Review Article

Biochemistry of fruit softening : an overview

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ABSTRACT

Softening is a developmentally programmed ripening process, associated with biochemical changes in cell wall fractions involving hydrolytic processes resulting in breakdown of cell-wall polymers such as cellulose, hemicelluloses and pectin etc. Various hydrolytic reactions are brought about by polygalacturonase, pectin methyl esterase, pectate lyase, rhamnogalacturonase, cellulase and β-galactosidase etc. Besides these enzymes, expansin protein also plays an important role in softening. Textural changes during ripening help in determining the shelf life of a fruit. An understanding of these changes would help in formulating procedures for controlling fruit softening vis-à-vis enhancing shelf life of fruits. In the present review an attempt has been made to coalesce recent findings on biochemistry of fruit softening. **[Physiol. Mol. Biol. Plants 2009; 15(2) : 103-113]** *E-mail : randhirbiotech@yahoo.com*

Key words : *Softening, shelf life, pectin, degradation*

Abbreviations : *ACO – 1-aminocyclopropane-1-carboxylate oxidase, PL – pectate lyase, PME – pectin methylesterase, PG – polygalacturonase, RG – Rhamnogalacturonase, XET – xyloglucan endotransglycosylase*

INTRODUCTION

Fruits constitute a commercially important and nutritionally indispensable food commodity. Being a part of a balanced diet, fruits play a vital role in human nutrition by supplying the necessary growth regulating factors essential for maintaining normal health. One of the limiting factors that influence their economic value is the relatively short ripening period and reduced post-harvest life (Prasanna *et al.*, 2007). Softening is a developmentally programmed ripening process in many fruits, providing different textures with various fruits including juiciness, crispness, and stiffness (Seymour *et al.*, 2002). Temperate fruits can be classified into two categories according to their softening behavior and textural properties (Bourne, 1979). One group includes those fruits that soften greatly during ripening, acquiring a melting texture, whereas the other group comprises fruits that soften moderately and display a crisp fracturable texture. Strawberry (*Fragaria* × *ananassa*, Duch., cv Chandler) is included in the first group, joint to other economically important crops such as tomato (*Lycopersicon esculentum*) and avocado (*Persea americana*) (Jiménez-Bermúdez *et al.*, 2002).

Softening is a very important aspect of the ripening syndrome. Substantial research has been conducted to investigate the events that occur in a fruit following the appearance of the climacteric rise (Brummell and Harpster, 2001; Dumville and Fry, 2003; Trainotti *et al.*, 2006; Chourasia *et al.*, 2006). Fruit softening is probably caused by the cumulative effect of a range of modifications occurring in the networks of polymers making up the primary cell wall, all of which contribute in different ways to a loss of firmness and changes in textural qualities. Modifications in cell wall polymers during ripening are complicated and considered to involve the coordinated and interdependent action of a range of cell wall-modifying enzymes and proteins such as polygalacturonase (PG, EC 3.2.1.15), pectin methylesterase (PME, EC 3.1.1.11), β-galactosidase (EC

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3.2.1.23) xyloglucan endotransglycosylase (XET, EC 2.4.1.207) and expansin, (Brummell and Harpster, 2001). However, the pectin and xyloglucan contents and composition in fruit cell walls are different in various fruit species (Redgwell *et al.*, 1997; Wakabayashi, 2000), and the nature, timing, and extent of the modification of cell wall polysaccharides vary between species (Rose *et al.*, 1998; Brummell and Harpster, 2001). Therefore, the role of individual cell wall-modifying enzymes in fruit softening would be dissimilar in different fruit species. Fruit softening is a complex process that involves three sequential steps: loosening of cell wall mediated by expansins, depolymerization of hemicelluloses, and finally polyuronide depolymerization by polygalacturonase or other hydrolytic enzymes (Brummell *et al.*, 1999b). In this review, we have focused on giving an overview of the biochemistry of fruit softening.

