# PRIMARY CELL WALL METABOLISM DURING FRUIT RIPENING*

DAVID A. BRUMMELL

New Zealand Institute for Crop and Food Research Ltd,
Food Industry Science Centre, Batchelar Road, Palmerston North 5301, New Zealand
brummelld@crop.cri.nz

(Received for publication 27 October 2005; revision 20 January 2006)

## ABSTRACT

Fruit softening is an important part of the ripening process, and involves changes to cell turgor and primary cell wall structure. Most of the polysaccharide components of the cell wall are subjected to some degree of controlled degradation, resulting in a loosening and swelling of the wall structure, a weakening of cell wall strength, and reduced intercellular adhesion. Early ripening changes involve the degradation of the galactan/arabinan side chains of rhamnogalacturonan-I, demethylesterification of homogalacturonan, and depolymerisation of matrix glycans (hemicelluloses). Solubilisation of pectins increases during ripening, but depolymerisation of pectins is usually most pronounced late in ripening. Considerable variation in the extent of pectin depolymerisation and galactan/arabinan loss exists between species. Transgenic studies have shown that expansin may control cell wall loosening, and β-galactosidase may be important in increasing cell wall porosity. Suppression of either of these enzymes resulted in a retention of fruit firmness. Suppression of endo-polygalacturonase and pectin methylesterase had little effect on fruit firmness during ripening, but influenced fruit shelf life due to alterations in the integrity of the middle lamella, which affected intercellular adhesion. The enzyme(s) responsible for depolymerisation of matrix glycans have not been defined, and the identity of the ripening-related xyloglucanase remains obscure.

**Keywords**: cell wall disassembly; fruit softening; pectins; matrix glycans; expansin.

## INTRODUCTION

Fruit ripening consists of a number of biochemical and physiological changes which help to attract animal vectors that will disperse the seeds. In addition to the accumulation of pigments, sugars, organic acids, and aroma volatiles, a series of

---

* Based on a paper presented at 1st Joint New Zealand – German Symposium on Plant Cell Walls, 23–24 June 2005, Rotorua, New Zealand

textural changes occur which improve palatability. Some fleshy fruit (such as tomato, peach, strawberry, and kiwifruit) undergo considerable softening and achieve a smooth melting texture when ripe, which is thought to be due largely to a regulated dismantling of the primary cell wall. However, the accumulation of solutes in the cell wall space reduces turgor pressure, and this also contributes to textural changes (Shackel *et al.* 1991). Ripening changes occurring within the wall lead to a softening and weakening of the primary cell wall, some degree of cell wall swelling in most species (Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997), and a weakening of the pectin-rich middle lamella, which is largely responsible for maintaining intercellular adhesion (Jarvis *et al.* 2003).

During ripening, the principal theme is a dismantling of the primary cell wall structure, and a degradation of the polysaccharides of which it is composed. In most fruit species there is a depolymerisation of matrix glycans (formerly called hemicelluloses, *see below*) and usually of polyuronides, a loss of pectin side chains, and an increase in pectin solubilisation. Softening, and the extent of cell wall changes, are less in fruit that attain a crisp fracturable texture when ripe, such as apple, bell pepper, and watermelon. However, even within the melting-flesh fruit that soften extensively during ripening there are substantial differences in the type and extent of cell wall modifications occurring in different species. Weakening of both primary cell walls and middle lamellae is necessary to achieve softness, but primary cell walls must weaken considerably in order for cells to split open when tissue is bitten or chewed, releasing the intracellular juice (Harker *et al.* 1997). The ultimate result of cell wall changes is to produce a soft or crisp fruit with a juicy texture, although there may be some differences between species in the mechanism by which this result is achieved.

There have been many studies of fruit softening during ripening because of the importance of fruit in the human diet and the limitations caused to shelf life by the softening process. This brief review will cover the major cell wall changes that occur in most species, and discuss the role of various cell wall modifying enzymes in the softening process.

## CELL WALL CHANGES DURING SOFTENING

Other than in specialised cells or in vascular tissues, the cells of fruit flesh possess primary walls. Primary cell walls are unlignified and consist of cellulose microfibrils held together by a diverse array of matrix glycans (xyloglucan, substituted and unsubstituted xylans, and glucomannan) and pectins (homogalacturonan, xylogalacturonan, and two types of rhamnogalacturonan), together with some structural glycoprotein and phenolics (Carpita & Gibeaut 1993).

