane deterioration during senescence and chilling injury (140), and results from the activity of lipoxygenase, resulting in the formation of lipid peroxides and free radicals (151). In a study to analyze the relationship between sugar accumulation and changes in membrane lipid composition associated with membrane permeability in early stages of LTS, sucrose cycling and accumulation were greatest for Norchip, a LTS susceptible cultivar at 4°C as compared to LTS tolerant ND 860-2 (15). No significant changes were observed in phospholipid, galactolipid, free sterol levels, or phospholipid to free sterol ratio. However, the double bond index obtained from the fatty acid profiles of the total lipid fraction decreased significantly (decreased unsaturation) for Norchip tubers at 4°C over time. Free fatty acid and diene conjugation values fluctuated and increased over time for both Norchip and ND 860-2 stored at 4°C and 12°C, with greater amplitude of fluctuations observed for Norchip stored at 4°C. From the results, it has been suggested that these effects may be due to the high levels of lipid acyl hydrolase and lipoxygenase found in potato tubers, and the observed peroxidation products could relate low temperature stress and the resultant LTS to chilling injury and drought stress (15). However, Fauconnier et al. (152) could not observe a correlation between cold sweetening and membrane permeability or lipid saturation status. They studied the effect of three storage conditions: at 4°C, at 20°C with sprout inhibitors, and at 20°C without sprout inhibitors, and observed that during storage at 20°C without sprout inhibitor, the increase in membrane permeability is inversely correlated to sucrose accumulation. It was also observed that lipoxygenase activity and gene expression are not correlated with the fatty acid composition of the membrane. It was also observed that the lipoxygenase activity and fatty acid hydroperoxide content are low in older tubers, irrespective of the storage conditions and the varieties. Spychalla and Desborough (134) studied the antioxidant potential of potato tubers stored at 3 and 9°C and observed that tubers stored at 3° C had higher superoxide dismutase activities than their 9°C counterparts and demonstrated time dependent increases in superoxide dismutase, catalase, and α-tocopherol during the 40 week storage period. They also observed that low sugar clones had significantly higher levels of superoxide dismutase and catalase than high sugar clones but significantly lower levels of α-tocopherol. The increased antioxidant responses could be due to increased free radical production as manifested by the higher levels of superoxide dismutase, catalase, and peroxidase activities in seed tubers stored at 4°C for 20 months as against those stored for 8 months (129).

From the above discussion, it can be concluded that LTS is not the result of a single cause. The sugar balance in potato is regulated by many intermediate carbohydrate metabolic pathways, which are subject to genetic and environmental control. It might be possible that the low temperature effects on enzymes involved in carbohydrate metabolism result in an imbalance in the normal metabolism combined with its effect on membrane fluidity, thus diverting or leaking the intermediates, or both, in the biosynthetic or respiratory pathway for sucrose and reducing sugar production. Hence, even though genetic engineering has great potential in manipulating or improving crop productivity and the quality of horticultural crops, because of the complexity of LTS, in depth research on various molecular and biochemical properties and their correlation to LTS need to be conducted before we can fully exploit that potential. The existence of cultivars resistant to LTS with several molecular, biochemical, or compositional characteristics, or combinations of

these, might provide a better tool in understanding the mechanism that is responsible for resisting LTS in those cultivars. We can therefore be hopeful that the coordinated efforts of plant biochemists, molecular biologists and traditional plant breeders would help to better understand and control LTS, thus eliminating the use of chemical sprout inhibitors and their harmful safety issues.

## 16.3 TOMATO

Tomatoes rank second to potatoes in dollar value among all vegetables produced in the USA and in other parts of the world where they are grown (1). In terms of per capita consumption, processed tomato products lead all the other processed vegetables. The main factors in determining the postharvest deterioration of fruit and vegetable crops are the rate of softening of the fruit which influences quality, shelf life, wastage, infection by postharvest pathogens, and frequency of harvest, and which limit the duration of transportation and storage. Damage to the structure and function of the membrane affects the post harvest shelf life and quality of fruits, vegetables, and other food sources by causing leakage of ions from cellular storage compartments into the cytosol, thereby disrupting the homeostasis of the cell (153,154). A major problem faced by the fruit and vegetable fresh market and processing industry in the Northern Latitudes is the lack of a year round supply of high quality material. Even though cold storage can be used for long term storage of fruits and vegetables, in the case of tomatoes it is not effective due to the sensitivity of tomatoes to being chilled. Many factors affect the shelf life of tomato products, but our discussion will be based mainly on membrane changes during cold storage and the genetic manipulation to circumvent these factors.