Cell wall structure and softening - associated changes

The presence of carbohydrate rich cell wall is a distinguishing feature of a plant cell. The cell wall is approximately 30 % cellulose, 30 % hemicellulose, 35 % pectin and 5 % protein in dicotyledonous plants (Fry, 1988). In fruit cell wall, pectin content is higher and protein content lower (Knee and Bartley, 1981). Cosgrove (2001) presented the detailed structure of plant cell wall and its loosening.

Cellulose microfibrils are coated with and cross-linked together with hemicellulose and the spaces in these networks are filled with pectins, which also form a network (Brummell and Harpster, 2001). [In ripening fruits of tomato (*Lycopersicon esculentum* L. var 83-G-38), the amounts of cellulose and xyloglucan remained constant during tissue softening, but the relative molecular weight of xyloglucan decreased markedly and the Mr of cellulose declined slightly] (Maclachlan and Brady, 1994). Xyloglucan is the principal hemicellulose and present in both dicot and monocot cell walls, although it is present in far larger amounts in the walls of dicots (about 20 %) relative to those of monocots (about 2 %) (Darvill *et al.*, 1980). Depolymerization of hemicellulosic polysaccharides during fruit softening has been reported in various fruits, such as tomatoes, melons, strawberries, and avocados (Sakurai and Nevins, 1997; Rose *et al.*, 1998).

Pectins are the common and major components of primary cell wall and middle lamella, contributing to the texture and quality of fruits. Structurally, pectins are a diverse group of heteropolysaccharides containing partially methylated D-galacturonic acid residues with side chain appendages of several neutral polysaccharides. The galacturonic acid occurs in two major structural forms, linear homogalacturonan and a branched rhamnogalacturonan. The degree of polymerization/esterification and the proportion of neutral sugar residues/side chains are the principal factors contributing to their heterogeneity (Prasanna *et al.*, 2007). During fruit softening, pectins (Fischer and Bennett, 1991) and hemicelluloses (Wakabayashi, 2000) typically undergo solubilization and depolymerization that are thought to contribute to cell wall loosening and disintegration. Cell wall polysaccharide breakdown causes ripening-associated softening (Vicente *et al.*, 2007). Cell wall polysaccharide depolymerization and the expression of cell wall metabolism-related genes were examined in transgenic melon (*Cucumis melo* var. Cantalupensis naud) fruit with suppressed expression of the 1-aminocyclopropane-1-carboxylate oxidase (ACO) gene and fruits treated with ethylene and 1-methylcyclopropene (1-MCP). Softening was completely inhibited in the transgenic fruit but was restored by treatment with exogenous ethylene. Moreover, postharvest application of 1-MCP after the onset of ripening completely halted subsequent softening, suggesting that melon fruit softening is ethylene-dependent (Nishiyama *et al.,* 2007).

Cell wall disassembly in ripening fruit is highly complex, involving the dismantling of multiple polysaccharide networks by diverse families of wall-modifying proteins (Nishiyama *et al.,* 2007). A class of proteins called expansins are cell wall-localized proteins associated with numerous tissues and developmental stages undergoing changes in size and shape (Cosgrove, 2000). Expansins appear to operate by disrupting hydrogen bonds between cellulose microfibrils and xyloglucans that bind them to one another in plant cell walls (Whitney *et al.*, 2000). Expansins have been shown to play an important role in fruit softening (Rose *et al.*, 1997; Anjanasree and Bansal, 2003). The ripening-associated expansins might contribute to cell wall degradation by increasing the accessibility of other cell wall-modifying proteins, such as PG and cellulase to structurally important cell wall polymers (Rose and Bennett, 1999).

Role of cell wall degrading enzymes in softening

Major cell-wall degrading enzymes along with their functions and changes in their activities are included in Table 1 (Johnston *et al.*, 2002). Cellulase often referred to as EGase (Endo (1→4) β-D glucanase) hydrolyzes internal linkages of (1→4) β-D-linked glucan chains.

Table 1. Major cell wall modifying enzymes in ripening fruits.