Matrix glycans (formerly known as hemicelluloses) are composed principally of neutral sugar residues, have a backbone consisting entirely of neutral sugar

residues, and lack GalA. Xyloglucan has a 1,4-β-D-glucan backbone, regularly substituted with Xyl on three consecutive Glc residues out of four, plus occasional further decoration with Gal and/or Gal-Fuc. Xylan has a backbone of 1,4-β-D-xylan, and can be largely unsubstituted, or possess occasional single residue side chains of Ara and GlcA (glucuronoarabinoxylan). Glucomannan is present in lower amounts, and consists of 1,4-β-D-Glc and 1,4-β-D-Man as alternating residues or in short alternating regions. All of these glycan molecules have a linear backbone, which may both hydrogen bond to cellulose and be interwoven into the outer layers of 1,4-β-D-glucan chains of cellulose microfibrils. Xyloglucan in particular may span between cellulose microfibrils, tethering them together, and, as such, polysaccharides of this class are also known as cross-linking glycans.

Pectins are characterised by their high content of GalA. Homogalacturonan (HGA) is a large polymer of 1,4-α-D-GalA, and is initially secreted into the wall with most residues methylesterified. Xylogalacturonan has the same backbone as HGA, but about half of the GalA residues are substituted with single α-D-Xyl residues. Rhamnogalacturonan-II (RG-II) has the same backbone as HGA, but has four complex, highly conserved, side chains consisting of at least 12 different sugars. Side chains of different RG-II molecules are linked together by borate diesters. Rhamnogalacturonan-I (RG-I) has a backbone of alternating 1,2-α-D-Rha and 1,4-α-D-GalA residues, and possesses large side chains attached to approximately half of the Rha residues. These side chains are linear galactans, branched arabinans, or branched arabinogalactans. RG-I pectin side chains may be covalently attached to xyloglucan (Popper & Fry 2005), and are strongly associated with cellulose (Redgwell, Fischer, Kendal, & MacRae 1997), potentially cross-linking cellulose microfibrils or interlocking the xyloglucan-cellulose and pectin networks together (Vincken *et al.* 2003; Zykwinska *et al.* 2005).

During ripening, modifications occur to cell wall polysaccharides, and this affects the structure and properties of the wall. One of the earliest changes to be initiated is the loss of galactan side chains from RG-I. In tomato, cell wall Gal declined by almost half during green fruit maturation, prior to the breaker stage which is the beginning of ripening, and declined by another two-thirds during ripening (Kim *et al.* 1991). A substantial loss of Gal from the wall during ripening has been observed in most fruit species (Gross & Sams 1984). Loss of arabinan side chains from RG-I also occurs in most species (Gross & Sams 1984), either coincident with or immediately prior to softening (Brummell *et al.* 2004; Peña & Carpita 2004). RG-I arabinan/galactan is very firmly associated with cellulose, and requires extensive extraction in concentrated alkali for its solubilisation (Redgwell, Fischer, Kendal, & MacRae 1997). It is thought that the pectin network determines the porosity of the wall (Baron-Epel *et al.* 1988), and degradation of RG-I side chains is likely to increase porosity and enhance the diffusion of enzymes within the apoplast.

Demethylesterification of HGA also began prior to ripening in grape and peach (Barnavon *et al.* 2001; Brummell *et al.* 2004), and declined substantially during ripening in these species and in tomato and avocado (Koch & Nevins 1989; Wakabayashi *et al.* 2000). Removal of the methylester group by pectin methylesterase (PME) leaves a charged carboxylic acid group, which has profound consequences for the properties of HGA and cell wall structure. In the absence of $Ca^{2+}$, demethylesterified negatively-charged HGA may become more loosely attached to the wall due to electrostatic repulsion by other unesterified HGA, increasing pectin solubilisation and possibly contributing to wall swelling. In the presence of $Ca^{2+}$, unesterified regions of HGA aggregate together in localised regions of calcium-pectate gel, which can stiffen the wall (Jarvis 1984). Calcium-pectate cross-links between HGA molecules in the middle lamellae eventually form most of the links between adjacent cells in ripe fruit, since treatment with chelating agents such as CDTA can cause almost complete cell separation (Peña & Carpita 2004). Furthermore, methylesterified HGA cannot be depolymerised by endo-polygalacturonase (endo-PG), a common ripening-related enzyme. A requirement for at least partial demethylesterification of HGA by the prior action of PME may provide a limitation to the action of endo-PG *in vivo*, thus restricting HGA depolymerisation in the wall (Wakabayashi *et al.* 2000). Pectate lyase (PL), which depolymerises HGA by a different biochemical mechanism, may also require a demethylesterified substrate.