### 16.3.1 Role of Membrane in Shelf life

The development of fruit in many plants can be interpreted as following a two step process. During the first phase, the ovary or hypothalamus within the flower expands and develops into a full sized fruit. During the second phase, the full sized fruit undergoes ripening, a complex set of molecular and physiological changes in the fruit. The ripening process brings dramatic changes to the fruit: softening, biosynthesis of pigments, and increase in sugar content, flavor, and aroma. In climacteric fruits such as tomatoes, and many other fruits, ripening begins with increased respiration and ethylene biosynthesis (155). Fruit ripening can be considered as the beginning of senescence of the fruit (156).

Senescence can occur at various levels, from cellular to whole plant levels, and is regulated by genetic, hormonal, and environmental factors (140,157). The plant hormone ethylene plays a major role in the ripening and senescence processes, and extensive work has been conducted in the past two decades on the role of ethylene in fruit ripening and signal transduction. Genetic manipulation to increase the shelf life of fruits, especially tomatoes, has been extensively undertaken, resulting in several new transgenic varieties with improved storage and quality characteristics (158–164).

Senescence is characterized by membrane deterioration resulting from the catabolism of membrane lipids and proteins. The pathway of the catabolism of phospholipids has been elucidated from several senescing systems and involves the sequential action of enzymes that include phospholipase D (PLD, phosphatidyl choline hydrolase, EC 3.1.4.4, PLD), phosphatidate phosphatase (3-*sn*-phosphatidate phosphohydrolase, EC 3.1.3.4), lipolytic acyl hydrolase and lipoxygenase (linoleate, oxygen oxidoreductase, EC 1.13.11.12) (140). In most systems studied, the first step in the lipid catabolic pathway is the conversion of phospholipid to phosphatidic acid by PLD, even though phosphatidylinositol and

its phosphorylated forms may be acted upon by both phospholipase C and PLD (165,166). Phosphatidic acid does not accumulate, as it is immediately converted to diacylglycerol by phosphatidate phosphatase. Diacylglycerol is deacylated by lipolytic acyl hydrolase, liberating free fatty acids. Among the free fatty acids, unsaturated fatty acids with 1–4 pentadiene systems (18:2 and 18:3) serve as substrates for lipoxygenase, resulting in the formation of fatty acid hydroperoxides. Fatty acid hydroperoxides undergo a variety of reactions by virtue of their active unstable structure, including the generation of free radicals. The free radicals damage the protein as well as the membrane, giving rise to the characteristics features of senescence. The above reactions are deemed autocatalytic, as the reaction products increasingly contribute to the formation of gel phase and nonbilayer lipid structures resulting in the destabilization of the membrane and eventually in the loss of homeostasis. Similar changes occur in the membrane in response to chilling injury, but also involve the effect of low temperature on the catalytic activity of enzymes involved as well as the effect on the phase transition temperature of the lipids (see section 16.2.9). Here we will be emphasizing the role of PLD and lipoxygenase in enhancing the shelf life of tomatoes during cold storage.