Cell wall enzyme	Function	Activity during ripening
Cellulase (EGase) EC 3.2.1.4	Hydrolyse β-1,4 glucan linkages in cellulose and xyloglucan	Decreases
Xyloglucan-endotransglycosylase EC 2.4.1.207	Hydrolyse and/or transglycosylate xyloglucan	Decreases
Glycosidases (i.e., β-galactosidase EC 3.2.1.23)	Terminal removal of galactosyl residues from pectin and xyloglucan	Increases
Endo-polygalacturonase EC 3.2.1.15	Hydrolytic cleavage of α-1,4-galacturonosyl linkages in unesterified pectin	Increases
Pectate lyase EC 4.2.2.2.	Cleavage of de-esterified pectin	Increases
Pectin methyl esterase EC 3.1.1.11	Removal of methyl groups from esterified pectin	Increases
Rhamnogalacturonase A	Hydrolyse α-1,2 linkages between galacturonosyl and rhamnosyl residues in pectin	Not measured

EGase activity has been found in fruits of all species (Brummell *et al.*, 1994). Suppression or overexpression of endo-(1→4) β-D-glucanase activity had no detectable effect on fruit softening or the depolymerization of matrix glycans, and neither the substrate nor the function for this enzyme has been determined (Brummel and Harpster, 2001). EGase, in addition to the pectic enzymes, plays an important role in fruit softening (Pesis *et al.*, 1978). The localization of EGase in the regions of the fruit associated with abscission zones suggests the involvement of the enzyme in fruit separation as well as softening (Sexton *et al.*, 1997). Two EGases, Cel1 and Cel2, show increase in mRNA coincident with ripening (Lashbrook *et al.*, 1994), but antisense suppression of each of these genes separately did not detectably alter fruit softening (Brummell *et al.*, 1997; Lashbrook *et al.*, 1998).

The role of XET activity in softening is also obscure, and the activity responsible for xyloglucan depolymerization during ripening, a major contributor to softening, has not yet been identified (Brummel and Harpster, 2001).

Hemicellulose modification is brought about by cell wall degrading enzymes such as XET and EGase. XET cleaves internal linkage of the (1→4) β-D-glucan backbone of xyloglucan. XET activity may be involved specifically in xyloglucan modification, and although total XET activity was much higher in green rather than in red fruit (Faik *et al.*, 1998), the mRNA of one XET gene increased during ripening (Arrowsmith and de Silva, 1995). While two XETs, and two EGases were cloned from 'La France' fruit, only PC-XET1 expression was detected at the preclimacteric stage. It was further increased during fruit softening, and was affected in part by treatment with 1-MCP, a potent inhibitor of ethylene perception (Hiwasa *et al.*, 2003). This suggests that the expression of PC-XET1 is regulated by both developmental factors and ethylene. The increase of XET activity and its mRNA accumulation have also been shown to correlate with fruit softening in tomato and kiwifruit (Arrowsmith and de Silva, 1995).

β-Galactosidase removes terminal non-reducing β-D-galactosyl residue from β-D-galactoside. Seven tomato β-galactosidase (TBG) genes are expressed during fruit development (Smith and Gross, 2000), six are known to be expressed during ripening. β-Galactosidase proteins have also been purified and characterized from a number of fruits including kiwifruit (Ross *et al.*, 1993), coffee (Golden *et al.*, 1993), persimmon (Kang *et al.*, 1994), and apple (Ross *et al.*, 1994). Suppression of β-galactosidase activity early in ripening significantly reduces fruit softening, suggesting that the removal of pectic galactan side-chains is an important factor in the cell wall changes leading to ripening-related firmness loss (Brummel and Harpster, 2001). Down-regulation of tomato β-Galactosidase results in decreased fruit softening (Smith *et al.*, 2002).

Glycosidases such as β-hexosaminidase, α-mannosidase and α-galactosidase are believed to play a major role in textural softening during fruit ripening. The activities of β-hexosaminidase and α-galactosidase, which were low initially during fruit development, significantly increased during ripening in tomato. On the other hand, activity of α-mannosidase, which increased during fruit development, steadily increased further during ripening after a transient decrease (Jagadeesh *et al.*, 2004a). The activity of β-hexosaminidase in bell capsicum (*Capsicum annuum* var. Variata) increased slightly during fruit development, while it increased significantly during the ripening phase. On the other hand, α-mannosidase activity was prominent during fruit development compared to its activity during ripening. Enzyme activity of β-hexosaminidase was found to be always higher than that of α-mannosidase in this fruit. All these data suggested the presence of an inter-relationship between the two enzyme activities that would lend support to the novel implication of these two glycosidases in the textural softening associated with fruit ripening (Jagadeesh, 2004b).