Depolymerisation of matrix glycans has been observed in all fruit species studied, where it has been shown to begin early in ripening and usually is closely correlated with softening. Depolymerisation of xyloglucan, which can be specifically detected using an iodine binding assay, was slow but progressive, resulting in a loss of molecules with the highest molecular weight and an accumulation of mid-sized molecules (O'Donoghue & Huber 1992; Cutillas-Iturralde *et al.* 1994; Maclachlan & Brady 1994; Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir 1999; Harpster, Brummell, & Dunsmuir 2002). However, a downshift in molecular weight of the total matrix glycans implies that depolymerisation of other polysaccharides in this extract, such as arabinans/galactans originating as RG-I side chains and xylans, also occurred (O'Donoghue & Huber 1992; Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir 1999; Harpster, Brummell, & Dunsmuir 2002). Changes to matrix glycan molecular weight were relatively limited, with moderately-sized molecules remaining even at the over-ripe stage and no accumulation of small molecules, suggesting that loosening of the matrix glycan-cellulose network is controlled and that the cross-linking glycans are not extensively degraded. Loosening of the matrix glycan-cellulose network by expansin and the depolymerisation of glycans by hydrolases or transglycosylases may be an important part of cell wall swelling and may increase the access of depolymerases to their substrates later in ripening.

Although the depolymerisation of matrix glycans appears to occur similarly in most species, this is not so for the depolymerisation of chelator-soluble pectins (predominantly HGA). Polyuronide depolymerisation varied strikingly between species, being extensive in avocado, moderate in peach and tomato, slight in melon, and virtually undetectable in strawberry and banana (Huber 1984; Wade *et al.* 1992; Huber & O'Donoghue 1993; Rose *et al.* 1998; Brummell *et al.* 2004). In fruit which ripen to a melting texture, polyuronide depolymerisation generally either began slowly during ripening followed by a dramatic loss of large molecules late in ripening (e.g., tomato, avocado, and papaya — Huber & O'Donoghue 1993; Brummell & Labavitch 1997; Paull *et al.* 1999), or was essentially absent during the early ripening stages and occurred dramatically late in ripening (e.g., pepino and melting-flesh peach — O'Donoghue *et al.* 1997; Brummell *et al.* 2004). Softening did not correlate with polyuronide depolymerisation early in ripening, although the pronounced depolymerisation occurring late in ripening was usually accompanied by a substantial loss of firmness. Polyuronide depolymerisation was very slight in fruit that ripen to a crisp fracturable texture, such as apple and bell pepper (Yoshioka *et al.* 1992; Harpster, Brummell, & Dunsmuir 2002). Degradation of HGA, which is the major component of the middle lamella, causes a reduction in cell-to-cell contacts. Weakened intercellular adhesion is a normal part of the softening process, and contributes to the textural alterations occurring in ripe fruit.

Cellulose microfibrils do not appear to be degraded during ripening in most fruit (e.g., Maclachlan & Brady 1994), with the exception of avocado (Platt-Aloia *et al.* 1980).

## CELL WALL-MODIFYING ENZYMES

The changes to cell wall polysaccharides that bring about altered wall properties are believed to be due largely to the actions of a variety of ripening-related enzymes which are secreted into the cell wall space as ripening progresses. These enzymes modify both pectins and matrix glycans. Particular changes include removal of polymeric or single-sugar side chains, removal of methylester or acetyl groups from HGA, cleavage of polymeric backbones, and loosening of hydrogen bonding between cellulose microfibrils and glycans. Some examples of enzymes with ripening-related expression patterns and their probable functions and major substrates are shown below (Pharr *et al.* 1976; Domínguez-Puigjaner *et al.* 1997; Medina-Escobar *et al.* 1997; Bewley *et al.* 2000; Brummell & Harpster 2001; Chen & Paull 2003; Schröder *et al.* 2004).

(1) Endo-polygalacturonase (endo-PG): cleavage of HGA backbone at internal sites by hydrolysis.

(2) Exo-polygalacturonase (exo-PG): step-wise removal of single GalA residues from non-reducing end of HGA backbone.

(3) Pectin methylesterase (PME): removal of methylester groups from HGA, leaving charged carboxylic acid groups.

(4) Pectate lyase (PL): cleavage of HGA backbone at internal sites by β-elimination.

(5) β-Galactosidase: exo-acting enzymes removing galactan side chain of RG-I.

(6) α-Arabinosidase: exo-acting enzymes removing arabinan side chain of RG-I.