### 16.3.2 Phospholipase D Gene Family

Phospholipids provide the backbone for biomembranes and serve as rich sources of signaling messengers. Phospholipase D (PLD, EC 3.1.4.4) catalyzes the hydrolysis of structural phospholipids to generate phosphatidic acid and a free head group. PLD has been grouped into three classes based on their requirements for $Ca^{2+}$ and lipids in *in vitro* assays: the conventional PLD that is most active at 20 to 100 mM levels of $Ca^{2+}$ ; the polyphosphoinositide (PI) dependent PLD that is most active at micromolar levels of $Ca^{2+}$; and the phosphatidylinositol (Ptdln) specific PLD that is $Ca^{2+}$ independent (167). PLD has been cloned from a number of plants (168), animals (169), and fungi (170) and found to constitute a supergene family of many isoforms (171). The PLD isoforms from *Arabidopsis* have been divided into five groups: PLD$\alpha$, $\beta$, $\gamma$, $\delta$, and $\varepsilon$. (168). The PLD$\alpha$ gene product is responsible for the conventional PLD activity and differs physiologically from the PLD$\beta$, and PLD$\gamma$ isoforms based on $Ca^{2+}$ requirements and pH (172). From tomatoes, three PLD$\alpha$ forms and two PLD$\beta$ classes have been cloned (173). Different expression patterns were observed in different plant tissues and organs for each PLD. In fruit, PLD$\alpha$3 appeared to be transiently accumulated during early ripening, whereas PLD$\alpha$2 was accumulated throughout fruit development and maturation (173).

#### *16.3.2.1 Role of PLD in Senescence and Chilling*

PLD activities have been observed in many cellular functions during seed germination, aging, various abiotic and biotic stresses, and senescence (140,167,174). In membranes of tomato fruit stored at low temperature, an accumulation of phospholipid catabolites occurs due to differential effects of reduced temperature on the activities of lipid degrading enzymes (175). Destabilization of membranes has been suggested as one of the causes of chilling injury (176). In ripening tomato fruits, rigidification of microsomal membranes has been reported to activate PLD and increase membrane catabolism (177). Increased PLD activities during and after chilling are suggested to result in chilling injury in maize (139) and cucumber fruits (178). In *Arabidopsis*, cold stress increased the expression of PLD$\alpha$, but not PLD$\beta$ or PLD$\chi$, implying that PLD$\alpha$ has a role in plant responses to low temperature stress (174). Recently the many roles of PLD in signal transduction have been reported (167). In tomatoes, PLD$\beta$ is suggested to have a role in signal transduction due to its low abundance, activation by micromolar concentration of $Ca^{2+}$, a concentration

range that arises locally during signaling (173), whereas a metabolic role has been assigned for the various forms of PLDα. Our studies on the role of PLD during fruit ripening in cherry tomatoes showed that the soluble and membrane associated PLD activities increased during fruit development, which peaked at the mature green and orange stages (178a). It was reported by Jandus et al. (179) that PLD activity decreased slightly between the mature green and orange stages when the tomatoes ripened on the plants, but increased between the orange and red stages to values higher than at the mature green stage. However, when tomatoes were harvested at the mature green stage and left to ripen at room temperature, PLD activity decreased by about 40% between the mature green and red stages. It has been reported that phosphatidic acid increased as much as twofold while total phospholipids decreased about 20–25% during ripening of tomato pericarp (180,181). Treatment with lysophosphatidylethanolamine, which acts as a specific inhibitor of PLD activity (182) retarded senescence in tomato fruits and leaflets (183). From the observations on PLD activity at low temperature, ripening and senescence, it is conceivable that the quality of tomatoes, which is highly chilling sensitive, may be affected to a large degree by the modulation of PLD activity.

#### *16.3.2.2 Regulation of PLD Activity*

The activities of PLD are affected by a number of factors such as $Ca^{2+}$ concentration, substrate lipid composition, pH changes, and mastoparan, a tetradecapeptide G-protein activator (167). Sequence analysis indicates that plant PLDs contain a $Ca^{2+}$ to phospholipid binding fold, called the C2 domain at the N terminus. The C2 domains of PLDα and PLDβ have been demonstrated to bind $Ca^{2+}$, with PLDβ having a higher affinity for $Ca^{2+}$, whereas the $Ca^{2+}$ requirement of PLDα is influenced by pH and substrate lipid composition (167). PLDα is active at near physiological, micromolar $Ca^{2+}$ concentrations at an acidic pH of 4.5–5 in the presence of mixed lipid vesicles. PLDβ and PLDχ are optimally active under physiological micromolar concentration of $Ca^{2+}$ concentrations at neutral pH and may play an active role in signal transduction. The relative distribution of PLD between the soluble and membrane fractions changes during development and in response to stress (184,185). It has been shown that $Ca^{2+}$ binding increases the affinity of the C2 domains for membrane phospholipids (186). This shows that the C2 domain in PLD is responsible for mediating a $Ca^{2+}$ dependent intracellular translocation between the cytosol and membranes. An increase in cytosolic $Ca^{2+}$, as well as a decrease in cytosolic pH, has been reported to occur in response to stress (187), which are favorable conditions for the activation of PLDα. PLDs associated with microsomal membranes are correlated with stress induced activation of PLD mediated hydrolysis (184,185). The increased association of preexisting PLD in the cell with membranes may represent a rapid and early step in PLD activation during stress responses (185).

