KIWIFRUIT CELL WALLS: TOWARDS AN UNDERSTANDING OF SOFTENING?*

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ABSTRACT

Kiwifruit is an excellent model in which to study events in the cell wall that lead to fruit becoming soft. In kiwifruit, softening can be separated into distinct phases, with the phase associated with the most extensive and rapid loss of firmness being well-separated temporally from that associated with the respiratory climacteric and ethylene production. This contrasts with tomato, avocado, and many other fruit where softening changes, ethylene production, and the climacteric occur concurrently. The changes that occur in the kiwifruit cell wall during the softening process have been extensively characterised by chemical analyses, by histochemical and immunolocalisation techniques, and by monitoring the activity and expression of wall-associated enzymes.

Keywords: cell wall; kiwifruit; ripening; softening; *Actinidia deliciosa*.

INTRODUCTION

Kiwifruit were introduced to New Zealand at the beginning of the twentieth century, when a missionary, Isabel Fraser, brought a handful of seeds back from China. Little did she know that within 100 years, kiwifruit would sit atop New Zealand's list of horticultural exports, and be one of the most extensively studied fruit systems in the world.

New Zealand's most important export cultivars are currently *Actinidia deliciosa* [A. Chev.] C. F. Liang and A. R. Ferguson var. *deliciosa* 'Hayward' marketed as ZESPRI™ GREEN, and *Actinidia chinensis* Planch. 'Hort16A' marketed as ZESPRI™ GOLD. Until the release of 'Hort16A', all commercial kiwifruit cultivars in New Zealand could be traced back to the original seeds collected by Isabel Fraser.

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Kiwifruit can be picked at an unripe but physiologically mature stage. At this stage, fruit can be stored for months in controlled atmosphere and cold temperature (McDonald 1990). The ripening process is greatly minimised under these conditions (Arpaia *et al.* 1987), making it possible to ship the crop from New Zealand into faraway markets such as Europe or Japan. After transfer from storage, the fruit continues to soften and ripen in a similar way to air-stored fruit at equivalent firmness (Lallu *et al.* 2005).

Ripening is accompanied by a change in the texture of the fruit. Kiwifruit develops a soft melting texture when ripe, like peach, avocado, strawberry, or pear. In contrast, fruit such as apples, nashi pears, or watermelon, become crisp and juicy when ripe. The water-binding properties of the cell wall have a large impact on texture, which is a key factor influencing the acceptability of fruit to the consumer (Harker *et al.* 1997).

At HortResearch, we have been studying changes in the kiwifruit cell wall during the softening process by chemical analyses of isolated cell walls, by monitoring the activity and expression of wall-associated enzymes, and by using histochemical and immunolocalisation techniques to follow changes in cell wall constituents. Most of this work has been carried out on the cultivar 'Hayward'. In this review, we will give an overview of this work. The reader is referred to work by Brummell (2006) for a broader coverage of species in the fruit softening process.

KIWIFRUIT SOFTEN IN DISTINCT PHASES

After harvest, kiwifruit go through three distinct softening phases that are temporally well separated. In the first phase, fruit typically hold close to their initial harvest firmness and soften only slowly. In the second phase, fruit soften extensively and rapidly to about 20% of their harvest value. The last phase of softening is marked by the start of internal ethylene production. Fruit undergo the respiratory climacteric, become eating soft, and develop the characteristic flavours and aromas associated with ripe kiwifruit (Paterson *et al.* 1991).

The clear temporal separation of the softening process into distinct phases makes it possible to study events in the kiwifruit cell wall that initiate the softening process (Phase 1), and that occur as the fruit rapidly soften (Phase 2). These processes occur independently of ethylene production and the climacteric burst which take place in Phase 3. This clear separation of softening and ethylene production does not happen in most other fruit. In tomato (Crookes & Grierson 1983) and avocado (Platt-Aloia & Thomson 1981), for example, most of the softening changes occur co-incident with the climacteric burst and ethylene production.

Ethylene treatment of firm kiwifruit reduces the total softening time from approximately 3–4 weeks to 6–7 days. Apart from reducing the softening time,

ethylene also minimises the variability in the softening rate between individual fruit. Therefore, most of the cell wall work on 'Hayward' has been done on ethylene-treated fruit.

COMPOSITION OF KIWIFRUIT CELL WALL POLYSACCHARIDES

In kiwifruit, the chemical approach to examine changes to polysaccharides during the softening process has been "careful demolition" of the cell wall: polysaccharides from the insoluble cell wall material (CWM) were sequentially extracted by solvents of increasing strength such as CDTA, Na_2CO_3, 6 M GTC, and 4 M KOH (Redgwell *et al.* 1988, 1991; Redgwell, Melton, Brasch, & Coddington 1992). Purification and detailed compositional analyses of extracted cell wall polysaccharides gave insight into the underlying chemical changes during softening.

