Stewart Postharvest Review
An international journal for reviews in postharvest biology and technology

Molecular basis of cell wall degradation during fruit ripening and senescence

Willis O Owino[1*], Jane L Ambuko[2] and Francis M Mathooko[3]

[1] Gene Research Center, University of Tsukuba, Tennodai 1-1-1, Tsukuba City, 305-8572, Ibaraki, Japan.
[2] Laboratory of Pomology, Graduate School of Life & Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, 305-8572, Tsukuba City, Japan.
[3] Department of Food Science and Technology, Faculty of Agriculture, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000, Nairobi 00200, Kenya.

Abstract

Purpose of review: This review highlights progress made in the recent past in understanding the molecular basis of cell wall changes during fruit ripening and senescence.

Recent findings: Gene expression studies in several fruit have continued to show that isoforms of various classes of cell wall modifying enzymes and proteins are involved in cell wall disassembly during fruit ripening and senescence. However, manipulation of various genes encoding these enzymes in transgenic plants have also continued to challenge the central role of any individual cell-wall degrading enzyme in fruit softening, and it now appears that no single cell-wall modifying enzyme can be identified as being necessary and sufficient for textural changes accompanying fruit ripening. This may suggest that there might be a cooperative action between various enzymes (proteins) in controlling fruit ripening. Furthermore, it is possible that there are yet to be identified enzymes, which by acting at low amounts on particularly important chemical bonds, significantly contribute to textural changes during fruit ripening.

Limitations: Tomato is the model system of choice for studying the textural changes during ripening of fleshy fruit due to its commercial importance, a rich source of genetic and biochemical information, relative ease of gene transformation and availability of pleiotropic mutants. However, extrapolation of findings in tomato to other fruit species may not be true and therefore, fruit softening may have to be treated on a case-by-case basis.

Directions of future research: The individual roles of these enzymes and proteins are still being addressed in part through use of gene silencing techniques (antisense and co-suppression). Another technique is the use of "chimeric" genes to simultaneously silence several target enzymes in order to manipulate entire biochemical pathways. Proteomics strategies in tomato and other fruit will also be useful in characterising the fruit cell wall proteome to identify novel proteins and to reveal the suites of proteins that are co-expressed during fruit ripening and senescence. An additional approach in identifying genes regulating fruit texture is the use tomato pleiotropic mutations.

Key words: fruit; ripening; cell-wall modifying enzymes; gene expression

Abbreviations

EG	Endo Glucanase
EGase	Endo-1,4-β-glucanase
ESS	Ethylene Synthesis-suppressed
PG	Polygalacturonase
PME	Pectin Methylesterase
PL	Pectate Lyase
XTH	Xyloglucan Endotransglycosylase

***Correspondence to:** Willis O. Owino, Department of Food Science and Technology, Faculty of Agriculture, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000, Nairobi 00200, Kenya.
Tel/Fax: +81-298-537-734; email: willis@gene.tsukuba.ac.jp; willyjp2001@yahoo.com

Stewart Postharvest Review 2005, 3: 3
Published online 01 October 2005
DOI: 10.2212/spr.2005.3.3

Introduction

Fruit are an essential part of the human dietary intake and nutrition, supplying vital vitamins, minerals, fiber, lipids and other bioactive compounds for health promotion and disease prevention. Some fruit such as apricots, peaches, apples and berries are not only rich in vital nutrients and high in fiber, but also offer natural sweetness. As a bonus, the water found in fruit assists in curbing hunger by creating the feeling of "fullness." Fruit are normally harvested after attaining physiological maturity, when development is completed and growth has ceased. Ripening is then initiated and fruit acquire the organoleptic, aesthetic and edible characteristic qualities for consumption. During ripening fleshy fruit undergo profound changes in texture, colour, flavour and aroma.

The textural changes that occur during ripening of most fruit largely arise from the structural and compositional changes in the fruit cell wall. These changes include modifications of the structure and composition of the constituent polysaccharides that have been correlated with the expression of a range of hydrolases and transglycosylases, and the potential alteration of covalent and noncovalent interactions between polysaccharide classes [1**]. Fruit cell wall polysaccharides fall into three categories; pectins, hemicelluloses and cellulose. Pectins and hemicelluloses are components of the wall 'matrix' within which are embedded the skeletal, cellulosic microfibrils [2]. Additional minor cell wall constituents include a broad range of structural and enzymatic proteins, hydrophobic compounds and inorganic molecules. Solubilisation and depolymerisation of cell wall polysaccharides have been observed in most fleshy fruit, but the relative extent and timing can vary among species and even among different cultivars of a given species, causing different rates of softening.