#### *16.3.2.3 Antisense Suppression of PLD Activity*

In order to study the role of PLD in fruit ripening and senescence of fruits, we have developed transgenic tomatoes expressing antisense PLDα cDNA. The fruits from antisense Celebrity tomato (a fresh eating type) were smaller than the control fruits and showed a 30% decrease in PLD activity during development (178a). After storage for two weeks at room temperature, the control fruits developed wrinkles, indicative of senescence and dehydration, whereas the transgenic fruits appeared to be relatively normal. The transgenic fruits were also firmer, possessed a higher level of red pigmentation and increased level of soluble solids (178a). Transgenic Celebrity fruits showed a decrease in PLD expression as evidenced from Northern blot. Even though very few transcripts were detected at the mature

green, orange and red stages in the antisense PLD celebrity fruits, PLD activity was present at these stages suggesting a very low turnover rate of PLD, and that PLD synthesized at young or intermediate stages remains functional even at the red stage (178a). These results suggest that for effective inhibition of PLD using antisense suppression, PLD expression has to be reduced at an early stage of fruit development using an appropriate fruit specific promoter. In our experiments we have used a constitutive promoter (CaMV 35S) for tomato transformation. The antisense Celebrity fruits also showed low levels of PLD activity during ripening, suggesting that the natural senescence process was retarded, which was translated into increased firmness in these fruits. This observation was contrary to earlier results in *Arabidopsis* where antisense suppression of PLDα resulted in retardation of ABA and ethylene promoted leaf senescence, without affecting the natural senescence of leaves (188). It has been reported that the phospholipid content of tomato fruit declines during ripening (180). This decrease in phospholipid content could be due to a high PLD activity. It was interesting to note that antisense suppression of PLDα in an ornamental cherry tomato cultivar, Microtom, did not show any significant reduction of PLD activity (178a). However, ethylene climacteric of the transgenic fruits was delayed by nearly six days, as compared to the control fruits. In Microtom, PLD activity declined during ripening in the control fruits, whereas transgenic fruits retained much higher levels of PLD activity. This may be related to the delayed climacteric in the transgenic fruits, indicating a slower rate of deterioration. *In situ* localization of PLD by immunolabeling followed by electron microscopy also supports this observation. These results suggest that fruits from different cultivars may differ in their pattern of senescence and the relative role of PLD may differ between fruits and leaves. It is unclear why PLD activity in transgenic Microtom was higher compared to control plants during ripening, as opposed to the observation in Celebrity. It has been reported that in PLDα suppressed *Arabidopsis*, the expression and activities of other PLD isoforms are not altered (189) which means that the other PLD members cannot compensate for the loss of PLDα.

### 16.3.3 Lipoxygenase

Lipoxygenases (LOX, EC 1.13.11.12) are a class of enzymes that catalyze the hydroperoxidation of *cis-cis*-1,4-pentadiene moieties in polyunsaturated fatty acids and that occur widely in both plant and animal kingdom. LOX pathways in higher plants and mammals are different in two main respects: in mammals the main LOX substrates are arachidonic and eicosapentaenoic acids whereas linoleate and α-linolenate are the most important LOX substrates in plants; and the hydroperoxide metabolizing enzymes are different in plants and mammals (190). Although LOX isoforms occur in most plant cells, the tissue specific expression level of LOX within a plant can vary substantially depending on developmental and environmental conditions (191). Plant lipoxygenases have been implicated as having a role in the loss of membrane integrity associated with senescence, flavor and odor formation, response to pest attack, and wounding (192). Products of the LOX pathway such as traumatin, jasmonic acid, oxylipins, and volatile aldehydes, are supposed to play a key role in signal transduction in response to wounding, as antimicrobial substances in host–pathogen interactions, as regulators of growth and development, and as aromatic compounds that affect food quality (191).