(7) Endo-1,4-β-glucanase (EGase): cleavage of glucan backbone of xyloglucan or non-crystalline cellulose chains at internal sites. Sometimes imprecisely referred to as cellulase.

(8) Endo-1,4-β-xylanase (EXase): cleavage of xylan backbone of glucuronarabinoxylan or unsubstituted xylan at internal sites.

(9) Endo-1,4-β-mannanase (EMase): cleavage of mannan backbone of mannan, galactomannan, or glucomannan at internal sites by hydrolysis.

(10) Mannan transglycosylase (MTase): joining together of (galacto)glucomannan or mannan polysaccharides by transglycosylation. May be a reaction carried out by the EMase activity described above.

(11) Xyloglucan endotransglycosylase (XET): internal cleavage of glucan backbone of xyloglucan and rejoining the end to another xyloglucan molecule.

(12) Expansin: loosening of hydrogen bonding between cellulose microfibrils and matrix glycans.

(13) Various other glycosidases may also be present in ripening fruit from some species, including α-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, α-xylosidase, and β-xylosidase.

The type and relative abundance of the enzymes that accumulate during ripening vary considerably between species; for example, tomato shows unusually high levels of endo-PG activity, and avocado unusually high levels of EGase activity. In some species, endo-PG activity is very low, but PL activity is present, which also acts to cleave HGA backbones but via β-elimination rather than hydrolysis (Marín-Rodríguez *et al.* 2002). Plant cell wall-modifying enzymes are usually composed of large gene families, several of which are expressed in maturing unripe or in ripening fruit. The presence of multiple isoforms of particular enzymes may confound attempts to investigate function by transgenic manipulation, since an unaffected gene product may be able to compensate for the one that is suppressed, preventing observation of a phenotype. Owing to the commercial importance of fruit softening as the major determinant of shelf life, investigation of the role of particular enzymes has been an attractive target for biotechnology. A summary of transgenic manipulations to determine the roles of candidate genes in the fruit softening process is shown in Table 1.

TABLE 1–Effect on fruit softening and shelf life of genetic manipulation of ripening-related cell wall-modifying enzymes.

| Gene*† | KD or OE‡ | Species | Fruit phenotype | Reference |
|---|---|---|---|---|
| *SlPG* | KD | Tomato | Softening slightly reduced<br>Shelf life increased | Kramer *et al.* (1992)<br>Langley *et al.* (1994) |
| *SlPG* | OE | Tomato | Softening not affected | Giovannoni *et al.* (1989) |
| *SlPME* | KD | Tomato | Softening not affected<br>Shelf life reduced | Hall *et al.* (1993)<br>Tieman & Handa (1994) |
| *SlCel1* | KD | Tomato | Softening not affected | Lashbrook *et al.* (1998) |
| *SlCel2* | KD | Tomato | Softening not affected | Brummell, Hall, & Bennett (1999) |
| *FaCel1* | KD | Strawberry | Softening not affected | Woolley *et al.* (2001) |
| *CaCel1* | KD | Pepper | None reported | Harpster, Brummell, & Dunsmuir (2002) |
| *CaCel1* | OE | Tomato | Softening not affected | Harpster, Dawson, Nevins, Dunsmuir, & Brummell (2002) |
| *SlEXPA1* | KD | Tomato | Softening reduced | Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir (1999) |
| *SlEXPA1* | OE | Tomato | Softening increased | Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir (1999) |
| *Slβsub* | KD | Tomato | Softening increased | Chun & Huber (2000) |
| *SlTBG1* | KD | Tomato | None reported | Carey *et al.* (2001) |
| *SlTBG4* | KD | Tomato | Softening reduced | Smith *et al.* (2002) |
| *SlTBG6* | KD | Tomato | Fruit cracking increased | Moctezuma *et al.* (2003) |
| *FaPL-C* | KD | Strawberry | Softening reduced | Jiménez-Bermúdez *et al.* (2002) |

* Species abbreviations: *Ca*, *Capsicum annuum* (pepper); *Fa*, *Fragaria* × *ananassa* (strawberry); *Sl*, *Solanum lycopersicum* (tomato).

† Enzyme abbreviations: Cel, cellulase (more accurately endo-1,4-β-glucanase); EXP, expansin; PG, endo-polygalacturonase; PL, pectate lyase; PME, pectin methylesterase; βsub, β-subunit protein of endo-polygalacturonase; TBG, tomato β-galactosidase.

‡ Knock-down (post-transcriptional gene silencing) or over-expression.