A detailed analysis of polysaccharides present in the outer pericarp tissue (*see* Fig. 1 for definition of tissue zones in kiwifruit) of unripe kiwifruit showed that basically all polysaccharide types present in cell walls of other fruit were also present in kiwifruit (Redgwell *et al.* 1988). The CDTA- and Na_2CO_3-extracted polysaccharides were rhamnogalacturonans, with galactosyl residues or arabinogalactan side chains with varying degrees of branching. The pectin of the GTC- and KOH-extracted fractions also consisted of rhamnogalacturonans with large β-(1→4)-linked galactan side chains attached. These pectic polysaccharides are referred to as "pectic galactans". They also have a higher degree of branching than the CDTA- and Na_2CO_3-extracted pectin (Redgwell, Melton, Brasch, & Coddington 1992). There was an increase in branching of the pectin as it became more difficult to extract from the cell wall material during the extraction process (Redgwell, Melton, & Brasch 1992).

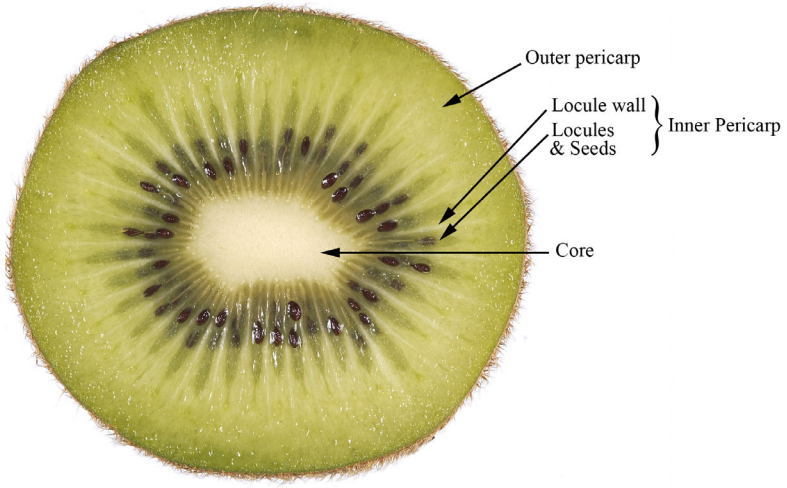

FIG. 1–Kiwifruit tissue zones.

The GTC- and KOH-extracted fractions also contained hemicelluloses. The major hemicellulose in kiwifruit cell walls is xyloglucan. Two minor hemicelluloses, a glucuronoxylan and a branched mannan, later purified and characterised as galactoglucomannan (Schröder *et al.* 2001), were present as well. There is also evidence that a polysaccharide of the rhamnogalacturonan II-type is associated with the pectin of kiwifruit (Redgwell *et al.* 1988). However, Dawson & Melton (1991) did not find rhamnogalacturonan II-type pectin in their kiwifruit study, but held the omission of a de-esterification step during the separation accountable for that.

The cell wall residue after KOH extraction contains the hemicelluloses xyloglucan and galactoglucomannan in small amounts, the pectic galactan, and cellulose (R.J.Redgwell unpubl. data). Cellulose is present in two crystalline forms (cellulose I_α and I_β), but their relative proportions are unknown (Newman & Redgwell 2002).

CELL WALL CHANGES DURING KIWIFRUIT SOFTENING

For most kiwifruit cell wall research, the outer pericarp of ethylene-treated 'Hayward' fruit has been used. The main events occurring in the cell wall during kiwifruit softening are pectin solubilisation, cell wall swelling, degradation of pectin, reduction in the molecular weight of xyloglucan, and dissolution of middle lamellae, all of which eventually lead to disintegration of the cell wall. These changes occur not only in kiwifruit but also in many other fruit that ripen to a soft melting texture (Brummel & Harpster 2001). A schematic representation of the key events during the softening process in relation to the timing of the softening phases of ethylene-treated kiwifruit is shown in Fig. 2 (modified from MacRae & Redgwell 1992).

Pectin "Softening" and Solubilisation

In firm fruit (Phase 1), pectin is generally water-insoluble in the cell wall, just as hemicelluloses and cellulose are. The first change in pectin was observed approximately 1 day after ethylene treatment, using nuclear spin relaxation experiments. Pectin retained in the cell wall started to "soften" — it changed from a relatively rigid (solid-like) state to a more mobile (liquid-like) state (Newman & Redgwell 2002). This "softening" of pectin is not a chemical but a physical change, as it cannot be picked up by chemical analyses. Pectin "softening" clearly precedes both pectin solubilisation and depolymerisation.