Mannan transglycosylase is a novel cell wall enzyme acting on mannan-based plant polysaccharides in primary cell walls of monocotyledonous and dicotyledonous plant fruits. High levels of the enzyme activity were present in flowers of some kiwifruit (*Actinidia*) species and in ripe tomato (*Solanum lycopersicum* L.) fruits. Low levels were detected in mature green tomato fruits and activity increased during tomato fruit ripening up to the red ripe stage. (Roswitha *et al.*, 2004).

Role of pectin degrading enzymes

Experiments with pectolytic enzymes have de-emphasized the role of pectins in fruit softening (Jiménez-Bermúdez *et al.*, 2002). Pectin degrading enzymes such as PG, PME, pectate lyase (PL), and rhamnogalacturonase (RG) are the most implicated in fruit-tissue softening (Prasanna *et al.*, 2007).

Polygalacturonase

PG are enzymes that catalyze the hydrolytic cleavage of galacturonide. It has been implicated as the primary agent of polyuronide degradation in ripening tomato fruit (Themmen *et al.*, 1982; Huber, 1983) and hence implicated as the primary enzyme regulating tomato fruit softening (Brady *et al.*, 1983).

The softening process and the modification of pectic polymers in fruit has been shown to correlate with the expression of PG genes (Hiwasa *et al.*, 2003). The activity of PG during ripening in climacteric fruits has been positively correlated with softening of the fruit tissue (Mehar and Nath, 2005). Mehar and Nath (2005) further cloned four partial cDNAs, *MAPG1* (acc. no. AF311881), *MAPG2* (acc. no. AF311882), *MAPG3* (acc. no. AF542382) and *MAPG4* (acc. no. AY603341) for PG genes and studied their differential expression during ripening in banana and concluded that softening during ripening in banana fruit results from the concerted action of at least four PG genes, which are differentially expressed during ripening.

Transgenic experiments showed that PG activity is largely responsible for pectin depolymerization and solubilization, but that PG-mediated pectin depolymerization requires pectin to be de-methyl-esterified by PME, and that the PG beta-subunit protein plays a role in limiting pectin solubilization (Brummel and Harpster, 2001). However, it substantially affects the textural properties (increased viscosity) of pastes, the integrity of stored fruits, and resistance to postharvest pathogens (Kramer *et al.*, 1992; Langley *et al.*, 1994). Antisense suppression of PG activity to 0.5 % of wild-type levels (Sheehy *et al.*, 1988; Smith *et al.*, 1988) resulted in only a modest reduction of polyuronide depolymerization (Brummell and Labavitch, 1997) and a very small increase in firmness later in ripening (Kramer *et al.*, 1992; Langley *et al.*, 1994). Furthermore, expression of a chimeric PG transgene in a nonsoftening mutant tomato fruit that normally lacks this activity resulted in polyuronide solubilization but did not restore softening (Giovannoni *et al.*, 1989). PG apparently plays a minor role in strawberry softening because little or no activity was found in fruits (Abeles and Takeda, 1990). The level of PG enzyme activity and polyuronide degradation have been correlated roughly with an elevated rate of tomato fruit softening, and on this basis PG was proposed to be a major determinant of tomato fruit softening. Giovannoni *et al.* (1989) demonstrated that high levels of PG enzyme activity and polyuronide degradation are not sufficient to induce softening in transgenic r/n (E8/PG)-2 fruit. Similar results in fruit from two other transformed plants, r/n (E8/PG)-1 and r/n (E8/PG)-3, were observed. Explanations for the lack of softening in propylenetreated rin (E8/PG) fruits indicated the possibility that perhaps insufficient levels of PG enzyme were generated by expression of the E8-PG chimeric gene. Sanwal and Payasi (2007) observed that delay in fruit softening in bananas on treatment with garlic plus sodium metabisulphite extract was accompanied by slowing of the rate of pectin

degradation. This was accomplished by the delay in the rate of increase in the activity of the enzyme involved in this process, namely PG.