Early work in tomato showed that endo-PG was only a minor contributor to fruit softening during ripening, although transgenic endo-PG-suppressed fruit showed an extended shelf life due to improved integrity of the middle lamella and consequent enhanced intercellular adhesion (Kramer *et al.* 1992; Langley *et al.*

1994). Reduced HGA demethylesterification by transgenic suppression of PME activity prevented the formation of calcium-pectate cross-links and weakened middle lamella integrity in over-ripe fruit, resulting in fruit almost falling apart as they entered the senescent phase (Tieman & Handa 1994). This confirms the importance of intercellular adhesion to extended fruit shelf life. Neither of these genes appeared to play a major role in fruit softening during normal ripening, although phenotypes were evident in long-term storage trials. However, enhanced mobility of endo-PG in the cell wall space caused by transgenic suppression of the endo-PG β-subunit (a tomato cell wall protein that binds strongly to endo-PG and may restrict its movement in the wall) resulted in softer fruit, showing that endo-PG does make some contribution to the softening process (Chun & Huber 2000). Correspondingly, in strawberry the post-transcriptional gene silencing of PL (which, like endo-PG, depolymerises HGA), resulted in firmer fruit (Jiménez-Bermúdez *et al.* 2002). Both of these observations suggest that depolymerisation of HGA, whether by endo-PG or PL, makes at least some contribution to fruit softening.

The roles of three different β-galactosidases have been investigated in tomato fruit ripening. Suppression of TBG1 (tomato β-galactosidase 1) had no measurable effect on galactose loss during ripening (Carey *et al.* 2001), but suppression of TBG4 early in ripening reduced galactose loss and transgenic fruit retained firmness later in ripening (Smith *et al.* 2002). It is possible that this is due to the retention of RG-I side chains that are normally degraded, which could both directly strengthen the wall and prevent the access of degradative enzymes to their substrate by diminishing the usual increase in wall porosity. Silencing of TBG6 would not be expected to affect firmness during ripening, since it is expressed in maturing fruit prior to ripening, but its suppression caused thickening of the cuticle and increased fruit cracking (Moctezuma *et al.* 2003). These experiments suggest that the widely observed degradation of RG-I galactan side chains plays an important role in the softening process.

Loosening of the xyloglucan-cellulose network by expansin may be required for normal fruit softening. Suppression of a tomato ripening-related expansin resulted in firmer fruit throughout ripening, while a three-fold over-expression of the protein caused a striking increase in softening (Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir 1999). This latter effect was correlated with an enhanced depolymerisation of matrix glycans in the overexpressing line. Since expansins do not themselves have hydrolytic or transglycosylation activity, this must have been due to increased wall loosening, allowing enzymes resident in the cell wall space access to substrates that were normally unavailable.

The identity of the enzymes that bring about depolymerisation of matrix glycans, particularly xyloglucan, is unknown. Suppression of ripening-related EGases in

tomato and strawberry had no effect on fruit firmness (Lashbrook *et al.* 1998; Brummell, Hall, & Bennett 1999; Woolley *et al.* 2001), although more than one gene family member is expressed during ripening in both these species, which may have obscured any phenotype. In bell pepper a single EGase gene (*CaCel1*) provides ripening-related EGase activity, yet silencing of this gene did not prevent depolymerisation of xyloglucan during ripening (Harpster, Brummell, & Dunsmuir 2002). This is consistent with biochemical data from avocado, in which EGase activity and xyloglucanase activity could be separated (O'Donoghue & Huber 1992). Furthermore, over-expression of the pepper gene in tomato increased EGase activity by up to 20-fold, yet no increase in the rate of depolymerisation of total matrix glycans or xyloglucan was observed (Harpster, Dawson, Nevins, Dunsmuir, & Brummell 2002). All of these observations indicate that EGase is not the enzyme that brings about xyloglucan depolymerisation during ripening, and the xyloglucanase responsible has yet to be discovered.

## CONCLUSIONS

Fruit softening is caused by a controlled degradation of cell wall polysaccharides and a loosening of the polymer networks of the wall, resulting in a swelling of the primary cell wall to become a softened, more hydrated, open structure. Some cell wall modifications begin before ripening is initiated, notably the loss of galactan side chains from RG-I. Early in ripening, cell wall galactan and arabinan are rapidly degraded, and a depolymerisation of matrix glycans begins, which is correlated with declining fruit firmness. Also during this time there is continued demethylesterification of HGA, and an increase in pectin solubilisation, both of which may be related to cell wall swelling. A depolymerisation of polyuronides can begin during early or mid ripening, but usually is most pronounced later in ripening, particularly in some melting-flesh fruit. However polyuronide depolymerisation may be very low or absent in some species, particularly in crisp fruit such as apple and bell pepper, but also in some melting-flesh fruit such as banana and strawberry. Different species appear to have evolved different ways of bringing about apparently similar changes to cell wall physical properties.