In vivo-solubilised pectin can be extracted from cell wall material with phenol:acetic acid:water (PAW), a solvent used to inactivate endogenous enzymes in the tissue. Pectin solubilised during the first softening phase and extracted with PAW has the same size and degree of branching as pectin extracted with Na_2CO_3 from cell wall

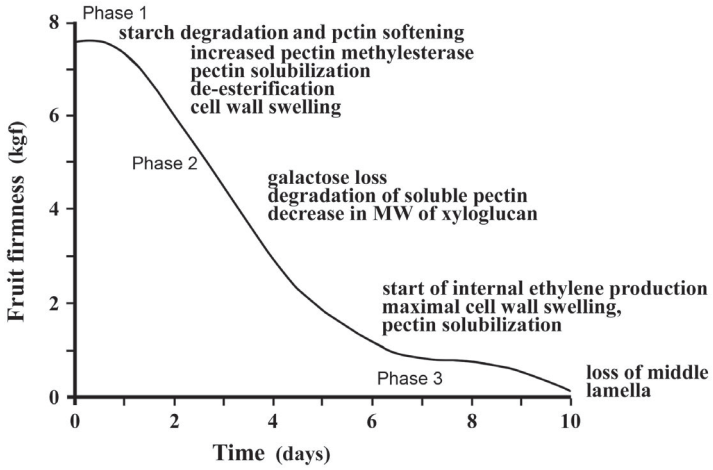

FIG. 2–Key events in kiwifruit softening.
Schematic representation of postharvest kiwifruit softening in relation to the timing of key events in the softening process of ethylene-treated 'Hayward' fruit (1 – 3 are softening phases) (modified from MacRae & Redgwell 1992).

material at harvest. Thus, this initial pectin solubilisation seems to occur without apparent degradation of pectin (Redgwell, Melton, & Brasch 1992). Overall, no neutral sugars are lost from the cell wall during this first softening phase.

Cell Wall Swelling

Microscopy observations showed that cell walls that were thin and compact in the unripe stage started to look diffuse (i.e., they appeared swollen and thicker with reduced stainability) as ripening proceeded. At eating softness, the cell walls had swollen to approximately three to four times their thickness at harvest (Hallett *et al.* 1992). This phenomenon accompanies softening in many other species that ripen to a soft, melting texture (Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997). Cell wall swelling can be even more clearly observed *in vitro* (Fig. 3A, modified from Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997). Isolated cell wall material suspended in water showed that cell wall swelling began towards the end of the first softening phase (1–2 days after ethylene treatment) and reached a maximum at eating softness. At the same time, the cell wall material also became more viscous, probably through changes in insoluble pectin. The degree of swelling and viscosity *in vitro* were not in synchrony, with viscosity being slightly advanced, indicating that changes in pectin might precede swelling.

Where Does the Solubilised Pectin Come From?

Pectin solubilisation (and cell wall swelling) became more pronounced in the second softening phase. When fruit is eating ripe, about 60% of the cell wall uronide

will have been solubilised from the cell wall. Where does the solubilised pectin come from? Redgwell, Melton, & Brasch (1992) observed that pectin solubilised in the first softening phase was similar to pectin extractable with Na_2CO_3 at harvest, and that the Na_2CO_3-fraction showed a marked decrease in yield during softening, thereby suggesting solubilised pectin might derive from the Na_2CO_3-extracted fraction. Furthermore, the molecular weight and degree of galactosylation of the Na_2CO_3-extracted fraction increased during softening (Redgwell *et al.* 1991). Redgwell, Melton, & Brasch (1992) speculated that smaller and less branched pectic polysaccharides were solubilised first, and that larger and more highly branched polysaccharides from "later" (more tightly bound) fractions were taking their place during the softening process. This means that a general movement of pectin towards "solubilisation" takes place during softening. Very tightly bound pectin (pectic galactan) may become less tightly bound pectin (extractable in Na_2CO_3) and then eventually pectin freely soluble in water.

How are Pectin Solubilisation and Cell Wall Swelling Related?

In vivo, cell wall swelling and pectin solubilisation were in synchrony (Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997), suggesting they may be connected or interdependent processes. Chemical removal of pectin from kiwifruit cell wall material supported the idea that swelling was associated with movement of water into the voids left in the cellulose-hemicellulose network by the solubilised pectin (Fig. 3B). No treatment, however, gave the same degree of cell wall swelling and release of (undegraded) pectin as the *in vivo* process, confirming a complex process that cannot be mimicked by simple chemical extractions.

Unpublished data of T.H.Koh and R.Schröder (HortResearch) support the involvement of expansin in the process of pectin solubilisation. Expansins are proteins that mediate cell expansion or growth in a unique and unknown mechanism.