The significant and often dramatic combination of cell wall loosening and degradation that occurs in practically all fruit species during ripening are collectively referred to as 'softening', but reflect multiple sensory attributes. Fruit softening results in negative quality attributes including high transportation and storage costs, short postharvest shelf-life, susceptibility to microbial infection and decreased consumer acceptability. As a result of the economic importance of fruit crop species, extensive research at the biochemical level has been devoted to elucidate the mechanisms responsible for the textural changes that occur during preharvest and postharvest ripening. However, the molecular basis of cell wall changes during fruit ripening is still poorly understood and remains an active area of study. An important purpose of research at molecular level is that, by using the knowledge obtained, it might be possible to interfere with some of the processes in order to curtail the extent of the softening process and, therefore, prolong the postharvest storage life of fleshy fruit.

Brummell and Harpster have comprehensively reviewed studies on fruit cell wall alterations in transgenic plants during ripening [2**]. The objective of the present review is to highlight the progress made in the recent past in understanding the molecular basis of cell wall changes during fruit ripening and senescence. The molecular studies on fruit cell wall degradation during ripening have focused on the expression and manipulation of genes encoding the cell wall enzymes outlined in the subsequent sections.

Polygalacturonase

Pectin is the most abundant class of macromolecules within the cell wall matrix. It is also abundant in the middle lamellae between primary cell walls where, being the major adhesive material between cells, it functions in regulating intercellular adhesion [3**]. Fruit cell walls are usually highly enriched with pectins. During fruit softening, pectins typically undergo solubilisation and depolymerisation that are thought to contribute to wall loosening and disintegration. Polygalacturonase (PG) catalyzes the hydrolytic cleavage of galacturonan linkages of pectin [2**]. PG is the most abundant and extensively studied of the pectin-degrading enzymes and many studies have focused on considerable pectin degradation that coincides with fruit softening in relation to expression of genes encoding PG and the activity of the PG enzyme. However, molecular genetic studies have revealed that even though PG is re-

sponsible for polyuronide depolymerisation and solubilisation, this makes only a partial contribution to fruit softening. However, the expression of the genes encoding PG together with the increase in the PG activity during ripening has been observed in several fruit.

The expression of PG genes increased strongly during ripening in 'Rocha' pears [4], peach [5*], and figs [6]. Comparative studies showed that different softening behaviours during ripening among three pear cultivars may be caused by different endo-PG activity and differential expression of PG genes [7*]. Differential expression of PG genes during ripening in banana fruit showed that *MAPG3* and *MAPG4* were regulated by ethylene whereas *MAPG2* was associated more with senescence [8]. In tomato, suppression of *LePG* alone did not significantly increase fruit firmness [9**]. However, simultaneous suppression of both *LeExp1* and *LePG* significantly reduced fruit softening during ripening. This increased firmness was evident at all stages of ripening from mature green (unripe) stage to the red ripe fruit, 21 days later, and the increased firmness was observed in fruit ripened on the plant or after harvesting at the mature green stage. Although fruit with suppressed expression of both *LeExp1* and *LePG* were firmer throughout ripening, the fruit continued to soften significantly. This indicates that other hydrolases with polymer-modifying functions or other proteins with unidentified influences on polymer structure or intermolecular associations were capable of completing the fruit softening process.

Pectate Lyases

The collapse of the hypothesis that PG represented the primary determinant of tomato fruit softening shifted attention to the isolation and functional analysis of alternative cell wall-associated or metabolising proteins [2**]. Alternatives to PG, which could play a major role in degrading pectins during fruit ripening, include pectate lyases (PLs). These are pectin degrading enzymes that randomly cleave β (1-4) linkages between galacturonosyl residues, generating 4, 5-unsaturated oligogalacturonates by β-elimination [3**. Until recently, it was thought that PLs were secreted mainly by plant pathogens and that their action resulted in the maceration of plant tissues. However, the tomato expressed sequence tags programme suggests a high level of PL-like gene expression in ripe tomato fruit indicating an important role of these enzymes in fruit softening [10]. The expression of PL genes has been observed in fruit such as banana [11,12], grapes [13], strawberry [14,15] and peach [5*]. In climacteric fruit, PLs appear to have the task of carrying out an early and coarse degradation of pectins, thus making them more susceptible to the subsequent attack by other degrading enzymes such as PGs. In non-climacteric fruit such as strawberry, PG apparently play a minor role in softening because little or no activity has been found in fruit. An antisense PL gene resulted in a high increase in firmness of full ripe strawberry fruit and reduced the postharvest softening, without affecting other fruit characteristics such as weight, colour, or soluble solids [16**]. This data suggest that PL plays a more important role in fruit softening than previously thought. Thus, this gene is an excellent candidate for biotechnological manipulation of fruit softening.