Cell wall changes are brought about by the ripening-related expression of many genes encoding cell wall-modifying proteins. Fruit softening is thus the result of the concerted action of numerous cell wall-modifying enzymes, and each one has its role to play. The sum of these small effects can be extensive softening and, eventually, tissue disintegration. It seems likely that there is considerable interaction between the enzymes bringing about cell wall disassembly. The dependence of endo-PG on the prior action of PME is well known, but wall loosening (and possibly swelling) mediated by expansin and pectin side chain removal by β-galactosidase and α-arabinosidase may be important for increasing cell wall porosity and

allowing the enhanced access of degradative enzymes to their substrates. The effects of enzymes acting early on the wall may thus be both direct, disassembling the wall structure, and indirect, increasing the accessibility of other enzymes to substrates. Removal of limitations to the action of resident degradative enzymes later in ripening allows the accelerating deterioration of the tissue, which will release the seeds.

## ACKNOWLEDGMENTS

I thank Erin O'Donoghue for useful comments on the manuscript.

## REFERENCES

BARNAVON, L.; DOCO, T.; TERRIER, N.; AGEORGES, A.; ROMIEU, C.; PELLERIN, P. 2001: 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 58*: 693–701.

BARON-EPEL, O.; GHARYAL, P.K.; SCHINDLER, M. 1988: Pectins as mediators of wall porosity in soybean cells. *Planta 175*: 389–395.

BEWLEY, J.D.; BANIK, M.; BOURGAULT, R.; FEURTADO, J.A.; TOOROP, P.; HILHORST, H.W.M. 2000: Endo-β-mannanase activity increases in the skin and outer pericarp of tomato fruits during ripening. *Journal of Experimental Botany 51*: 529-538.

BRUMMELL, D.A.; HARPSTER, M.H. 2001: Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology 47*: 311-340.

BRUMMELL, D.A.; LABAVITCH, J.M. 1997: Effect of antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. *Plant Physiology 115*: 717-725.

BRUMMELL, D.A.; HALL, B.D.; BENNETT, A.B. 1999: Antisense suppression of tomato endo-1,4-β-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Molecular Biology 40*: 615–622.

BRUMMELL, D.A.; DAL CIN, V.; CRISOSTO, C.H.; LABAVITCH, J.M. 2004: Cell wall metabolism during maturation, ripening and senescence of peach fruit. *Journal of Experimental Botany 55*: 2029–2039.

BRUMMELL, D.A.; HARPSTER, M.H.; CIVELLO, P.M.; PALYS, J.M.; BENNETT, A.B.; DUNSMUIR, P. 1999: Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell 11*: 2203–2216.

CAREY, A.T.; SMITH, D.L.; HARRISON, E.; BIRD, C.R.; GROSS, K.C.; SEYMOUR, G.B.; TUCKER, G.A. 2001: Down-regulation of a ripening-related β-galactosidase gene (TBG1) in transgenic tomato fruits. *Journal of Experimental Botany 52*: 663–668.

CARPITA, N.C.; GIBEAUT, D.M. 1993: Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal 3*: 1–30.

CHEN, N.J.; PAULL, R.E. 2003: Endoxylanase expressed during papaya fruit ripening: purification, cloning and characterization. *Functional Plant Biology 30*: 433–441.

CHUN, J.-P.; HUBER, D.J. 2000: Reduced levels of β-subunit protein influence tomato fruit firmness, cell-wall ultrastructure, and PG2-mediated pectin hydrolysis in excised pericarp tissue. *Journal of Plant Physiology 157*: 153–160.

CUTILLAS-ITURRALDE, A.; ZARRA, I.; FRY, S.C.; LORENCES, E.P. 1994: Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycosylase. *Physiologia Plantarum 91*: 169–176.

DOMINGUEZ-PUIGJANER, E.; LLOP, I.; VENDRELL, M.; PRAT, S. 1997: A cDNA clone highly expressed in ripe banana fruit shows homology to pectate lyases. *Plant Physiology 114*: 1071-1076.

GIOVANNONI, J.J.; DELLAPENNA, D.; BENNETT, A.B.; FISCHER, R.L. 1989: Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. *Plant Cell 1*: 53–63.