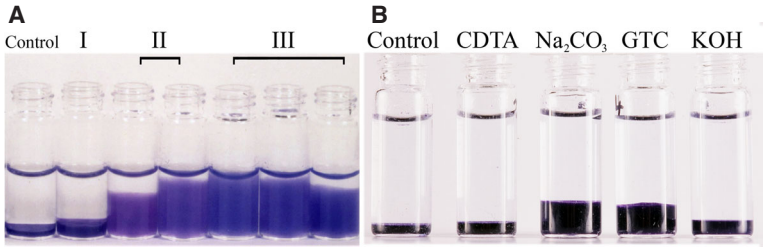

FIG. 3–Cell wall swelling *in vitro*.
3A: swelling of cell wall material isolated from kiwifruit at harvest (contl) and during the three different phases of softening .
3B: *in vitro* swelling of cell wall material obtained from 'Hayward' fruit at harvest (contl) and after sequential extraction with CDTA, Na_2CO_3, GTC, and 4 M KOH (visual representation of data from Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997).

In a current model, they induce cell expansion by disrupting hydrogen bonding between cellulose and xyloglucan, components of the major load-bearing network in the plant cell wall, thereby temporarily loosening the cell wall (McQueen-Mason & Cosgrove 1995). Their presence in softening fruit has been well described (*see* Brummell & Harpster 2001); their function, however, is unknown. In kiwifruit, we have shown that purified expansin is able to release pectin from unripe cell wall material (Koh & Schröder unpubl. data), suggesting that expansin action may lead to pectin solubilisation. This might occur by expansin wedging itself between polymers of the hemicellulose network, thereby enabling pectin to diffuse out and become soluble, without apparent degradation. The voids left by the pectin could then be filled with water that becomes manifest as cell wall swelling.

Role of Xyloglucan During Cell Wall Swelling

Xyloglucans are thought to be part of a cellulose-hemicellulose framework, stabilising the cell wall. During softening, the average molecular weight of xyloglucan was reduced from approximately 500 to 300 kDa (KOH-extracted) or 185 to 115 kDa (GTC-extracted) (Redgwell *et al.* 1991), thereby making this framework weaker and promoting cell wall swelling. No change in composition or linkages accompanied this decrease. The xyloglucan network seems to add stability to the swollen cell wall, as treatment with 4 M KOH, which solubilises and removes hemicelluloses from the cell wall residue, caused swollen cell walls to collapse (Fig. 3B, reproduced from Redgwell, MacRae, Hallett, Fischer, Perry, & Harker 1997).

Using ^{13}C NMR, Newman & Redgwell (2002), however, found that ripening processes in kiwifruit did not affect the nature of cellulose crystallites or the nature of polysaccharides adhering to surfaces of cellulose crystallites even in the softest fruit. By means of labelling with gold-conjugated exoglucanase, it was shown that cellulose remained intact and labelled densely across the cell wall at all stages of softening (Sutherland *et al.* 1999).

Galactoglucomannan, a minor hemicellulose component in kiwifruit cell walls, is also potentially able to participate in the hemicellulose-cellulose framework of the cell wall. However, galactoglucomannans did not seem to undergo any changes during softening. Their molecular weight and sugar composition remained the same throughout the softening procedure. An increase in yield towards the end of softening was attributed to better extraction due to cell wall loosening, and not to galactoglucomannan synthesis (Redgwell pers. comm.).

Galactose Loss and Pectic Galactan

In the second softening phase the cell wall started to lose galactose until, by the end of softening, only about 30% of the galactose present at harvest remained in the cell

wall (Redgwell, Melton, & Brasch 1992). Most cell wall galactose was associated not with the pectin of the CDTA- or Na_2CO_3-extracted fractions, but with pectin extracted with 4 M KOH or what remained bound within the cell wall residue. These pectic polysaccharides or "galactans" contained, overall, twice as much galactose as galacturonic acid, with domains that contained up to 10 times as much. It is from these galactan side chains that most of the galactose was lost during kiwifruit softening (Redgwell, Fischer, Kendal, & MacRae 1997). Therefore, in kiwifruit galactose loss and pectin solubilisation and hydrolysis affect different pectins. Galactose loss is associated mainly with insoluble pectin in the cell wall, whereas hydrolysis of pectin to monomers and oligomers is only associated with solubilised pectin.