Pectin Methylesterases

Pectin is secreted into the cell wall in a highly methylesterified form and subsequently de-esterified in muro by pectin methylesterases (PMEs) [17**]. PMEs may play important roles in determining the extent to which demethylated polygalacturonans are accessible to degradation by PGs, releasing galacturonic acid (exo-PG) or oligogalacturonate (endo-PG), and the availability of homogalacturonan carboxylic groups for Ca^{2+} binding resulting in supramolecular assemblies and gels. The formation of these calcium-mediated pectin gels significantly affects the mechanical properties of the cell wall and adds rigidity to the wall [3**, 2**]. In most fruit, PME is expressed before ripening and is down-regulated by ethylene as ripening begins. PMEs play little role in fruit softening during ripening, but substantially affect tissue integrity [2**]. PME genes were recently isolated and expression patterns determined in strawberry [18], peach [5*] and grapes [19]. These data indicate that PME transcripts accumulate prior to fruit softening. In straw-

berry, low level accumulation of *FaPE1* mRNA was observed at the late stages of fruit ripening and, in particular during senescence. The inhibition of *FaPE1* following the endogenous ethylene burst would increase the degree of methyl esterification of pectic polysaccharides and thus reduce pectin stabilization mediated by calcium cross-links. The inability to form calcium cross-bridges would lead to cell separation and have a negative effect on fruit integrity [18]. Low PME expression could have been responsible for maintaining a strong cell wall observed in the *Cnr* tomato mutant, further implicating PME as playing an important role in maintaining fruit cell wall integrity [20**].

β-Galactosidase

Another possible contributor to wall loosening and relevant to fruit softening is the cleavage of the pectin–xyloglucan cross-links by enzymes such as β-galactosidase. β-Galactosidases of a number of fruit types were reported to possess β-galactanase activities, functioning possibly, as exo-glycanases [2**]. The mechanism by which β-galactosidase with β-galactanase activity can substantially affect modification of both pectin and hemicellulose is unclear and needs further investigation. Molecular approaches have been used to study the role of β-galactosidase in fruit development and ripening. β-Galactosidases from fruit sources occur in multiforms, seemingly encoded by multi-gene families; however, not all members of the gene family were ripening related [6, 21, 22, 23]. Down-regulation of a ripening-related β-galactosidase cDNA reduced ß-galactosidase enzyme activity and free galactose content, and increased tomato fruit firmness thus confirming a role for this gene's product in determining fruit firmness [24**]. In tomato, ethylene positively regulated mRNA abundance of *TBG4* in a time-dependent manner, but down-regulated *TBG5* and *TBG6* mRNAs [25]. Antisense suppression of a *TBG6* in transgenic tomato plants resulted in increased fruit cracking, reduced locular space, and a doubling in the thickness of the fruit cuticle [26**]. The decreased locular space phenomena observed in TBG6 transgenic lines can be useful in developing 'meatier' tomatoes, which could have significant impact in the fresh-cut slice industry. Further study in the genetic and molecular origins of increased cuticle thickness, without the deleterious effects of increased cracking, may be helpful in developing fruit with improved resistance to water loss and infection by pathogens. In 'La France' pear fruit, the β-galactosidase genes can be divided into three classes based on ethylene response; positively regulated, negatively regulated and the constitutive genes [23]. Controlling the expression of the genes positively regulated by ethylene may serve to regulate the rate and extent of softening during ripening of 'La France' pear fruit ripening and thereby improve the quality and postharvest handling of the fruit.

α-L-Arabinofuranosidases

The release of galactosyl and arabinosyl residues during softening has been observed in many kinds of fruit. The release of these sugar residues is assumed to increase the sensitivity of enzymatic degradation or accessibility of other glycan hydrolases. Terminal arabinosyl residues are widely distributed in pectic and hemicellulosic polysaccharides such as arabinan, arabinogalactan, arabinoxylan, arabinoxyloglucan and glucuronoarabinoxylan [27]. α-L-Arabinofuranosidases catalyze the hydrolysis of terminal nonreducing -L-arabinofuranosyl residues from various pectic and hemicellulosic homo- (arabinans) and heteropolysaccharides (arabinogalactans, arabinoxylans, arabinoxyloglucans, glucuronoarabinoxylans, etc.) as well as from different glycoconjugates [27]. α-L-Arabinofuranosidases activity is detectable throughout preripening development control and ethylene synthesis-suppressed (ESS; antisense- ACC synthase transgene-expressing) fruit, but that the large increase in the extractable α-L arabinofuranosidase activity exhibited by ripening-controlled fruit occurred only in the ESS fruit if they were given postharvest ethylene treatment [28]. α-L-Arabinofuranosidase activity has also been observed to increase in fruit such as avocado [29], persimmon [30] and peach [31].

Recently α-L-arabinofuranosidase was purified from Japanese pear fruit and its corresponding cDNA (*Pp ARF2)* clone isolated [32**]. The pear *Pp ARF2* is a new member of the glycosidase hy-

drolase family 3, according to the primary structure of the enzyme and its activity against native substrates and its gene was highly expressed in pear fruit. In fig fruit, *Fc-Arabf1* gene was highly induced at the onset of fruit ripening [6]. Many of the physiological changes that occur during fruit ripening are in response to ethylene production. In tomato, *LeARF1* mRNA was detected in ripe fruit of *Nr2, nor* and *rin*, but not in ripe fruit of the Nr mutant and the *LeARF1* mRNA was negatively regulated by ethylene [33**]. These gene expression studies show that α-L-arabinofuranosidase may play an important role in alteration of fruit texture during ripening. However, the mechanism by which α-L-arabinofuranosidase affects modification of the cell wall polysaccharides is unclear and needs further investigation.