Pectate lyase

PL otherwise known as pectate transeliminases, catalyse the eliminative cleavage of de-esterified pectin, which is a major component of the primary cell walls of many higher plants (Carpita and Gibeaut, 1993). The degradation of pectins by PL occurs by a β-elimination reaction in contrast to the hydrolytic mechanism of PG. PL like sequences from higher plants were first reported from tomato pollen (Wing *et al.*, 1989). Genes encoding PL (*pels*) have also been reported from style (Budelier *et al.*, 1990), rag weed (Griffith *et al.*, 1991), *Zea mays* (Turich, 1993), cedar pollen allergen cry j I (Sone *et al.*, 1994), *Zinnia elegans* (Domingo *et al.*, 1998), grape (Nunan *et al.*, 2001), and opium poppy latex (Pilatzke-Wunderlich and Nessler, 2001). Recently two *pel* genes have been reported from strawberry and these are strongly and predominantly expressed in the full ripe stage (Benitez-Burraco *et al.*, 2003).

Pel genes recently isolated from ripe strawberry has been proposed as a new candidate for pectin degradation, contributing to the loss of fruit firmness (Medina-Escobar *et al.*, 1997). The expression of this gene is restricted to ripening fruits and is inhibited by auxin treatment. Similarly, Domínguez-Puigjaner *et al.* (1997) isolated a gene with homology to PL in banana whose expression was induced during ripening of this climateric fruit. PL has been extensively studied in pathogenic bacteria, which secreted this enzyme causing depolymerization of pectins in the middle lamella and primary cell walls of higher plants, and consequently the maceration of plant tissues (Henrissat *et al.*, 1995). In bananas, the expression of two distinct PL-like genes (*Pel I and Pel II*) has been detected during ripening. Both show different levels of expression in ripening pulp and peel, with *Pel I* predominating. An active PL protein was produced by expression of banana *Pel I* in yeast. More importantly, for the first time from fruit tissue, PL activity has been obtained directly from banana pulp with a substantial increase in activity during ripening (Maŕin-Rodriguez, 2003, Payasi and Sanwal, 2003). Additionally, a PL sequence from strawberry has also been expressed in yeast giving an active protein, although the authors were unable to observe any endogenous enzyme activity in the fruits themselves (Medina-Escobar *et al.*, 1997). More recently PL gene expression has been manipulated in transgenic strawberry fruits and suppression of the PL mRNA during ripening resulted in significantly firmer fruits (Jiménez-Bermúdez *et al.*, 2002). The highest reduction in softening occurring during the transition from the white to the red stage. *Pel* gene is an excellent candidate for biotechnological improvement of fruit softening in strawberry (Jiménez-Bermúdez *et al.*, 2002). Chourasia *et al.* (2006) proposed that expression of PL may be closely associated with pectin degradation during ripening and play an important role in mango softening. Sesmero *et al.* (2007) observed the antisense inhibition of pectate lyase gene expression in strawberry fruit and found two independent lines showing a reduction in pectate lyase mRNA transcript level of 90 % (Apel 14) and 99 % (Apel 23). At harvest, ripen fruits from these two lines were significantly firmer than control.