The pectic galactan largely remained in the cell wall residue even after extraction with 4 M KOH (Redgwell, Melton, & Brasch 1992). The strong alkali solution is used to extract hemicelluloses from the cell wall because this solvent breaks the hydrogen bonds that attach hemicelluloses to cellulose microfibrils. The pectic galactan seems to be held in the cell wall simply by entanglement. Treatment of the cell wall residue with polygalacturonase from tomato released some of the galactan chains, implying that the pectic galactan must be attached to the hemicellulose-cellulose framework via the pectic backbone, leaving the galactan side chains free (Redgwell, Melton, Brasch, & Coddington 1992; Redgwell, Fischer, Kendal, & MacRae 1997). To determine whether pectic galactan was held in the cell wall by covalent cross-linking to the cellulose-hemicellulose framework, cell wall residue after 4 M KOH extraction was treated with purified fungal cellulases. The cellulase mixture degraded surrounding cellulose and hemicelluloses and thereby released pectic galactan. Compositional analyses of the barely water-soluble polysaccharide (> 2000 kDa) gave galactose and galacturonic acid and small amounts of xylose and glucose. Linkage analyses, however, indicated that these trace monomers were probably present because of contaminating traces of xyloglucan rather than because they belonged to the pectic galactan molecule (Koh & Schröder unpubl. data). Interestingly, neither polygalacturonase nor cellulases were able to release all galactan in the cell wall residue.

Degradation of Pectin and Disintegration of the Cell Walls

Degradation of solubilised pectin and loss of middle lamellae are processes that are initiated in the second softening phase but peak in the last softening phase (Phase 3). These processes contribute to the disintegration of the cell wall that is completed at the end of Phase 3.

Hydrolysis of solubilised pectin produced wall-derived monosaccharides and oligosaccharides during softening. Galactose and galactose-containing

oligosaccharides were investigated more closely (Redgwell *et al.* 1990). Small amounts of these oligosaccharides were detected at harvest, but in the ripe stage only free galactose increased. However, the amount of free galactose and galactose-containing oligosaccharides could not account for the total amount of galactose lost from the cell wall. This indicated that metabolism of wall-derived galactose had occurred postharvest, to avoid accumulation of potentially toxic galactose (Thorpe *et al.* 1999).

Breakdown of middle lamellae was visible by microscopy in samples taken at the end of softening Phase 2 (Hallett *et al.* 1992). CDTA-extracted pectin, which is thought to make up the middle lamellae gluing the cells together, also decreased in yield and molecular weight during softening (Redgwell *et al.* 1990), confirming the microscopy data. Interestingly, Sutherland *et al.* (1999) found no "typical" middle lamellae using immunolabelling with JIM5 antibodies, lectin labelling, and cationic colloidal gold labelling. In firm and in ripe fruit, unesterified galacturonic acid residues, thought to be a characteristic of middle lamellae pectin, were scattered throughout the cell wall, and the density seemed to be greatest at the cell wall/plasmalemma boundary. "Typical" middle lamellae were visible only near an intercellular space or when bordering an intercellular space, where the label was packed in a thin layer.

During the softening process, the shape of cells changes as a consequence of tissue packing. Cells in unripe fruit have an angular polyhedral shape with only limited intercellular space visible. As softening progressed, cell profiles rounded and became more spherical, and air-filled intercellular space increased in the outer pericarp and in the core (Hallett *et al.* 1992). A unique feature of kiwifruit in the last softening stage is the accumulation of electron-dense bodies between plasmalemma and the cell wall, and in lesser amounts in the middle lamella regions (Hallett *et al.* 1992). These deposits are most likely protein, or complexes with polysaccharides or lipids, and have not been detected in other fruit to date.

Contribution of Polysaccharide Synthesis to Cell Wall Changes During Softening

Redgwell (1996) labelled kiwifruit by $^{14}CO_2$ exposure, and examined their capacity to synthesise cell wall polysaccharides immediately after harvest and when they were ripe. The fruit showed incorporation of radioactivity into cell wall material during ripening, but over 90% of the radioactivity was in cell-wall-associated protein. Radioactivity was detected in galactose, glucose, and mannose from the cell wall material, but there was no ripening-related increase of labelled components. It was concluded that although mature and ripe kiwifruit were able to synthesise cell wall polysaccharides during softening, this did not appear to be ripening related.