Expansins

The expansins comprise a family of proteins that appear to be involved in the disruption of the noncovalent bonds between cellulose microfibrils and cross-linking glycans. Their role in the modification of the fruit cell wall has been evaluated by manipulating the expression of expansin genes in transgenic tomato plants [34]. It was shown that reduced expression of *LeExp1* in transgenic tomato plants resulted in considerable decreases in polyuronide degradation. *LeExp*1-suppressed fruit of some lines were 13–23% firmer than controls, depending upon the ripening stage, shelf-life was increased by approximately 10 days in boxes and 5 days in clam shells. The increase in firmness of *LeExp1*-suppressed fruit cannot be explained entirely by reduced polyuronide depolymerisation. *LeExp1* is thought to have a direct effect on some aspects of cell wall loosening during ripening which contributes to fruit softening, probably including increasing the accessibility of depolymerases such as endo-1,4-β-glucanases (EGases) to matrix glycans, with an additional and perhaps more indirect effect on the accessibility of a pectinase such as PG to polyuronide substrates. A reduction in both the direct effect of *LeExp1* on wall loosening and the indirect effect on pectin disassembly may together be responsible for the substantially improved shelf-life of *LeExp1*-suppressed fruit. While several expansins show a general increase in expression levels during the later stages of fruit development, some isoforms show a greater association with softening than others. Enhanced expansin gene expression during ripening has recently been reported in tomato [35], peach [5*, 36], pear [37], banana [38], olive [39], watermelon [40] , apricot (41) and fig [6]. The large number of expansins isoforms in fruit such as pear [37], poses intriguing questions regarding the function of these proteins and the significance of their redundancy. Furthermore, individual expansins could have different substrate specificities, there may be differences in their biochemical modes of action, and they could act in different cell types.

Other fruit cell wall modifying enzymes

In many fruit the depolymerisation of cell-wall hemicellulose is a common characteristic of fruit ripening. Some of the enzymes that might be involved in the breakdown of fruit cell wall matrix glycans include EGases and xyloglucan endotransglycosylases (XTHs) [2**]. In 'Masui Dauphine' figs, *Fc-Cel1* mRNA was up-regulated by ethylene similar to other ripening-related Egases isolated from avocado, tomato and pepper [42]. The *Fc-XTH1* mRNA was down-regulated by ethylene whereas *Fc-Cel1* and *Fc-XTH2* were ethylene independent indicating that XTHs and EGases from fig fruit comprise gene families with divergent members showing differential regulation during fruit ripening. However, suppression of *cel1* in transgenic strawberry had no appreciable effect on endo glucanase (EG) activity and fruit firmness [43**]. The unchanged level of EG activity in fruit with lowered *cel1* expression could be explained by the presence of strawberry *Cel2*. In this case, the activity of Cel2 would be likely to increase in order to compensate for the expected loss of Cel1 activity. The relative contribution of Cel1 and Cel2 enzymes to the total EG activity in normal ripe strawberry fruit is unknown. These transgenic studies did not reveal the role of Cel1 in fruit softening but the absence of any effect of *cel1* suppression on fruit texture implicated Cel2 in the softening process. Transgenic suppression of *Cacel* in bell pepper indicated that EGases of the CMCase types are not necessary for the ripen-

ing-related depolymerisation of either xyloglucan or matrix glycan polymers [44**].

Furthermore, constitutive overexpression of *CaCel1* in transgenic tomato did not cause depolymerisation of tomato fruit xyloglucan *in vivo*, suggesting that fruit softening is not limited by the amount of EGase activity present during ripening [45**].

Endo-(1,4)-β-Mannanase is another cell wall hydrolase identified some time ago in ripening tomato but which has received less attention. Mannanase activity was observed to increase during the latter stages of ripening in tomato but no corresponding increase in *LeMAN4* mRNA was observed [46]. This suggests that either this gene is subject to post-transcriptional regulation or that the *LeMAN4* protein remains inactive or sequestered until the later stages of ripening. It is also plausible that the natural substrate of tomato fruit mannanase is not a cell wall mannan.

β-D-Xylosidase is a cell wall enzyme responsible for the hydrolysis of xylans liberating ß-D-xylosyl residues. *LeXYL2* was expressed abundantly during fruit development and *LeXYL1* was expressed during fruit ripening. The abundance of *LeXYL1* and *LeXYL2* mRNAs was not influenced by treatment with 1-methylcyclopropene, indicating that the expression of the two ß-D-xylosidase genes is independent of ethylene action [33**].