Pectin methyl esterase

PME de-esterifies polyuronides by removing methyl groups from the C-6 position of the galacturonic acid residue of pectins. PME is transcribed by multigene family in tomato (Turner *et al.*, 1996; Gaffe *et al.*, 1997). PME activity increases during strawberry ripening (Barnes and Patchett, 1976), but according to Huber (1984), this increment is not sufficient by itself to account for the large changes that occurred in water-soluble polyuronides. Thus, pectin degradation and softening of ripe strawberry must be mediated by a different cell wall hydrolytic enzyme. However, as observed in tomato (Brummell *et al.*, 1999b), it is possible that this enzyme is not the only component that determines the loss of firmness. The slight reduction of the decrement in firmness that occurs during the transition from the green to the white stage in apel fruits suggests that other cell wall-degrading mechanisms are taking place in unripe fruit. EGase (Harpster *et al.*, 1998; Llop-Tous *et al.*, 1999; Trainotti *et al.*, 1999) and expansin (Civello *et al.*, 1999) genes are candidates for cell wall degradation at these stages. Suppression of PME activity does not affect firmness during normal ripening, and suppression of beta-subunit protein accumulation increases softening (Brummel and Harpster, 2001). Suppression of PME activity had little effect on fruit firmness or ripening characteristics, but resulted in significant favorable changes in the soluble solids content of raw juice and serum viscosity, paste viscosity, and serum separation of processed juice (Tieman *et al.*, 1992; Thakur *et al.*, 1996) which is correlated with reduced polyuronide depolymerization during processing (Brummel and Harpster, 2001). PME is the most implicated in fruit-tissue softening (Prasanna *et al.*, 2007).

Rhamnogalacturonase

RG is able to release a galacturonic acid residue from the nonreducing end of RG chains but not from homogalacturonan (Mutter *et al.*, 1998). Activity of RG has been found in apples, grapes and tomatoes (Gross *et al.*, 1995). RG, a recently identified hydrolase in *Aspergillus aculeatus*, can degrade the pectic backbone and is potentially important in fruit softening and fungal decay (Gross *et al.*, 1995). RG is now being implicated in fruit softening (Prasanna *et al.*, 2007).

Starch metabolism during softening

Fruit starch reserves can be an important contributor to the sugar content of some ripe fruits (Souleyre *et al.*, 2004). The conversion of starch to sugars is the most remarkable chemical change occurring in banana pulp during ripening (Palmer, 1971). During ripening the starch content declines from 20-23 % in unripe fruit to 1-2 % in fully ripe fruit and at the same time the soluble sugars increase from less than 1 % to 20 % (Forsyth, 1980, Agarvante *et al.* 1990). Kojima *et al.* (1994) suggested the coordinated degradation of pectic and hemicellulosic polysaccharides and starch to be the main cause for the pulp softening process. Sanwal and Payasi (2007) further observed starch content to decrease progressively throughout the postclimacteric ripening of banana fruit. The decrease in starch content was accompanied by the increase in soluble sugars. The degradation of starch to sugars during the storage of ripe banana was accompanied by the increase in β-amylase activity. The increase rate of starch degradation with the increase in β-amylase activity suggests the major role of β-amylase in starch degradation and subsequent fruit softening, particularly in banana and other starch containing fruits.

Free radical mediated cell wall softening

Fruit softening also involves oxidative as well as hydrolytic degradation (Dumville and Fry, 2003). A role for H_2O_2 in the softening of pears was first suggested by Brennan and Frenkel (1977), who found that H_2O_2 concentrations increase at the onset of ripening. Treatment of pear tissues with xanthine or glycollate, which are substrates for enzymes that convert O_2 to superoxide ($\cdot O_2$–) or H_2O_2 respectively, promoted. Softening first and subsequently ethylene production. It is believed that the inhibition of endogenous glycollate oxidase by 2-pyridyl hydroxymethane sulphonate, which decreased H_2O_2 formation, restricted softening. Membrane deterioration was associated with ripening in blackberry fruits (Wang and Jiao, 2001). Tomato fruit ripening was accompanied by increased H_2O_2 concentrations and the oxidation of lipids and proteins (Jiménez *et al.* 2002). Changes in membranes during ripening have also been demonstrated by electron microscopy and by chemical analyses (Brady 1987). Damage to membrane lipids may be initiated by lipoxygenases, which are often induced early during ripening (Griffiths *et al.* 1999). Such membrane damages could provide the basis for the frequently reported increases in membrane leakiness in ripening fruits evidenced for example by intracellular re-distribution of K^+ (Almeida and Huber 1999), amino acid efflux, increased hydraulic conductivity, loss of turgor (Brady 1987), and accumulation of oligosaccharides in the apoplast (Dumville and Fry, 2003). The hydroxyl radical ($\cdot OH$), the most reactive known compound, if present in the apoplast, OH would cleave polysaccharides and might therefore bring about lead to wall-loosening (Schopfer, 2001; Schweikert *et al.* 2002). The formation of apoplastic $\cdot OH$ could occur by reactions (i) to (iii):