#### *16.3.3.1 Role of Lipoxygenase in Membrane Deterioration*

Lipid peroxidation is an inherent feature of senescence and generates a variety of activated oxygen species such as singlet oxygen and the alkoxy and peroxy radicals. Alkoxy and peroxy radicals are formed directly as decomposition products of lipid peroxides and singlet

oxygen is formed through the interaction of lipid peroxy radicals (157). Lipid peroxidation is initiated either by the action of reactive oxygen species or enzymatically by the action of lipoxygenases (LOX). LOX appears to play an important role in the deterioration of membranes during senescence by initiating lipid peroxidation, and also by forming activated oxygen independently (193). Increasing data suggests that LOX is activated by various stresses such as wounding (194), water deficit (195), thermal stresses (196), and ozone stress (196). It is known that the primary site of action of various stresses is the biomembrane (151), which results in the liberation of free linoleic and linolenic acids (197). Some results also suggest the direct oxygenation of membrane lipids and biomembranes by LOX (195,198–200). The role of LOX in membrane deterioration is evident from its association with membrane in tomato fruits (192,201) and carnation petals (202) as a membrane bound LOX can attack the membrane lipids more readily than a soluble one. This is supported from the observation that LOX can oxygenate esterified polyenoic acids in complex lipids and biomembranes, in addition to free polyenoic fatty acids such as linoleic and linolenic acids (190). Yamauchi et al. (198) reported the oxygenation of dilinolenoyl monogalactosyldiacylglycerol in dipalmitoylphosphatidylcholine liposomes by a crude soybean LOX preparation containing all the three isozymes, thus demonstrating that plant LOX catalyzes the oxygenation of both free polyunsaturated fatty acids and monogalactolipids. Brash et al. (199) observed that soybean LOX-1 oxidizes fatty acid residues within phoshatidylcholine and other phospholipids such as phosphatidylinositol lipids and_phosphatidylethanolamine. Similar results were reported by Kondo et al. (200) from their studies on LOX action in soybean seedlings and Maccarrone et al. (195) on soybean LOX during water deficit. It has been proposed that the role of soybean cotyledon LOX during the early stages of seedling growth is the disruption of storage cell membranes, enhancing their permeability (203). These evidences corroborate the role of LOX in membrane deterioration. However, there is controversy regarding the role of LOX in senescence, as its activity increases during senescence in *Pisum sativum* foliage (204), whereas in soybean cotyledons, total LOX activity has been shown to decrease with advancing senescence (205). The occurrence of LOX activity in young and expanding tissues as well as the observation that soybean LOX is not induced in senescing tissues, argues against the role of LOX in senescence (191). However, soybean seedlings subjected to water stress showed an increase in specific activity of their major lipoxygenases, LOX-1 and LOX-2, which was paralleled by the increase of LOX content and mRNA, indicating that osmotic stress modulates the expression of LOX genes at the transcriptional level (195). Osmotic stress also increased the oxidative index of biomembranes by increasing the hydroperoxide content of the lipid ester fraction. Water deficit has been reported to impair cell membrane functioning (206). Based on the observed enhancement of both LOX activity and membrane oxidative state in response to water deficit, the authors suggested that their results corroborate the hypothesis of a role of LOX in plant membrane deterioration. Studies of the effects of thermal injury (heat shock and cold) and ozone treatment on LOX activity of soybean seedlings have shown that cold stress decreased the specific activities of LOX1 and LOX2, which is attributed to at least in part to a down regulation of gene expression at the translational level (196). Both heat shock and ozone treatment enhanced the LOX-specific activities, acting at the level of transcription of the genes. It is proposed that LOX-1 and LOX-2 are involved in the thermotolerance of soybeans and in the precocious aging induced by ozone. It has been suggested by the authors that cold, heat shock, and ozone can ultimately act on cell membranes. This corroborates the hypothesis for a major role of LOX in the control of membrane integrity. It has been observed that during ripening in tomato fruit pericarp, two distinct LOXs were identified based on their pH optima and their sensitivity to the LOX inhibitor nordihydroguaiaretic acid (207). Both these activities increased sharply during early ripening stages, and decreased after the fruit had ripened

fully. It has been shown that the LOX require free fatty acids as their substrate and the timing and extent of peroxidative reactions initiated by LOX are determined by the availability of these substrates which are made available through the action of lipolytic acyl hydrolase (157,201). All these results suggest that a method of enhancing shelf life and quality of fruits could be by the regulation of LOX activity.