α-Galactosidase is one of the exoglycosidases, capable of hydrolysing α-1,6 linked α-galactoside residues. α-Galactosidases are known to remove galactosyl moieties from stored galactomannan polysaccharides in germinating seeds and can be used to change the rheological properties of these important plant gums [47]. Despite their wide distribution and diversity, α-galactosidases seem to be less abundant in fruits compared with other plant organelles. α-Galactosidases activity increased substantially during postharvest storage in grapes, whereas the unripe grape showed a "stagnancy" for 10–15 days prior to the increase [48]. In another study, even though the activity could be detected, α-galactosidase cDNA could not be isolated from grapes [13]. α-Galactosidase activity was observed to increase during ripening in tomato [49]. α-Galactosidase activity was ethylene regulated in Ceccona apricot whereas in San Castrese apricot its activity was ethylene independent [50] and ethylene affected the abundance α-galactosidase transcripts in water-melon [40]. Even though the presence of α-galactosidases has been observed in a number of fruit species its physiological role in fruit softening is yet to be established.

Conclusions

The cell wall degradation during fruit softening of fleshy fruit is a physiological process that is still being thoroughly studied in many laboratories all over the world. Such huge interest is due to both a desire for deeper knowledge and to the relevant economic implications of this process. Fruit softening during ripening is a complex process mainly contributed to by the action of a variety of cell wall modifying enzymes and proteins. Within a single species, each of these classes of plant cell wall enzymes and proteins involved in cell wall disassembly contains isoforms that show specific expression during fruit ripening. However, the central role of any individual cell wall enzyme in fruit softening has been challenged and it now appears that no single enzyme can be identified as being necessary and sufficient for fruit softening.

The individual roles of these enzymes and proteins is still being addressed in part through use of gene silencing techniques (antisense and co-suppression) to manipulate the activities of these enzymes in genetically modified plants and determine the effect on cell wall degradation and fruit quality. Another technique is the use of "chimeric" genes to simultaneously silence several target enzymes. This technique could be used to manipulate various biochemical pathways. While several families of cell wall-modifying proteins have been identified to date that contribute to cell wall modification processes, numerous new classes remain to be discovered and preliminary studies suggest that most extracellular proteins cannot yet be assigned a biochemical function. Proteomics strategies are being utilised to characterise the cell wall proteome in tomato fruit, to identify novel proteins and to reveal the suites of

proteins that are co-expressed during fruit ripening. Another approach in identifying genes regulating fruit texture is the use tomato pleiotropic mutations. Recent research has focused on the tomato *Cnr* mutation, which has a particularly dramatic effect on fruit texture [20**, 51**]. *Cnr* is a dominant mutation that arose spontaneously in a commercial population of cv. Liberto. This single gene mutation results in a non-ripening fruit phenotype with characteristics such as firm fruit with much reduced cell-to-cell adhesion and complete abolition of carotenoid biosynthesis in the pericarp with obvious mealy texture when ripe. There is a limited amount of information available on gene expression in *Cnr* mutant. Studies on the cell wall of *Cnr* mutant and the isolation and characterization of other such pleiotropic mutants will provide further insights into the cell wall degradation during fruit ripening. Parallels drawn between such mutants and normal fleshy fruit will aid in unraveling the molecular basis of fruit cell wall loosening and degradation accompanying fruit softening.

References

Papers of interest have been highlighted as

*Marginal importance

**Essential reading

1 Rose JKC, Catala C, Gonzalez-Carranza ZH, Roberts JA. Cell wall disassembly. In: Rose JKC. (Ed). The Plant cell wall, Annual plant review. Blackwell publishing, Oxford, UK. 2003. pp 264–324.

**This book provides an overview of the current understanding of plant cell walls, drawing on the recent advances of plant molecular biology. It incorporates the identification of a rapidly growing number of genes and the proteins responsible for plant wall synthesis, restructuring, degradation and wall-associated signal transduction.

2 Brummell DA and Harpster MH. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology 2001: 47(1-2):311–400.

**This is an excellent review on the chemistry of fruit cell wall polymers, cell wall enzymes and the manipulation of genes encoding these enzymes in transgenic plants.

3 Willats WG, McCartney L, Mackie W, Knox JP. Pectin: cell biology and prospects for functional analysis. Plant Molecular Biology 2001:47(1-2):9–27.

**This review surveys what is known about the structure and function of pectin domains. It discusses the current knowledge of biosynthetic enzymes, plant and microbial pectinases and the interactions of pectin with other cell wall components and the impact of molecular genetic approaches in terms of the functional analysis of pectic polysaccharides in plant growth and development.

4 Fonseca S, Monteiro L, Barreiro MG and Pais MS. Expression of genes encoding cell wall modifying enzymes is induced by cold storage and reflects changes in pear fruit texture. Journal of Experimental Botany 2005: 56 (418):2029–2036.

5 Trainotti L, Zanin D and Casadoro G. Cell wall-oriented genomic approach reveals a new and unexpected complexity of the softening in peaches. Journal of Experimental Biology 2003: 54(389):1821–1832.