i. $Cu^{2+} + ½AH_2 \rightarrow Cu^+ + ½A + H^+$

ii. $O_2 + AH_2 \rightarrow H_2O_2 + A$

iii. $Cu^+ + H_2O_2 \rightarrow Cu^{2+} + \cdot OH + \cdot OH$

where AH_2 = ascorbate and A = dehydroascorbate; (iii) is a Fenton reaction and will also operate with other transition metal ions, e.g. Fe^{2+} (Halliwell and Gutteridge 1999). Reactions (i) to (iii) proceed readily under apoplastically relevant conditions of temperature and pH *in vitro*. Since the above three reactions require Cu^{2+}, ascorbate and O_2. Their presence in the apoplast will ensure the accurrence of these reactions (Fry *et al.,* 2002).

Role of expansin in fruit cell wall metabolism and softening

Expansins, are proteins with no apparent hydrolytic enzymatic activity (Cosgrove, 2000). Rose *et al.* (1997) suggested that expansins may enhance the accessibility of wall polymers to enzyme action, thereby accelerating wall hydrolysis. A probable mechanism of action of expansin is depicted in Figure 1. According to this model the expansin protein is hypothesized to disrupt the bonding of the glycans to the microfibril surface (Cosgrove, 2000). Expansin protein is directly correlated with fruit softening and has additional indirect effects on pectin depolymerization, showing that this protein is intimately involved in the softening process. However, the molecular mechanism by which expansins loosen cell walls has not yet been worked out on molecular terms (Neela *et al.*, 2006).

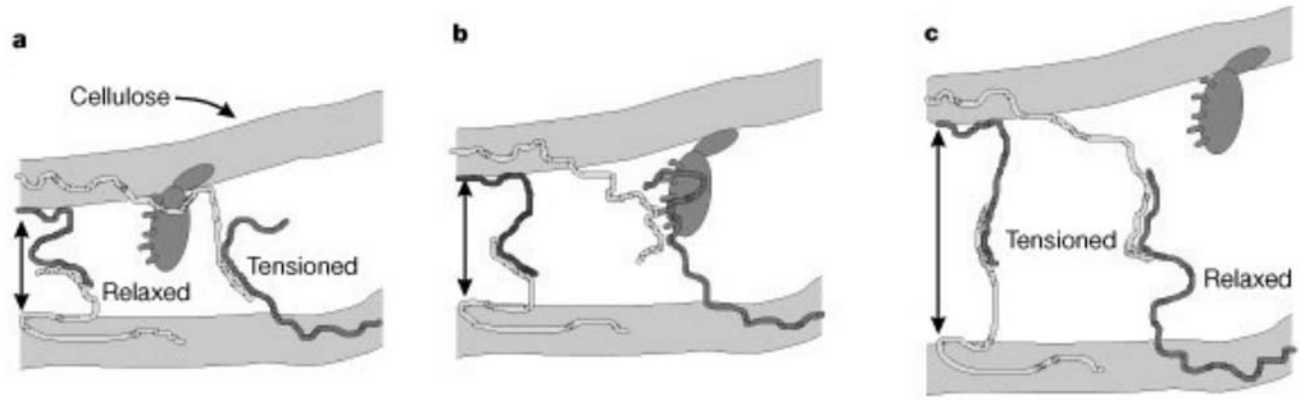

Fig. 1. Cellulose microfibrils are connected to each other by glycans (strands) that can stick to the microfibril surface and to each other. The expansin protein is hypothesized to disrupt the bonding of the glycans to the microfibril surface (a) or to each other (b). Under the mechanical stress arising from turgor, expansin action results in a displacement of the wall polymers (c) and slippage in the points of polymer adhesion (compare a and c) (Cosgrove, 2000).