GROSS, K.C.; SAMS, C.E. 1984: Changes in cell wall neutral sugar composition during fruit ripening: a species survey. *Phytochemistry 23*: 2457–2461.

HALL, L.N.; TUCKER, G.A.; SMITH, C.J.S.; WATSON, C.F.; SEYMOUR, G.B.; BUNDICK, Y.; BONIWELL, J.M.; FLETCHER, J.D.; RAY, J.A.; SCHUCH, W.; BIRD, C.; GRIERSON, D. 1993: Antisense inhibition of pectin esterase gene expression in transgenic tomatoes. *Plant Journal 3*: 121–129.

HARKER, F.R.; STEC, M.G.H.; HALLETT, I.C.; BENNETT, C.L. 1997: Texture of parenchymatous plant tissue: A comparison between tensile and other instrumental and sensory measurements of tissue strength and juiciness. *Postharvest Biology and Technology 11*: 63–72.

HARPSTER, M.H.; BRUMMELL, D.A.; DUNSMUIR, P. 2002: Suppression of a ripening-related endo-1,4-β-glucanase in transgenic pepper fruit does not prevent depolymerization of cell wall polysaccharides during ripening. *Plant Molecular Biology 50*: 345–355.

HARPSTER, M.H.; DAWSON, D.M.; NEVINS, D.J.; DUNSMUIR, P.; BRUMMELL, D.A. 2002: Constitutive overexpression of a ripening-related pepper endo-1,4-β-glucanase in transgenic tomato fruit does not increase xyloglucan depolymerization or fruit softening. *Plant Molecular Biology 50*: 357–369.

HUBER, D.J. 1984: Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. *Journal of Food Science 49*: 1310–1315.

HUBER, D.J.; O'DONOGHUE, E.M. 1993: Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. *Plant Physiology 102*: 473–480.

JARVIS, M.C. 1984: Structure and properties of pectin gels in plant cell walls. *Plant Cell and Environment 7*: 153–164.

JARVIS, M.C.; BRIGGS, S.P.H.; KNOX, J.P. 2003: Intercellular adhesion and cell separation in plants. *Plant Cell and Environment 26*: 977–989.

JIMENEZ-BERMUDEZ, S.; REDONDO-NEVADO, J.; MUNOZ-BLANCO, J.; CABALLERO, J.L.; LOPEZ-ARANDA, J.M.; VALPUESTA, V.; PLIEGO-ALFARO, F.; QUESADA, M.A.; MERCADO, J.A. 2002: Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. *Plant Physiology 128*: 751–759.

KIM, J.; GROSS, K.C.; SOLOMOS, T. 1991: Galactose metabolism and ethylene production during development and ripening of tomato fruit. *Postharvest Biology and Technology 1*: 67–80.

KOCH, J.L.; NEVINS, D.J. 1989: Tomato fruit cell wall. 1. Use of purified tomato polygalacturonase and pectinmethylesterase to identify developmental changes in pectins. *Plant Physiology 91*: 816–822.

KRAMER, M.; SANDERS, R.; BOLKAN, H.; WATERS, C.; SHEEHY, R.E.; HIATT, W.R. 1992: Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. *Postharvest Biology and Technology 1*: 241–255.

LANGLEY, K.R.; MARTIN, A.; STENNING, R.; MURRAY, A.J.; HOBSON, G.E.; SCHUCH, W.W.; BIRD, C.R. 1994: Mechanical and optical assessment of the ripening of tomato fruit with reduced polygalacturonase activity. *Journal of the Science of Food and Agriculture 66*: 547–554.

LASHBROOK, C.C.; GIOVANNONI, J.J.; HALL, B.D.; FISCHER, R.L.; BENNETT, A.B. 1998: Transgenic analysis of tomato endo-β-1,4-glucanase gene function. Role of *cel1* in floral abscission. *Plant Journal 13*: 303–310.

MACLACHLAN, G.; BRADY, C. 1994: Endo-1,4-β-glucanase, xyloglucanase and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. *Plant Physiology 105*: 965–974.

MARIN-RODRIGUEZ, M.C.; ORCHARD, J.; SEYMOUR, G.B. 2002: Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany 53*: 2115–2119.

MEDINA-ESCOBAR, N.; CARDENAS, J.; MOYANO, E.; CABALLERO, J.L.; MUNOZ-BLANCO, J. 1997: Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology 34*: 867–877.

MOCTEZUMA, E.; SMITH, D.L.; GROSS, K.C. 2003: Antisense suppression of a β-galactosidase gene (TBG6) in tomato increases fruit cracking. *Journal of Experimental Botany 54*: 2025–2033.