*In this study expression of various peach ESTs was analysed in fruits at different developmental stages by using the macroarray technique. The analysis of the expression patterns indicated that six out of the nine genes related to the weakening of the cell walls encode pectin-degrading enzymes. The degradation of pectins appears to be finely tuned with different genes being expressed according to highly co-ordinated patterns. Only three enzymes thought to act on hemicelluloses were found. The expression patterns of cell wall genes supported the idea that pectin degradation prevails over the degradation of hemicelluloses.

6 Owino WO, Nakano R, Kubo Y and Inaba A. Coordinated expression patterns of genes encoding cell wall modifying enzymes during ripening in distinct anatomical tissue regions of the fig (*Ficus carica* L.) fruit. Postharvest Biology and Technology 2004: 32(3): 253-261.

7 Hiwasa K, Nakano R, Hashimoto A, Matsuzaki M, Murayama H, Inaba A and Kubo Y. European, Chinese and Japanese pear fruits exhibit differential softening characteristics during ripening. Journal of Experimental Botany 2004: 55(406):2281–2290.

*This is a comparative study on the softening characteristics of three types of pear fruit, namely, European pear 'La France', Chinese pear 'Yali', and Japanese pear 'Nijisseiki'. The results of the study suggest that different softening behaviours during ripening among the three pear fruits may be caused by different endo-PG activity and different expression of PG genes.

8 Asif HM and Nath P. Expression of multiple forms of polygalacturonase gene during ripening in banana fruit. Plant Physiology and Biochemistry 2005: 43(2):177–184.

9 Powell AL, Kalamaki MS, Kurien PA, Gurrieri S and Bennett AB. Simultaneous transgenic suppression of *LePG* and *LeExp1* influences fruit texture and juice viscosity in a fresh market tomato variety. Journal of Agricultural and Food Chemistry 2003: 1(25):7450–7455.

**Suppression of both *LePG* and *LeExp1* expression produced

transgenic tomato plants which were significantly firmer throughout ripening and were less susceptible to deterioration during long-term storage.

10 Marin-Rodriguez MC, Orchard J and Seymour GB. Pectate lyases, cell wall degradation and fruit softening. Journal of Experimental Botany. 2002: 53(377):2115–2119.

11 Pua EC, Ong CK, Liu P, Liu JZ. Isolation and expression of two pectate lyase genes during fruit ripening of banana (*Musa acuminata*). Physiologia Plantarum 2001: 113 : 92–99.

12 Marin-Rodriguez MC, Smith DL, Manning K, Orchard J and Seymour GB. Pectate lyase gene expression and enzyme activity in ripening banana fruit. Plant Molecular Biology 2003: 51(6):851–857.

13 Nunan KJ, Davies C, Robinson SP and Fincher GB. Expression patterns of cell wall-modifying enzymes during grape berry development. Planta 2001: 214(2):257–264.

14 Redondo-Nevado J, Movano E, Medina-Escobar N, Caballero JL and Munoz-Blanco J. A fruit-specific and developmentally regulated endopolygalacturonase gene from strawberry (*Fragaria x ananassa* cv. Chandler). Journal of Experimental Botany 2001: 52(362):1941–1945.

15 Benitez-Burraco A, Blanco-Portales R, Redondo-Nevado J, Bellido ML, Moyano E, Caballero JL and Munoz-Blanco J. Cloning and characterization of two ripening-related strawberry (*Fragaria x ananassa* cv. Chandler) pectate lyase genes. Journal of Experimental Botany 2003: 54(383):633–645.

16 Jimenez-Bermudez S, Redondo-Nevado J, Munoz-Blanco J, Caballero JL, Lopez-Aranda JM, Valpuesta V, Pliego-Alfaro F, Quesada MA and Mercado JA. Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiology 2002: 128 (2):751–759.

**In this study antisense expression of strawberry pectate lyase gene resulted in total suppression of pectate lyase genes in ripe fruit. Post harvest softening in transgenic plants was reduced indicating that pectate lyase gene is an excellent candidate for biotechnological improvement of fruit softening in strawberry.

17 Micheli F. Pectin methylesterases: cell wall enzymes with important roles in plant physiology. Trends in Plant Science 2001: 6(9):414–419.

**This is an interesting review on the mechanism and function of pectin methylesterases in plants.

18 Castillejo C, de la Fuente JI, Iannetta P, Botella MA and Valpuesta V. Pectin esterase gene family in strawberry fruit: study of *FaPE1*, a ripening-specific isoform. Journal of Experimental Botany 2004: 55(398):909–918.

19 Barnavon L, Doco T, Terrier N, Ageorges A, Romieu C and Pellerin P. Involvement of pectin methyl-esterase during the ripening of grape berries: partial cDNA isolation, transcript expression and changes in the degree of methyl-esterification of cell wall pectins. Phytochemistry 2001: 58(5):693–701.

20 Eriksson EM, Bovy A, Manning K, Harrison L, Andrews J, De Silva J, Tucker GA and Seymour GB. Effect of the Colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening. Plant Physiology 2004: 136(4):4184–4197.