#### *16.3.3.2 Tomato fruit Ripening and Lipoxygenases*

Studies on LOX genes during tomato fruit ripening using a low ethylene producing fruit containing an ACC oxidase (*ACO1*) sense suppressing transgene, and tomato fruit ripening mutants such as *Never-ripe* (*Nr*), and *ripening-inhibitor* (*rin*), have demonstrated that expression of three LOX genes *TomloxA, TomloxB* and *TomloxC* is regulated differentially during fruit ripening, and that ethylene and a separate developmental component are involved (208). The expression of *TomloxA* declines during ripening and this is delayed in the *ACO1* transgenic low ethylene and *Nr* fruit, indicating that this phenomenon is ethylene regulated. Transcript abundance also declines during *rin* fruit development indicating that developmental factors also influence the expression of *TomloxA*. *TomloxB* expression increases during fruit ripening, which is also stimulated by ethylene. TomloxC gene expression is up regulated in the presence of ethylene and during ripening. The principal substrates of LOX in tomato fruit are the linoleic and linolenic acids, and the action of the 13-lyase on the 13-fatty acid hydroperoxide products of these substrates results in the production of hexanal and hexenal respectively. Release of these aldehydes following disruption of the tissue results in the production of the typical aroma characteristic of fresh tomatoes (209). As the ripening process continues, the thylakoid membranes break down as the chloroplasts are transformed into chromoplasts. It has been suggested that LOX may be the trigger for the chloroplast to chromoplast transition (210), and the polyunsaturated fatty acids in the thylakoid may be acted upon by LOX, and subsequently by a lyase, to release hexanal and hexenal, which in turn influence the flavor and aroma characteristics of the fruit (209). It has been suggested that the various LOX genes in tomato fruit may aid either in the defense mechanisms early in unripe fruit, or flavor and aroma generation and seed dispersal mechanism in the later developmental stages (208).

#### *16.3.3.3 Regulation of LOX by Genetic Manipulation*

With the genetic tools available today, it is possible to identify the role or function of a particular gene or gene product either by over expressing or down regulating the gene. Overexpression of LOX2 gene from soybean embryos fused with the enhancer of alfalfa mosaic virus under the control of a duplicated CaMV 35S promoter in transgenic tobacco increased the fatty acid oxidative metabolism as evidenced by a 50–529% increase in $C_6$ aldehyde production (211). The impact on $C_6$ aldehyde formation was greater than the effect on production of fatty acid hydroperoxides, which is consistent with other studies indicating the greater involvement of soybean embryo LOX2 in generating $C_6$ aldehydes than that of other well characterized LOX isozymes. To evaluate the role of LOX in the onset of plant defense, transgenic tobacco plants expressing the antisense tobacco LOX cDNA were developed which showed strongly reduced elicitor and pathogen induced LOX activity (212). A linear relationship was observed between the extent of LOX suppression and the size of the lesion caused by the fungus, *Phytophthora parasitica*. The antisense plants also showed enhanced susceptibility toward the compatible fungus *Rhizoctonia solani*. The authors suggested that their results demonstrate the strong involvement of LOX in the establishment of incompatibility in plant–microorganism interactions, consistent with its role in the defense of host plants. Antisense tomato plants were developed with