Correlation of Events (the Role of External Ethylene)

Redgwell, Fischer, Kendall, & MacRae (1997) found a strong correlation existed between pectin solubilisation, cell wall swelling and softening, but no similar correlation was found for galactose loss. In ethylene-treated fruit, galactose loss occurred when pectin solubilisation, cell wall swelling, and softening were already well under way. This result also indicated that galactose loss and pectin solubilisation were independent processes since removal of galactose side chains from pectin was evidently not necessary for its solubilisation. Investigations on cell wall changes during on-vine softening confirmed these findings. Kiwifruit left on the vine to soften also showed pectin solubilisation, swelling, and galactose loss but the relative timing of the events differed from softening in ethylene-treated fruit (Redgwell & Percy 1992). Cell wall swelling and pectin solubilisation still seemed correlated but galactose loss started beforehand, at a firmness stage when ethylene-treated fruit showed no evidence of galactose loss at all. This suggested that none of the key cell wall changes measured dictates the initiation and development of the others (Redgwell & Percy 1992). Interestingly, treatment of kiwifruit discs with an inhibitor of ethylene biosynthesis inhibited softening and pectin solubilisation but had no influence on galactose loss. This result suggested that galactose loss and pectin solubilisation in ripening kiwifruit were separate processes and that galactose loss was independent of internal ethylene production (Redgwell & Harker 1995).

ENZYMES IN THE SOFTENING PROCESS

β-galactosidase

A β-galactosidase from kiwifruit has been purified (Ross *et al.* 1993) and shown to release galactose from a variety of kiwifruit polysaccharide fractions, including cell wall material, Na_2CO_3-extracted pectin, galactan, and the hemicelluloses xyloglucan and galactoglucomannan. Although the enzyme was active against all those potential substrates, the activity of the enzyme *in vitro* was far too low to account for the galactose loss observed during softening. Surprisingly, β-galactosidase activity remained constant during the period of greatest galactose loss (softening Phase 2) in kiwifruit ripening (Wegrzyn & MacRae 1992; Bonghi *et al.* 1996), with activity rising only late in Phase 3 (Bonghi *et al.* 1996). However, it should be noted that enzyme activity was measured against synthetic substrates in these studies.

Given the large amount of galactose lost from the kiwifruit cell wall during softening, a β-galactosidase expressed in softening Phase 2 would be a good candidate for manipulation in transgenic kiwifruit, especially given that suppression of a β-galactosidase isoform in tomato resulted in reduced galactose loss and firmer fruit during storage (Smith *et al.* 2002).

Xyloglucan Endotransglucosylase/hydrolase (XTH)

Xyloglucan endotransglucosylase/hydrolase (XTH) enzyme was detected at low levels in fruit at harvest and increased as fruit ripened, especially in core tissue (Redgwell & Fry 1993; Percy *et al*. 1996). Distribution of the xyloglucan endotransglucosylase (XET) activity in ripe kiwifruit is shown in Fig. 4, using a tissue print technique developed by Fry (1997). Highest xyloglucan endotransglucosylase activity can clearly be seen in core and vascular bundles, with lesser amounts in the rest of the fruit (Schröder & Atkinson unpubl. data). Xyloglucan endotransglucosylase/hydrolase gene expression was detected in fruit throughout softening, with expression of two genes, *AdXET5* and *AdXET6*, peaking in softening Phase 3 (Schröder *et al.* 1998).

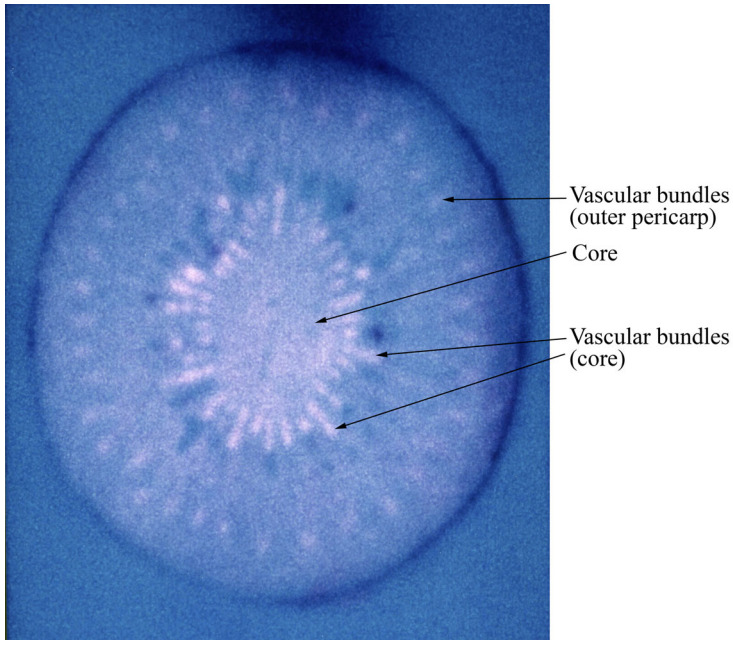

FIG. 4–Distribution of xyloglucan endotransglucosylase activity in ripe kiwifruit. Tissue prints of kiwifruit were labelled with fluorescent xyloglucan-derived oligosaccharides according to the technique of Fry (1997). Highest xyloglucan endotransglucosylase activity (white) is observed in the core and vascular bundles of core and outer pericarp.