**This study reports on the changes in enzyme activity associated with cell wall disassembly in Cnr and the effect of the mutation on the program of ripening-related gene expression. The study demonstrates that the Cnr mutation has a profound effect on many aspects of ripening-related gene expression.

21 Smith DL and Gross KC. A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. Plant Physiology 2000: 123(3):1173–1183.

22 Trainotti L, Spinello R, Piovan A, Spolaore S and Casadoro G.. Beta-Galactosidases with a lectin-like domain are expressed in strawberry. Journal of Experimental Botany 2001: 52(361):1635–1645.

23 Mwaniki MW, Mathooko FM., Matsuzaki M, Hiwasa K, Tateishi A, Ushijima K, Nakano R, Inaba A and Kubo Y. Expression characteristics of seven members of the β-galactosidase gene family in 'La France' pear (*Pyrus communis* L.) fruit during growth and their regulation by 1-methylcyclopropene during postharvest ripening. Postharvest Biology and Technology 2005: 36(3) 253–263.

24 Smith DL, Abbott JA and Gross KC. Down-regulation of tomato beta-galactosidase results in decreased fruit softening. Plant Physiology 2002: 129(4):1 755–1762.

**Antisense suppression of TBG4 produced one antisense line which had red-ripe fruit that were 40% firmer than controls. Fruit from this antisense line also had the lowest TBG4 mRNA and exo-galactanase levels and the highest wall galactosyl content during the early stages of ripening, implicating an involvement of this gene product in cell wall modification leading to fruit softening.

25 Moctezuma E, Smith DL and Gross KC. Effect of ethylene on mRNA abundance of three β-galactosidase genes in wild type and mutant tomato fruit. Postharvest Biology and Technology 2003: 28(2):207–217 .

26 Moctezuma E, Smith DL and Gross KC. Antisense suppression of a beta-galactosidase gene (TBG6) in tomato increases fruit cracking. Journal of Experimental Botany 2003: 54(390):2025–2033.

**In this study suppression of TBG6 resulted in increased fruit cracking, reduced locular space, and a doubling in the thickness of the fruit cuticle in the transgenic phenotypes.

27 Saha BC. α-L-arabinofuranosidases: biochemistry, molecular biology and application in b iotechnology. Biotechnology Advances 2000: 18: 403–423.

28 Sozzi GO, Greve LC, Prody GA and Labavitch JM. Gibberellic acid, synthetic auxins, and ethylene differentially modulate alpha-L-Arabinofuranosidase activities in antisense 1-aminocyclopropane-1-carboxylic acid synthase tomato pericarp discs. Plant Physiology 2002: 129 (3):1330–1340.

29 Tateishi A, Inoue H and Yamaki S. Fluctuation of the activities of three β-galactosidase isoforms from avocado (*Persea Americana*) fruit with fruit ripening and different activities against cell wall polysaccharides. Journal of the Japanese society for Horticultural science 2001 70: 586–592.

30 Xu CG, Nakatsuka A, Kano H and Itamura H. Changes on ethylene production and activities of cell wall degrading enzymes during rapid fruit softening of Japanese persimmon 'Saijo'. Journal of the Japanese society of horticultural science 2003: 72: 460–462.

31 Brummell DA, Dal Cin V, Crisosto CH and Labavitch JM. Cell wall metabolism during maturation, ripening and senescence of peach fruit. Journal of Experimental Botany 2004: 55(405):2029–2039.

32 Tateishi A, Mori H, Watari J, Nagashima K, Yamaki S and Inoue H. Isolation, characterization, and cloning of {alpha}-L-Arabinofuranosidase expressed during fruit ripening of Japanese pear. Plant Physiology 2005: 138 (3):1653–64.

**This study reports on the purification of α-L-Arabinofuranosidase and isolation of a related cDNA from Japanese pear fruit (*Pyrus pyrifolia*). The enzyme did not release xylose from arabinoxylan and xylan. The only activity observed was the hydrolysis of the arabinosyl residue from native polysaccharides, whereas it showed bifunctional activity against artificial substrates. According to the expression pattern and properties of the enzyme, it is a new member of the glycoside hydrolase family 3 isolated from fruit, and it may be responsible for modification of the cell wall architecture during fruit softening.

33 Itai A, Ishihara K and Bewley JD. Characterization of expression, and cloning, of beta-D-xylosidase and alpha-L-arabinofuranosidase in developing and ripening tomato (Lycopersicon esculentum Mill.) fruit. Journal of Experimental Botany 2003: 54(393):2615–2622.

**Brummell DA, Howie WJ, Ma C and Dunsmuir P. Postharvest fruit quality of transgenic tomatoes suppressed in expression of a ripening-related expansin. Postharvest Biology and Technology 2002: 25(2):209–220.

34 Suppression of the expression of LeExp1 in transgenic tomatoes improved the shelf-life and processing properties of the fruit.