Transgenic work has shown that the cell wall changes leading to fruit softening and textural changes are complex, and involve the coordinated and interdependent activities of a range of cell wall-modifying proteins (Brummel and Harpster, 2001). Expansins are encoded by large multigene families (Cosgrove *et al.*, 1997; Brummell *et al.*, 1999a), and the expression of expansin mRNA and protein is correlated with growth in many tissues of the plant, including hypocotyls (McQueen-Mason *et al.*, 1992), coleoptiles (Li *et al.*, 1993), leaves (Keller and Cosgrove, 1995), roots (Wu *et al.*,1996), internodes (Cho and Kende, 1997), and green fruits (Brummell *et al.*, 1999a). The tomato genome has a relatively large family of expansin genes (Rose and Bennett, 1999).

The role of the ripening-specific expansin, EXP1 protein in fruit softening and cell wall metabolism was investigated by suppression and overexpression of EXP1 in transgenic tomato plants (Brummel *et al.*, 1999a). Suppression and overexpression of EXP1 protein had opposite effects on fruit softening, indicating a role for EXP1 in this process. But unexpectedly they also caused indirect changes in different cell wall components. Overexpression of recombinant EXP1 protein in mature green fruit evoked extensive hemicellulose depolymerization and considerable softening, in the absence of polyuronide depolymerization. Thus, polyuronide depolymerization is not essential for at least one major component of softening. Fruit overexpressing EXP1 continue to soften during ripening, even though changes to the hemicellulose molecular mass profile are complete at the mature green stage, implying that mechanisms of softening other than hemicellulose breakdown occur as the fruit ripens. The magnitude of the difference in softness between overexpressing fruits and controls diminishes progressively with ripening, presumably as hemicelluloses of control cell walls also become increasingly depolymerized. In contrast, strong suppression of EXP1 protein accumulation increases fruit firmness to a similar extent throughout ripening. Suppression of EXP1 protein, however, does not prevent hemicellulose depolymerization, which proceeds at wild type levels, even though indirect effects on polyuronide disassembly are evident later in ripening (Brummel *et al.*, 1999a).

EXP1 protein is responsible for one component, perhaps by a loosening of non covalent linkages between unidentified polymers at the hemicellulose–microfibril interface, as suggested by McQueen-Mason and Cosgrove (1995). This is accompanied by the second and major component of softening, consisting of the depolymerization of structurally important hemicelluloses brought about by an independent mechanism that does not require EXP1 protein or for which only very small amounts of EXP1 protein are sufficient. A third aspect of firmness loss is polyuronide depolymerization, the later stages of which are dependent on the EXP1-mediated relaxation of the wall structure necessary to allow PG or other enzymes access to polyuronide substrate sites. This would have the effect of ensuring that cell wall loosening and the complete fruit softening process precede the final component of polyuronide disassembly, which is involved in fruit deterioration. Expansins can cause extensive and prolonged cell wall loosening in the absence of active cell wall hydrolases (McQueen-Mason and Cosgrove 1994). Ripening-related

expansin protein abundance is directly correlated with fruit softening and has additional indirect effects on pectin depolymerization, showing that this protein is intimately involved in the softening process It is suggested that the ripening-related expansin, may restrict or control the activities of other ripening-related enzymes necessary for the fruit softening process. (Brummel and Harpster, 2001).

CONCLUSION

Fruit softening is the process which occurs as a result of hydrolysis of various cell wall components including cellulose, hemicellulose, pectin and protein. The hydrolysis of these components is brought about by the action of PG, PME, PL, EGase, RG and β-galactosidase. The role of these enzymes has now been well confirmed by applying molecular techniques involving over and under expression of enzyme activity viz. alteration in the process of hydrolysis. Expansins are specialized group of proteins which also have been implicated in the process of softening due to their effect enhancing the accessibility of wall polymers to enzyme action, thereby accelerating wall hydrolysis. The exact role of expansin in softening process still needs to be ascertained by use of molecular tools.

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