During softening, the average molecular weight of xyloglucan decreases, thereby weakening the xyloglucan-cellulose network. How can xyloglucan endotransglucosylase/hydrolase accomplish this? The protein corresponding to *AdXET6* has been purified from ripe kiwifruit core. *In vitro,* the enzyme was able to act as a transglucosylase in the presence of xyloglucan-derived oligosaccharides (XGOs)

as well as a hydrolase if only xyloglucan was present (Schröder *et al.* 1998). Like hydrolysis, transglucosylation of xyloglucan with short-chain xyloglucan-derived oligosaccharides also reduces the average molecular weight of xyloglucan. Therefore, this enzyme is able to reduce the molecular weight of xyloglucan during kiwifruit softening either by transglucosylation or by hydrolysis. However, if xyloglucan endotransglucosylase/hydrolase were to reduce the average molecular weight of xyloglucan during softening via transglucosylation, the presence of xyloglucan-derived oligosaccharides *in vivo* is necessary. Extraction of oligosaccharides from unripe, medium ripe and ripe outer pericarp of kiwifruit showed that xyloglucan-derived oligosaccharides were present at the unripe stage and medium stage. In the ripe stage, however, their presence drastically increased (Schröder & Redgwell unpubl. data). Since xyloglucan endotransglucosylase/hydrolase from kiwifruit core had a much higher transglucosylase than hydrolase activity (Schröder *et al.* 1998), the average molecular weight of xyloglucan is most likely reduced by transglucosylation of xyloglucan with the *in vivo* xyloglucan-derived oligosaccharides.

Mannan Transglycosylase

Recently, a new enzyme activity was discovered, mannan transglycosylase. Like xyloglucan endotransglucosylase, this enzyme activity was able to act as a transglycosylase using mannan-derived polysaccharides and mannan-derived oligosaccharides instead of xyloglucan and xyloglucan-derived oligosaccharides (Schröder *et al.* 2004). Although the substrate for the *in vitro* assays, galactoglucomannan, has been purified from kiwifruit outer pericarp (Schröder *et al.* 2001), only low mannan transglycosylase activity was detected in unripe or ripe kiwifruit outer pericarp or core (Schröder *et al.* 2004).

Polygalacturonase (PG)

Three polygalacturonase genes have been characterised from kiwifruit (Wang *et al.* 2000): *CkPGA* and *CkPGB* were expressed only in softening Phase 3 fruit, whilst *CkPGC* was expressed throughout all three softening phases and expression increased significantly late in Phase 2 before reaching a maximum in Phase 3 (*see* Fig. 2). Bonghi *et al.* (1996) and Wegrzyn & MacRae (1992) both detected very low levels of polygalacturonase enzyme activity in softening Phases 1 and 2. However, Bonghi *et al.* (1996) reported an increase in polygalacturonase activity in Phase 3, whilst Wegrzyn & MacRae (1992) reported a decrease. Western blot analyses using an antibody raised against polygalacturonase from ripe tomato indicated that polygalacturonase protein was found in fruit from the middle of softening Phase 2 onwards, but not in fruit in softening Phase 1 or very early Phase 2 (Koh & Schröder unpubl. data). Therefore, polygalacturonase activity in kiwifruit softening is most

likely related to pectin degradation and not to solubilisation, since enzyme activity and protein are not present at the time pectin solubilisation starts, but are found when pectin degradation occurs towards the end of Phase 2 and in Phase 3.

Pectin Methylesterase (PME)

Pectin methylesterase and pectin methylesterase inhibitor protein have been isolated from ripe kiwifruit (Ciardiello *et al.* 2004; Balestrieri *et al.* 1990). Pectin methylesterase activity increased during ethylene treatment (Phase 1) then dropped rapidly to low levels as fruit softened (Wegrzyn & MacRae 1992). Although recombinant pectin methylesterase inhibitor protein from kiwifruit has been extensively characterised (Di Matteo *et al.* 2005), little is known of the role of pectin methylesterase or pectin methylesterase inhibitor protein on kiwifruit cell walls *in vivo*.

COMPLEXITY IN KIWIFRUIT SOFTENING

Differential softening can be observed between kiwifruit tissues, within a tissue, and within cell walls. The characteristic tissue zones within a kiwifruit are the outer pericarp, inner pericarp, seed plus locule area, and core (Fig. 1). They differ in their chemical composition (MacRae, Bowen, & Stec 1989), rate of softening (MacRae, Lallu, Searle, & Bowen 1989), firmness at harvest (Jackson & Harker 1997), and cell size and packaging (Hallett *et al.* 1992).