O'DONOGHUE, E.M.; HUBER, D.J. 1992: Modification of matrix polysaccharides during avocado (*Persea americana*) fruit ripening: an assessment of the role of Cx-cellulase. *Physiologia Plantarum 86*: 33–42.

O'DONOGHUE, E.M.; SOMERFIELD, S.D.; DE VRE, L.A.; HEYES, J.A. 1997: Developmental and ripening-related effects on the cell wall of pepino (*Solanum muricatum*) fruit. *Journal of the Science of Food and Agriculture 73*: 455–463.

PAULL, R.E.; GROSS, K.; QIU, Y.X. 1999: Changes in papaya cell walls during fruit ripening. *Postharvest Biology and Technology 16*: 79–89.

PENA, M.J.; CARPITA, N.C. 2004: Loss of highly branched arabinans and debranching of rhamnogalacturonan I accompany loss of firm texture and cell separation during prolonged storage of apple. *Plant Physiology 135*: 1305–1313.

PHARR, D.M.; SOX, H.N.; NESBITT, W.B. 1976: Cell wall-bound nitrophenylglycosides of tomato fruits. *Journal of the American Society for Horticultural Science 101*: 397–400.

PLATT-ALOIA, K.A.; THOMSON, W.W.; YOUNG, R.E. 1980: Ultrastructural changes in the walls of ripening avocados: transmission, scanning, and freeze fracture microscopy. *Botanical Gazette 141*: 366–373.

POPPER, Z.A.; FRY, S.C. 2005: Widespread occurrence of a covalent linkage between xyloglucan and acidic polysaccharides in suspension-cultured angiosperm cells. *Annals of Botany 96*: 91–99.

REDGWELL, R.J.; FISCHER, M.; KENDAL, E.; MacRAE, E.A. 1997: Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta 203*: 174-181.

REDGWELL, R.J.; MacRAE, E.A.; HALLETT, I.; FISCHER, M.; PERRY, J.; HARKER, R. 1997: In vivo and in vitro swelling of cell walls during fruit ripening. *Planta 203*: 162–173.

ROSE, J.K.C.; HADFIELD, K.A.; LABAVITCH, J.M.; BENNETT, A.B. 1998: Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. *Plant Physiology 117*: 345–361.

SCHRODER, R.; WEGRZYN, T.F.; BOLITHO, K.M.; REDGWELL, R.J. 2004: Mannan transglycosylase: a novel enzyme activity in cell walls of higher plants. *Planta 219*: 590–600.

SHACKEL, K.A.; GREVE, C.; LABAVITCH, J.M.; AHMADI, H. 1991: Cell turgor changes associated with ripening in tomato pericarp tissue. *Plant Physiology 97*: 814–816.

SMITH, D.L.; ABBOTT, J.A.; GROSS, K.C. 2002: Down-regulation of tomato β-galactosidase 4 results in decreased fruit softening. *Plant Physiology 129*: 1755–1762.

TIEMAN, D.M.; HANDA, A.K. 1994: Reduction in pectin methylesterase activity modifies tissue integrity and cation levels in ripening tomato (*Lycopersicon esculentum* Mill.) fruits. *Plant Physiology 106*: 429–436.

VINCKEN, J.-P.; SCHOLS, H.A.; OOMEN, R.J.F.J.; McCANN, M.C.; ULVSKOV, P.; VORAGEN, A.G.J.; VISSER, R.G.F. 2003: If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. *Plant Physiology 132*: 1781–1789.

WADE, N.L.; KAVANAGH, E.E.; HOCKLEY, D.G.; BRADY, C.J. 1992: Relationship between softening and the polyuronides in ripening banana fruit. *Journal of the Science of Food and Agriculture 60*: 61–68.

WAKABAYASHI, K.; CHUN, J.-P.; HUBER, D.J. 2000: Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. *Physiologia Plantarum 108*: 345–352.

WOOLLEY, L.C.; JAMES, D.J.; MANNING, K. 2001: Purification and properties of an endo-β-1,4-glucanase from strawberry and down-regulation of the corresponding gene, *cel1*. *Planta 214*: 11–21.

YOSHIOKA, H.; AOBA, K.; KASHIMURA, Y. 1992: Molecular weight and degree of methoxylation in cell wall polyuronide during softening in pear and apple fruit. *Journal of the American Society for Horticultural Science 117*: 600–606.

ZYKWINSKA, A.W.; RALET, M.C.J.; GARNIER, C.D.; THIBAULT, J.F.J. 2005: Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiology 139*: 397–407.