35 Hoeberichts FA, Van Der Plas LHW and Woltering EJ. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. Postharvest Biology and Technology 2002: 26(2):125–133.

36 Hayama H, Ito A, Moriguchi T and Kashimura Y. Identification of a new expansin gene closely associated with peach fruit softening. Postharvest Biology and Technology 2003: 29(1):1–10.

37 Hiwasa K, Rose JK, Nakano R, Inaba A, and Kubo Y. Differential expression of seven alpha-expansin genes during growth and ripening of pear fruit. Physiologia Plantarum 2003: 117(4):564–572.

38 Trivedi PK and Nath P. *MaExp1*, an ethylene-induced expansin from ripening banana fruit. Plant Science. 2004: 167 (6): 1351–1358.

39 Ferrante A, Hunter DA and Reid MS. Towards a molecular strategy for improving harvesting of olives (*Olea europaea* L.). Postharvest Biology and Technology 2004: 31 (2):111–117.

40 Karakurt Y, Huber DJ. Ethylene-induced gene expression, enzyme activities, and water soaking in immature and ripe watermelon (*Citrullus lanatus*) fruit. Plant Physiology. 2004: 161(4):381–388.

41 Mbeguie-A-Mbeguie D, Gouble B, Gomez R, Audergon JM, Albagnac G and Fils-Lycaon B. Two expansin cDNAs from *Prunus armeniaca* expressed during fruit ripening are differently regulated by ethylene. Plant Physiology and Biochemistry 2002: 40(5):445–452.

42 Owino, WO, Yuge H, Nakano R, Kubo Y and Inaba, A. Expression analysis of genes encoding cell wall modifying enzymes during ripening of Fig Fruit (*Ficus Carica* L. Cv Masui Dauphine). Acta Horticulturae 2004: 632:265–269.

43 Woolley LC, James DJ and Manning K. Purification and properties of an endo-beta-1,4-glucanase from strawberry and down-regulation of the corresponding gene, *Cel1*. Planta. 2001: 214(1):11–21.

**To assess the role of the Cell enzyme in fruit softening, *Cel1* cDNA corresponding to fruit-specific and ripening-enhanced strawberry gene was down-regulated in transgenic plants. The EG activity and firmness of transgenic fruits were indistinguishable from those of control fruit. The expression of a second gene, *Cel2*, encoding a different strawberry EG was unaltered in transgenic fruits suggesting that the presence of the Cel2 in transgenic plants may have prevented the specific down-regulation of cell from revealing its role in fruit softening.

44 Harpster MH, Brummell DA and Dunsmuir P. Suppression of a ripening-related endo-1,4-beta-glucanase in transgenic pepper fruit does not prevent depolymerization of cell wall polysaccharides during ripening. Plant Molecular Biology 2002: 50(3):345–355.

**The results of this study showed that activities other than CaCel1 are responsible for the depolymerization of matrix glycans occurring during ripening in pepper.

45 Harpster MH, Dawson DM, Nevins DJ, Dunsmuir P and Brummell DA. Constitutive overexpression of a ripening-related pepper endo-1,4-beta-glucanase in transgenic tomato fruit does not increase xyloglucan depolymerization

or fruit softening. Plant Molecular Biology: 2002: 50 (3):357–369.

**The data from this study suggest that degradation of a proportion of matrix glycans other than xyloglucan does not result in fruit softening, and that fruit softening is not limited by the amount of EGase activity present during ripening.

46 Carrington CMS, Vendrell M and Dominguez-Puigjaner E. Characterisation of an endo-(1,4)-beta-mannanase (*LeMAN4*) expressed in ripening tomato fruit. Plant Science 2002: 163(3):599–606.

47 Gao Z and Schaffer AA. A Novel Alkaline α-Galactosidase from Melon Fruit with a Substrate Preference for Raffinose. Plant Physiology 1999: 119: 979–988.

48 Kang HC and Lee SH. Characteristics of an α-galactosidase associated with grape flesh. Phytochemistry 2001: 58(2):13–219.

49 Jagadeesh BH, Prabha TN and Srinivasan K. Activities of glycosidases during fruit development and ripening of tomato (*Lycopersicum esculantum* L.): implication in fruit ripening. Plant Science 2004: 166(6):1451–1459.

50 Botondi R, DeSantis D, Bellincontro A, Vizovitis K and Mencarelli F. Influence of ethylene inhibition by 1-methylcyclopropene on apricot quality, volatile production, and glycosidase activity of low- and high-aroma varieties of apricots. Journal of Agricultural and Food Chemistry 2003: 51(5):1189–1200.

51 Seymour GB, Manning K, Eriksson EM, Popovich AH and King GJ. Genetic identification and genomic organization of factors affecting fruit texture. Journal of Experimental Botany 2002: 53(377):2065–2071.

**This review focuses on the texture characteristics of the Cnr mutant. A possible framework for the molecular regulation of fruit texture is discussed and quantitative genetic approaches to determining the generic attributes of fruit texture are explored