TomloxA under the control of fruit specific promoter 2A11 and ripening specific promoter of polygalacturonase (213). Reduced levels of endogenous *TomloxA* and *TomloxB* mRNA (2–20% of wild type) were detected in transgenic fruit containing 2A11 promoter compared to nontransformed plants, whereas the level of mRNA for *TomloxC* was unaffected. LOX enzyme activity was also reduced in these transgenic plants. However, no significant changes were observed in flavor volatiles. The transgenic plants with PG promoter were less effective in reducing endogenous LOX mRNA levels. The authors concluded that either very low levels of LOX are sufficient for the generation of $C_6$ aldehydes and alcohols, or a specific isoform *TomloxC* in the absence of *TomloxA* and *Tomlox*B is responsible for the production of these compounds. Transgenic tomato and tobacco plants were developed by transformation using the chimeric gene fusions of *TomloxA* and *TomloxB* promoter with $\beta$-glucuronidase (GUS) reporter gene. GUS activity in *tomloxA-gus* plants during seed germination peaked at day 5 and was enhanced by methyl jasmonate whereas no GUS activity was detected in *tomloxB-gus* seedlings (214). During fruit development, GUS expression in *tomloxA-gus* tobacco fruit increased 5 days after anthesis and peaked at 20 days after anthesis. In *tomloxB-gus* tobacco GUS activity increased at 10 days after anthesis and peaked at 20 days after anthesis. In *tomloxA-gus* tomato fruit, GUS activity was observed throughout fruit ripening, with highest expression at the orange stage, and the expression was localized to the outer pericarp during fruit ripening. In *tomloxB-gus* fruit, GUS activity was detected at the mature green stage, while expression was localized in the outer pericarp and columella. It has been shown that antisense transgenic potato plants with reduced levels of one specific 13-LOX isoform (LOX-H3) largely abolished the accumulation of proteinase inhibitors upon wounding, indicating that this LOX-H3 plays an important role in the regulation of wound induced gene expression (215).

The genetic manipulation studies on LOX show a specific role of LOX in various developmental processes and defense mechanisms. However, considering the presence of various isoforms in tomatoes and the roles they play, it is very important to characterize the specific role of each isoform before regulating its expression for a specific intent. For example, from the results of Beaudoni and Rothstein (214), it appears that antisense suppression of *TomloxA* may be helpful in enhancing the shelf life of the tomato fruit during storage without affecting the flavor of the fruit.

### 16.3.4 Cell Wall Metabolism and Fruit Softening

Tomato fruit ripening is a highly regulated developmental process requiring expression of a large number of gene products (216). Enzymes involved in the degradation of cell walls, complex carbohydrates, chlorophyll, and other macromolecules must be coordinately expressed with enzymes that make the fruit desirable nutritionally and aesthetically. Polyglalacturonases [PGs, poly (1→4-α-D-galacturonide) glycanohydrolases] are enzymes that catalyze the hydrolytic cleavage of galacturonide linkages in the cell wall, and are the most widely studied among cell wall hydrolases. PG has been implicated as an important enzyme in fruit softening based on its appearance during ripening, corresponding to the increase in fruit softening. In a number of cultivars, there is a correlation between levels of PG activity and the extent of fruit softening; it degrades isolated fruit cell walls *in vitro* in a manner similar to that observed during ripening and several ripening mutants that have been described with delayed or decreased softening are deficient in PG activity (217). Results on the genetic manipulation of PG has suggested that PG activity alone is not sufficient to affect fruit softening (218), and other enzymes such as pectin-methylesterase, $\beta$-galactosidase, and expansins are involved in fruit softening. This discussion is not intended to cover this area, as it has been reviewed recently by Brummell and Harpster (218) and through another chapter in this book.

## 16.4 CONCLUSION

Fruit color, texture, nutritional value, and flavors are the most important parameters that affect the quality of fruits and vegetables. The post harvest storage conditions of fruits and vegetables also affect these parameters as well as the shelf life, and the type of fruit and vegetables determines the extent of the effect. In the case of potatoes and tomatoes, it is apparent from the above discussion that chilling induced changes affect the above parameters even though preharvest factors and cultivar identity also contribute to this effect. While damages to membranes are attributed to be responsible for these changes in both crops, in the case of potatoes, carbohydrate metabolism also plays a major role. Our understanding of the genes involved in the membrane deteriorative processes as well as carbohydrate metabolism have resulted in the alleviation of these effects to a certain extent. Considering the roles of PLD and LOX in the membrane deteriorative pathway, it might be possible to enhance the shelf life of tomato by generating a double transgenic plant with suppressed activities of PLD and LOX.

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