The tissue zones differ with respect to the cell wall changes that occur during softening. Although the tissue zones contained a range of polysaccharides that were remarkably similar (Redgwell *et al.* 1988), cell wall breakdown triggered by ethylene did not occur simultaneously, with softening being more pronounced in the outer and inner pericarp than the locule plus seed area and core tissue (Redgwell *et al.* 1990). The changes in composition of cell wall material were also similar, albeit the extent of change was different for each tissue. The outer pericarp, for example, lost about twice as much galactose as the inner pericarp or core during softening. Uronic acid changes also differed between these tissue zones. The locule walls tended to resemble the core chemically and physiologically. They had similar composition at each stage with less change in composition during softening (Redgwell *et al.* 1988, 1990). Morphologically, cell walls of the outer pericarp, locule wall, and core showed extensive swelling, with varying thickness between cells and even within the same cell. Cell walls in the locules of the inner pericarp, however, showed only minimal thickening in ripe fruit (Hallett *et al.* 1992), and cells from the outer pericarp swelled at an earlier stage than those of the core.

Differential softening also occurs in cell walls within a tissue zone. The outer pericarp consists of two distinct populations of cells. From anthesis onwards, large cells can be seen alongside small cells. Various staining techniques and

immunolocalisation tools, however, did not distinguish between the two cell types. This changed in ripe fruit. Cell walls of large cells retained the structural characteristics of unripe fruit, whereas cell walls of small cells altered markedly. Large cells can be segregated intact from very ripe kiwifruit. The partitioning of cell wall material from large cells and the sugar composition were different from that of whole tissue (I.Hallett, P.Sutherland, E.A.MacRae, HortResearch, unpubl. data).

Within the same cell wall region, differential cell wall changes also occur. Plasmodesmata can be observed as small groups in discrete pit fields. In ripe fruit, these pit fields were different from the rest of the wall (Hallett *et al.* 1992). They were much thinner, comparable to plasmodesmata and wall areas in unripe fruit, and retained their structural and staining characteristics. Using immunolocalisation, Sutherland *et al.* (1999) showed an absence of cellulose or xyloglucan labelling, and identified specific pectin labelling, and the presence of callose. Therefore, plasmodesmatal regions are likely to be unaffected by cell-wall-degrading enzymes. This retention of unaltered plasmodesmatal regions would not only permit cell–cell interaction throughout softening but may also allow a degree of tissue integrity to be maintained as fruit softens.

FUTURE DIRECTIONS

This review clearly demonstrates the considerable progress that has been made in the past 10 years towards understanding the processes that occur during kiwifruit softening. But what of the future? We believe progress will accelerate, as tools developed for other plant species and tools developed specifically for kiwifruit are applied to cell wall research. In genomics research, HortResearch has developed a proprietary database of >130 000 expressed sequence tags (ESTs) from kiwifruit that were derived from a range of tissues and *Actinidia* species (Atkinson & MacRae in press). These databases have allowed identification of many cell wall gene homologues in kiwifruit — including, for example, large classes of expansins, xyloglucan endotransglucosylase/hydrolases, and β-galactosidases.

To understand global gene expression changes during softening and how expression is affected by different post-harvest storage treatments, HortResearch has developed a 17 k microarray of kiwifruit genes (Atkinson & MacRae in press). Genes on the array were derived primarily from *A. chinensis* and *A. deliciosa;* however, sequences from additional *Actinidia* species were also included. These additional *Actinidia* species exhibit a range of potentially interesting ripening and softening behaviours (White *et al.* 2004). *Actinidia eriantha* develops a peelable skin as the fruit ripen, *A. arguta* ripens in less than 10 days at 20°C *vs* 20–25 days for *A. deliciosa* and *A. chinensis* (Jackson & Harker 1997), whilst some small-fruited genotypes tend to remain firm even towards the end of the ripening process (White *et al.* 2004).

To determine the *in planta* role of cell-wall-related genes from the kiwifruit expressed sequence tags database, a high throughput functional genomics platform is used at HortResearch. Transformation systems are utilised for four *Actinidia* species (*A. deliciosa*, *A. chinensis, A. arguta,* and *A. eriantha*). Transgenic plants down-regulated in cell-wall-related genes including ACC oxidase, polygalacturonase, and transcription factors involved in peelability have been produced. Genes are also placed on an *A. chinensis* framework map (Fraser *et al.* 2004) to identify linkage between post-harvest storage/softening behaviour and genes involved in cell-wall-related processes.

The ultimate goal will be to understand the changes in the cell wall that lead to the physical changes observed in postharvest storage, and then to manipulate them on demand. Is this realistic? Maybe not in 10 years' time when the next kiwifruit cell wall review is written, but 10 years after that…?

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